



Green and biodegradable composite films with novel antimicrobial performance based on cellulose



Yuehan Wu^a, Xiaogang Luo^b, Wei Li^a, Rong Song^a, Jing Li^a, Yan Li^a, Bin Li^a, Shilin Liu^{a,*}

^a College of Food Science & Technology, Huazhong Agricultural University, Wuhan 430070, China

^b Key Laboratory of Green Chemical Process of Ministry of Education, School of Chemical Engineering and Pharmacy, Wuhan Institute of Technology, Wuhan 430073, China

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ABSTRACT

In order to obtain a safe and biodegradable material with antimicrobial properties from cellulose for food packaging, we presented a facile way to graft chitosan onto the oxidized cellulose films. The obtained films had a high transparent property of above 80% transmittance, excellent barrier properties against oxygen and antimicrobial properties against *Escherichia coli* and *Staphylococcus aureus*. The antimicrobial properties, mechanical properties, and water vapor permeability of composites are essential characteristics in determining their applicability as food-packaging materials. Moreover, using a sausage model, it was shown that the composites exhibited better performance than traditional polyethylene packaging material and demonstrated good potential as food packaging materials. The results presented a new insight into the development of green materials for food packaging.

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1. Introduction

Food packaging materials with proper mechanical strength, barrier properties against water, water vapor, thermal stability, recyclability, and biodegradability as well as functional properties such as antimicrobial activities are highly desirable for food safety and extending the shelf-life of packaged foods. Currently, the materials used in packaging industries are dominated by petroleum based plastic materials such as polypropylene (PP), polyethylene (PE) or polystyrene (PS) (Fabra, López-Rubio, & Lagaron, 2014). The usage of these kinds of materials not only consumes non-renewable petrochemicals, but also results in environmental pollution (Benbettaieb, Karbowiak, Brachais, & Debeaufort, 2016; Rochman et al., 2013). Furthermore, the plastic compounds and additives could migrate from packaging materials and have potential risks to food safety and human health (Bang et al., 2012; Guart, Bono-Blay, Borrell, & Lacorte, 2014; Guart et al., 2013). Therefore, research works have been focused on the development of biodegradable packaging materials using biopolymers to substitute petroleum based plastic packaging materials. A variety of biopolymers such as starch, agar, carrageenan, and gelatin have been used for this purpose (Rhim, Park, & Ha, 2013). However, their poor antibacterial, mechanical and processing properties with high production costs have been the main limitation in using the

biopolymer films for food packaging application (Kanmani & Rhim, 2014).

Cellulose, the most abundant biopolymer which can be extracted from wood, cotton, leaves and many other sources, is a natural linear organic compound consisting of d-glucopyranose units connected by β -1,4-glycosidic bonds (Johar, Ahmad, & Dufresne, 2012; Khan et al., 2012; Sheltami, Abdullah, Ahmad, Dufresne, & Kargarzadeh, 2012). Cellulose is environmental friendly and biodegradable, it has been successfully used as a packaging material for food preservation (Ghaderi, Mousavi, Yousefi, & Labbafi, 2014; Lu, Xiao, Zhang, & Gong, 2014). However, pristine cellulose lacks intrinsic antimicrobial properties which is useful for instance in the development of antimicrobial materials. Examples of such materials include cellulose nanocomposites with antimicrobial properties by the incorporation of inorganic nanoparticles (Jia, Mei, Cheng, Zhou, & Zhang, 2012; Martins et al., 2012; Yang, Xie, Deng, Bian, & Hong, 2012) or by adequate grafting of reactive groups onto cellulose nanofibers (Barbiroli et al., 2012; Rouabhia et al., 2014). Several techniques have also been reported to immobilize cationic antibacterial groups onto cellulose film surfaces, including tethering antimicrobial polymers onto cellulose film surface via chemical coupling reactions, surface initiated polymerization of monomers having antibacterial moieties, and synthesis of surface-tethered antimicrobial polymer brush via post-polymerization modifications, and self-assembled polyelectrolytes on the cellulose matrix (Abkenar & Malek, 2012; Dong, Qian, Zhao, He, & Xiao, 2014; Gomes, Mano, Queiroz, &

* Corresponding author.

E-mail address: slliu2013@mail.hzau.edu.cn (S. Liu).

Gouveia, 2013; Teli & Sheikh, 2012). A major challenge for these methods was that the reaction processes were complex and multi-step.

We have put an intensive research work on the cellulose dissolving and preparation of functional materials from the dissolved solution (Liu, Yan, Tao, Yu, & Liu, 2012; Liu, Yu, Wu, Li, & Li, 2014; Wu, Zhang, Li, & Liu, 2014). In this work, we presented a facile way to prepare highly transparent antimicrobial films by grafting chitosan onto the oxidized cellulose matrix. This work was inspired by the chemical structure and novel antibacterial properties of chitosan. Chitosan is an ammonium salt, and the amino group in chitosan could link with aldehyde group in cellulose matrix through the Schiff base reaction. The obtained composite films had integrated the merits of cellulose and chitosan, they were characterized with the preservation of sausage and obvious antibacterial performance was observed. The results added new insight into the potential of this processing method for the development of biodegradable food packaging applications.

2. Experimental

2.1. Chemicals

Native cellulose (Cotton linter, α -cellulose $\geq 95\%$) was supplied by Hubei Chemical Fiber Co. Ltd. (Xiangfan, China), and its viscosity-average molecular weight (M_v) was about 1.07×10^5 , which was determined in cadoxen at 25 °C. Sodium periodate, chitosan and other chemicals with analytical grade were purchased from Shanghai Aladdin Industrial Inc. Deionized water was used for the experiments.

2.2. Preparation of regenerated cellulose (RC) films and composite films

The native cellulose was dissolved by the freezing-thawing method (Liu et al., 2014). Briefly, powder-like filter paper (Native cellulose) was dispersed into aqueous lithium hydroxide/urea solution (8.7 wt%/12.0 wt%), and then put it in a refrigerator for 12 h, after frozen, took it out and thawed at room temperature to obtain a transparent cellulose solution (4 wt%). The resultant cellulose solution was subjected to centrifugation at 7000 rpm and 4 °C for 8 min to eliminate some bubbles in the viscous solution. The viscous bubble-free solution was cast on a glass plate and the thickness of the solution was controlled to be about 1 mm, and then immersed it into coagulation bath containing 5 v% H_2SO_4 to regenerate for 10 min. The regenerated cellulose films (RC) were washed with deionized water and then immersed into Na_5IO_6 solution (5 wt%) for 0.5, 1, 2 and 3 h to obtain oxidized cellulose (OC) films. The OC films were immersed into 2.5 wt% chitosan solution for 3 h, then washed with deionized water and dried at ambient conditions to obtain OC/CS composite films. The process was presented in Scheme 1. The composite films prepared from cellulose with different oxidation time were named for CS-0.5, CS-1, CS-2, CS-3, respectively.

2.3. Determination of oxidation degree

The oxidation degree (OD) of the cellulose films was determined according to the reported method based on the Schiff base reaction between aldehyde groups and hydroxylamine hydrochloride (Cheng et al., 2014). The oxidation degree was calculated through the following equation:

$$OD(\%) = \frac{161(V_2 - V_1)}{C_{(HCl)} \times 10^{-3}} \times 100 \quad (1)$$

where V_1 is the amount of hydrochloric acid for sample titration in mL, V_2 is the amount of hydrochloric acid for control titration in mL, $C_{(HCl)}$ is the concentration of hydrochloric acid in mol/L, and 161 is the average molecular weight when glucose units were translated into 50% dialdehyde.

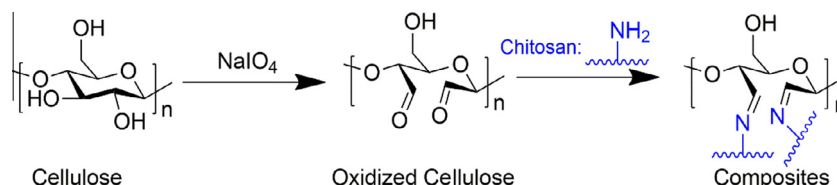
2.4. Characterization

The Fourier-transform infrared (FT-IR) spectra of the samples were characterized with FT-IR spectrometer (Nicolet Nexus 470). The samples were ground into powders, and mixed with KBr and pressed to form a sample disk for the tests. The optical transmittance of the films was carried out on an UV-Visible spectrometer (Shimadzu UV-1700) from 300 to 800 nm using air as a reference, the thickness of the cellulose, and composite films was about 33 μm . The surface morphology of the samples was characterized by scanning electron microscopy (SEM) (Hitachi S4800, Japan). X-ray photoelectron spectroscopy (XPS) was performed by using an axis ultra DLD apparatus (Kratos, UK). The mechanical properties of the films were characterized with a tensile tester (CMT 6503, Shenzhen SANS Test machine Co. Ltd., China) according to ASTM/D638-91 with a speed of 2 mm min⁻¹. Thermogravimetric analysis (TGA) was carried out on a Pyris TGA linked to a Pyris diamond TA Lab System (Perkin-Elmer Co., USA) at a heating rate of 10 °C min⁻¹ from 40 to 700 °C under nitrogen atmospheres. The swelling ratio (Q) of the films was characterized by a gravimetric method (Li, Wu, Liang, Li, & Liu, 2014). A known amount of dried film was immersed in distilled water at room temperature. The weight of the film was directly measured after gently wipe off the water on the surface at different times. The swelling ratio (Q) was calculated using the following equation:

$$Q = \frac{W_h - W_d}{W_d} \quad (2)$$

where W_h was the weight of the swollen film and W_d was the weight of the dried sample. Three replicates for each sample were performed to obtain an average value of Q .

The water vapor transmission rate (WVTR) of the films was calculated in accordance with ASTM E96-00 standard test method (ASTM, 2010). Saturated aqueous solutions with different salts were used to establish different relative humidity (RH) inside the test chamber and weighing bottles were used in the test. Certain amount of anhydrous $CaCl_2$ was added to the weighing bottle to maintain 0% RH in the bottle, the test film was placed over the mouth of the weighing bottle and then, molten paraffin was



Scheme 1. The process for the preparation chitosan grafted cellulose composites through the oxidation of regenerated cellulose.

applied to seal the film over the rim of the weighing bottle. The weighing bottle was placed in sealed jars containing pure water (100% RH), saturated Na_2SO_4 (93% RH), NaCl (75% RH) or MgCl_2 (33% RH) solution in 30 °C. The RH in the jar was kept by placing 1000 mL of distilled water in the bottom of the jar. The WVP was determined from the weight gain of the permeation cell every 12 h until a constant rate of weight gain was attained. WVTR and water vapor permeability (WVP) were calculated from Eq. (3):

$$\begin{aligned} \text{WVTR} &= \frac{\Delta w}{\Delta t A} \\ \text{WVP} &= \frac{\text{WVTR} L}{\Delta p} \end{aligned} \quad (3)$$

where WVTR was in grams per second squared meter, $\Delta w/\Delta t$ was rate of water gain in grams per 12 h, A was the exposed surface area (m^2), L was the mean thickness (m) of samples, and Δp was the difference in partial water vapor pressure between the two sides of film in Pascal.

The oxygen permeability rates of the cellulose film and composite films were, respectively, determined at 23 °C and 40% RH conditions using an oxygen permeation analyzer (VAC-V3, Lacbthink Instrument, Jinan, China) according to the Chinese National Standard GB/T 1038–2000. The oxygen permeability (P_g) was calculated using the following equation:

$$P_g = \frac{\Delta p}{\Delta t} \times \frac{V}{S} \times \frac{T_0}{p_0 T} \times \frac{D}{(p_1 - p_2)} \quad (4)$$

where P_g is the oxygen permeability ($\text{cm}^3 \text{ cm}/\text{cm}^2 \text{ s Pa}$), $\Delta p/\Delta t$ (Pa/h) is the average value of the pressure change of the low pressure chamber in unit time after oxygen permeation is stable, V (cm^3) is the volume of the low-pressure chamber, T_0 is standard temperature (273.15 K), S (m^2) is the test area of the test film, p_0 is standard pressure ($1.01 \times 10^5 \text{ Pa}$), T (K) is the temperature of test, $(p_1 - p_2)$ is the pressure difference between the two sides of test film (Pa). D is the thickness of test film (cm). The thickness of the films was measured immediately before testing. Average thickness was calculated from five measurements.

All permeation data were determined for at least three samples in order to confirm the reproducibility of the experimental results.

2.5. Antimicrobial activity

The antimicrobial activity was characterized according to the reported method with minor modification (Zhou, Liu, Qi, & Zhang, 2006). *Escherichia coli* and *Staphylococcus aureus* were selected as the represented bacteria of gram-negative bacterium and gram-positive bacterium. They were cultured in nutrient broth medium at 37 °C for 24 h before usage. The mid-exponential growth phase bacterium after being cultured overnight was diluted with physiological saline to approximately 10^7 CFU/mL . The RC film and the composite films were cut into disk with diameter of 25 mm, and then ultraviolet sterilized for 30 min. The disks were put in sterile nutrient agar plate and inoculated with 500 μL of bacterial suspension, respectively. After inverted cultured avoid light at 37 °C for 24 h, the agar plates were examined and the numbers were counted manually.

2.6. Application of antimicrobial packaging on sausage

Sausage was purchased from local supermarket. 200 g sausage was cut into 10 equal small cubes by weight and the divided into three parts as test group and two control groups. The test group was packaged with CS-2 film while the other two control groups were packaged with RC film or PE wrap. The sausages was sampled and tested on days of 0, 1, 4 and 10 for microbial and physicochemical properties. The sample was homogenized with 180 mL non-ammonia purified water and impregnated for 30 min. Then resul-

tant solution was filtered to obtain sausage infusion. The pH of the infusion was measured by a digital pH meter (Mettler-Toledo Co., Ltd.). Total volatile basic nitrogen (TVB-N) was measured by stream distillation method through a semi micro steam distillation device (Cai, Chen, Wan, & Zhao, 2011). For determination of the total number of bacteria in sausage, serial dilutions from 10^{-1} to 10^{-5} were prepared; the microbial were cultured on plate count agar (PCA) at 37 °C for 24–48 h.

3. Results and discussion

The synthesis route of the composites was presented in Scheme 1. Periodate is a specific oxidant capable to oxidize the vicinal hydroxyl groups at carbon atoms 2 and 3 in an anhydroglucose unit of cellulose to two aldehyde groups and meanwhile breaking the C–C bond between the carbon atoms 2 and 3. This cleavage results in the formation of the two aldehyde groups per glucose unit, forming 2,3-dialdehyde cellulose (Guigo, Mazeau, Putaux, & Heux, 2014). After the dialdehyde cellulose films were immersed into the chitosan solution, the amino group of chitosan combined with aldehyde groups in dialdehyde cellulose by C=N bond through Schiff base reaction.

The successful oxidation of cellulose was confirmed with FT-IR. Fig. 1a shows the FT-IR image of OC films. The strong and broad band centered at $\sim 3400 \text{ cm}^{-1}$ was assigned to the stretching mode of –OH groups involved in different hydrogen bonding interactions.

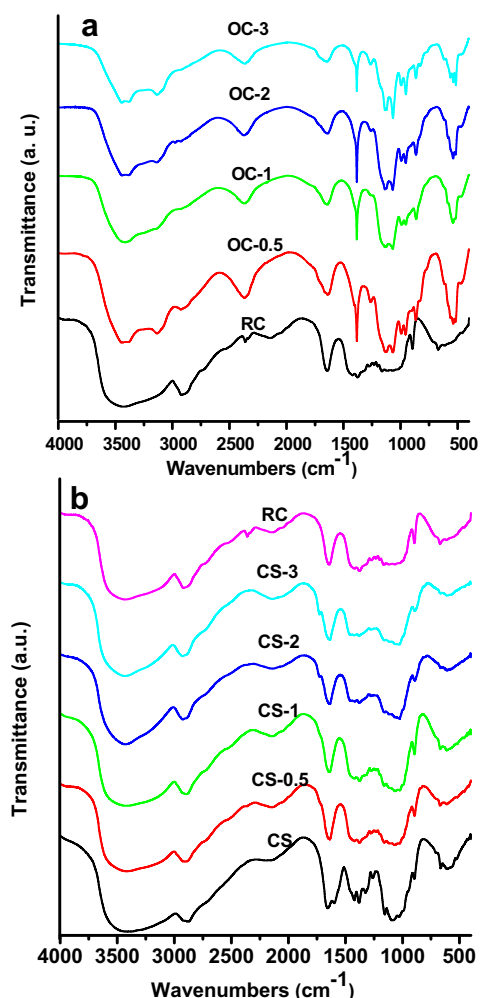


Fig. 1. FT-IR spectra of the oxidized cellulose films (a) and the composite films (b).

They were correlated with -OH of cellulose and molecular water (in the H-O-H deformation region, at $\sim 1648\text{ cm}^{-1}$). Oxidized cellulose exhibited two characteristic FT-IR bands near 1740 and 880 cm^{-1} , which were attributed to C=O stretching and the formation of hemiacetal bonds between newly achieved aldehyde groups and their neighboring hydroxyl groups. The oxidation degree of the oxidized cellulose films for 0.5, 1, 2 and 3 h were 3.43%, 5.05%, 13.92% and 18.94%, respectively. It indicated that the oxidation degree of the cellulose films increased with the increasing oxidation time. The aldehyde groups of dialdehyde cellulose have high reactivity towards further modification such as to react with the amino group of chitosan via Schiff base reaction.

The RC film prepared from this solvent exhibited a porous structure consisting of fibrils that tangled with each other and formed interconnected networks. This unique structure was ascribed to the phase separation of the cellulose solution during the regeneration process, where solvent-rich regions contributed to the formation of pores (Liu et al., 2011). When the RC films were treated with NaIO_4 , the microstructure was totally different from that of RC, as shown in Fig. 2. For the RC film treated with NaIO_4 for short time, the cellulose fibrils could be observed from the surface morphology, while some compact structure was also present. The original structure of cellulose molecular was destroyed by oxidation performance of NaIO_4 , the formed aldehyde groups resulted in the increasing the H-bonding interactions between the network structures of the films. With the increasing of the oxidation time, the number of aldehyde groups increased led to increasing the H-bonding, the surface of the films became more and more rough, and film-like morphologies were observed.

After the grafting of chitosan to the oxidized cellulose through Schiff base reaction, the composite films exhibited a different morphology (Fig. 3) when compared to that of RC or OC films. The composite films exhibited smooth structure and it was hard to detect pore on the surface of the composite films, indicating OC film reacted with chitosan, and the grafted chitosan covered the matrix of the OC. As shown in Fig. 2b, the FT-IR image confirmed that chitosan were successfully crosslinked on the OC matrix. Compared

with OC films, the absorption peak appeared at 1740 cm^{-1} which was ascribed to the stretching vibration of the aldehyde group became weak. Meanwhile, two significant variation of OC/CS films at 1640 cm^{-1} and 1530 cm^{-1} that was assigned to the -C=N- band and amide II band was observed, separately. There was an obvious variation at $\sim 1100\text{ cm}^{-1}$, which could be ascribed to the -C-N- band of OC/CS films. To further verify the FT-IR results, the XPS analysis was used to investigate the elemental compositions of the composite films. S3 showed the XPS image of CS-3 that the C 1s and O 1s peaks could be easily observed. The C 1s peak in 286.1 eV was attributed to the sp^2 -type C=N bonds, while peak near 399.6 eV was $\text{sp}^3\text{ N-C}$ bonds. (Mahdavi, Abedini, Bagherpour, & Heidari, 2015). The results indicated that the chitosan had been grafted on the OC films.

The pure RC film had a swelling ratio of about 1.2, while the CS-0.5 and CS-1 had the swelling ratio of about 0.4, and with the increasing of oxidation, the swelling ratio of CS-2 and CS-3 film was about 0.9. It was obvious that the water uptake ability of the composite films decreased compared with the RC films. This indicated that after oxidation, the OC film exhibited a more compact structure, and after loading of the chitosan in the OC matrix, the pores in OC matrix were occupied by the grafted chitosan, there was less space in the polymeric matrix to accommodate water. S2 shows the water vapor transmission rate (WVTR) and the water vapor permeability (WVP) of the composite films. The WVTR and the WVP were also characterized at different relative humidities of 33%, 75%, 93% and 100% at 30°C . The WVP value of the RC film and composite films increased with the increasing of the relative humidity. It indicated that there was a slight increase in the WVP of the composite films when compared with that of RC, however, for practical applications, the WVP of the composite and cellulose films also could be improved through surface coating method.

The mechanical behavior of the RC and the composite films was investigated by using tensile testing at room temperature. Fig. 4a shows the stress-strain curves of the materials. The tensile strength and Young's modulus were showed in S2. It was obvious

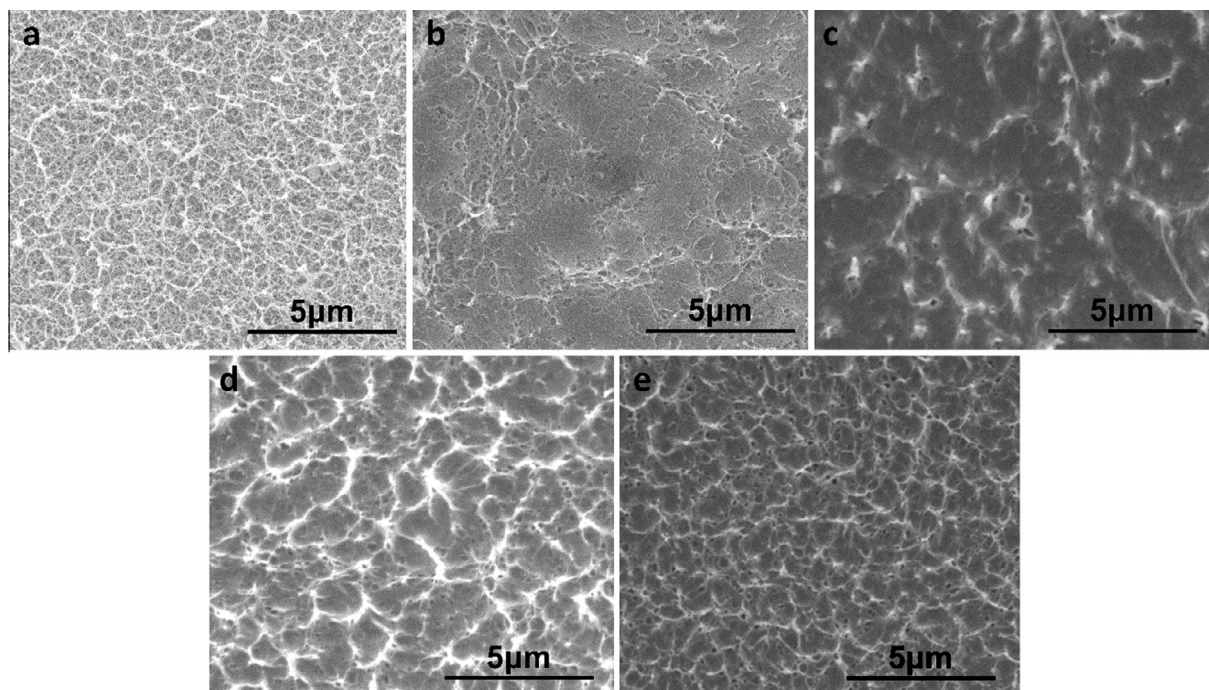


Fig. 2. SEM images of the surface morphologies of the pure cellulose film (a) and the cellulose films with different oxidation time of 0.5 h (b), 1 h (c), 2 h (d) and 3 h (e).

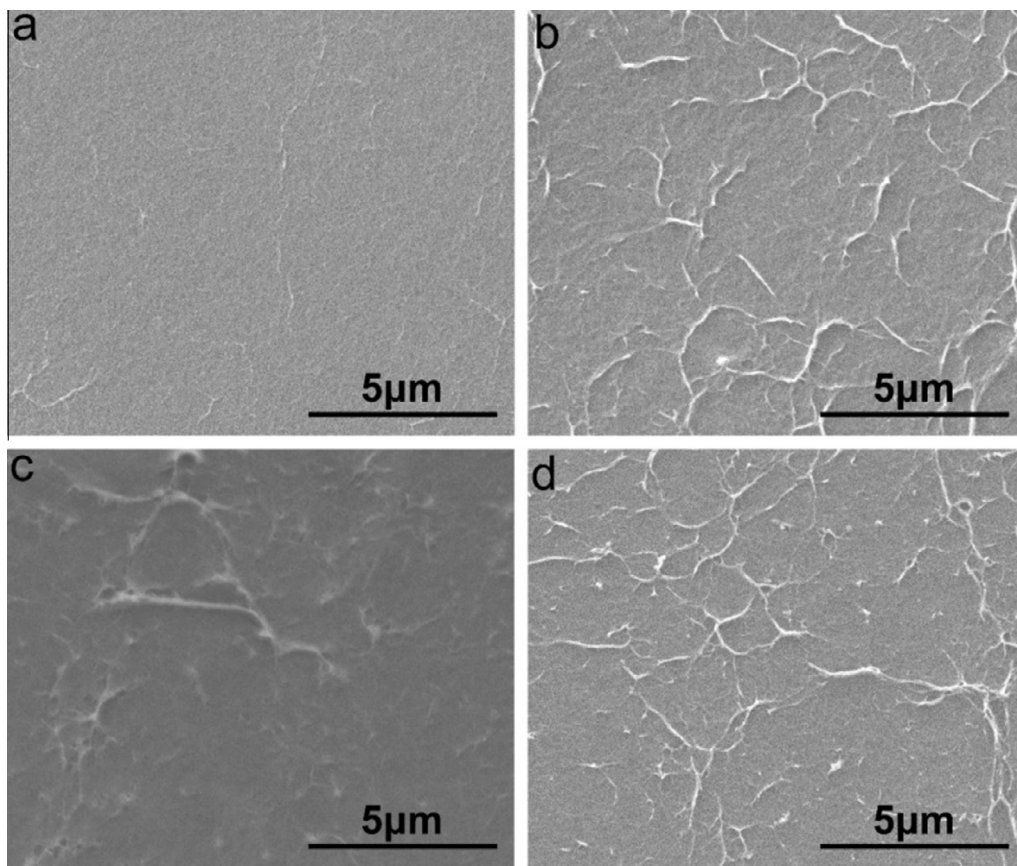


Fig. 3. SEM images of the surface of CS-cellulose composite films with different time of oxidation of 0.5 h (a), 1 h (b), 2 h (c) and 3 h (d).

that the tensile strength of the composites were much higher than the RC films. It indicated that the mechanical property of the composite films was not destroyed by the oxidation process of cellulose for the grafting reaction. The increased mechanical strength of composite films was important for their potential application as food packaging materials. The transmittance of the RC and composite films were shown in Fig. 4b. The RC film and composite films were optically transparent; the transmittances of CS-2, CS-3 were about 90%, while for the RC, CS-1 and CS-0.5, it was about 80% at 550 nm. It was well known that the surface reflection had a negative impact on the transmittance of the composite films that resulted from the difference in the refractive index. The relationship between light reflection and RI was described by using the following equation:

$$\Gamma = [(n_R - n_F)/(n_R + n_F)]^2$$

where, Γ was the reflective coefficient and n_R and n_F were RIs of chitosan and cellulose, respectively. Therefore, the higher the difference between RIs of the chitosan and cellulose, the higher the reflective coefficient obtained for the composites. With the grafting of chitosan onto the cellulose matrix, the cellulose/air interfaces were replaced by three types of interfaces, i.e., cellulose/chitosan, cellulose/air, and chitosan/air in the composite films. The increased interfaces would have a negative influence on the transmittance of the composite films. However, on the other hand, the loaded chitosan had an effect on the microstructure of the composites, resulting in a denser structure for the composite films, as it was shown in Fig. 3. The diffuse reflection of the light in the films decreased. Therefore, the composite films would have good optical transmittance.

S1 shows the thermal stability the composites, it was determined by using thermogravimetry in N_2 atmosphere. It indicated

that the composite films exhibited good thermal stability. There was a small weight loss below 100 °C for the films which resulted from the release of moisture from the samples. There was a slight mass loss until 250 °C and on further heating there was a sharp weight loss occurred at 300 °C for the composite films.

The oxygen permeability (P_g) of the films was determined at 23 °C and 40% RH conditions. The composite films had excellent oxygen barrier property that the oxygen permeability were very low. The oxygen permeability of CS-0.5, CS-1, CS-2, CS-3 were 8.93×10^{-15} , 7.63×10^{-15} , 5.43×10^{-15} and 4.32×10^{-15} $\text{cm}^3 \cdot \text{cm}/\text{cm}^2 \cdot \text{s} \cdot \text{Pa}$, respectively. The pure cellulose films had little oxygen barrier property, the oxygen permeability of RC films were beyond the detection limit of the oxygen permeation analyzer. The RC films were a porous structure that gas was easy to cross it. After grafting with chitosan, the pores were blanketed the pores that oxygen were hard to cross the composites. The excellent barrier properties of the composite films would be of great relevance for their application.

Chitosan had stronger antibacterial activity against gram negative bacteria than gram positive bacteria (Cruz-Romero, Murphy, Morris, Cummins, & Kerry, 2013), and the composite film showed the same phenomenon. The antimicrobial activity of composites films against *E. coli* and *S. aureus* was investigated, and the results were shown in Fig. 4c. RC film had no antimicrobial activity, and the bacteria grew rapidly on its matrix. It is obvious that the number of bacteria on CS-1 was three orders of magnitude difference less than the number of bacteria on RC films, it indicated the bacteria grew very slowly or even stop growing. The bacteria on CS-2 and CS-3 films declined rapidly that the composites killed more than 99% bacteria attached on them. It indicated that the composite membrane had good antibacterial properties to *E. coli* and *S. aureus*. The antibacterial performance increased with the increas-

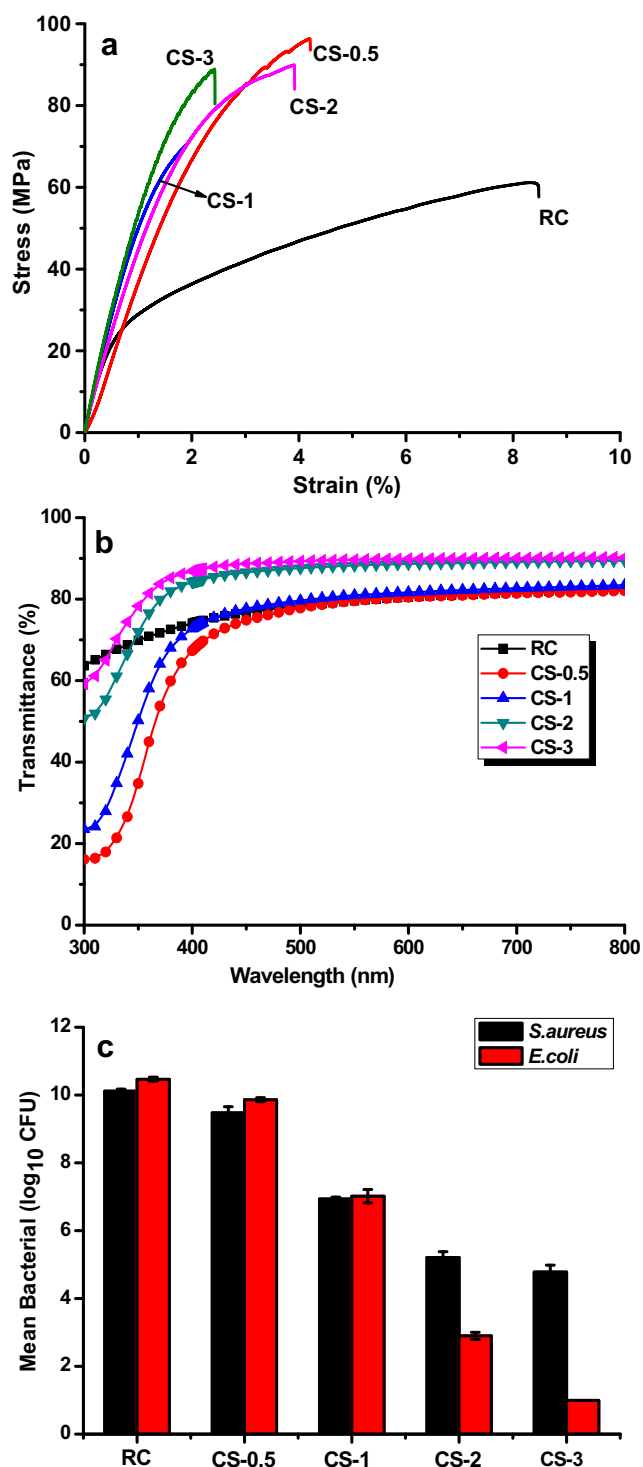


Fig. 4. Optical transmittance spectra in the wavelength from 300 to 800 nm (a), tensile stress-strain behaviors (b) and antimicrobial ability (c) of the cellulose and the composite films with different oxidation degree after being dried at ambient conditions.

ing the content of grafted chitosan. For antimicrobial test, the used concentration of bacteria was much higher than the total bacteria on fresh food or in the air, the result indicated that the composites had potential application in antibacterial packaging materials.

Fig. 5 shows the antibacterial performance of the composite films used as packaging materials for sausage, and the comparison between PE and RC was also shown. The pH of sausage decreased

from 6.8 to 6.2 over the 10 days storage period, which could be attributed to the accumulation of basic compounds such as ammonia due to microbial action. However, the pH value for the test group changed more slightly than other groups. Fig. 5a shows the total bacteria number in sausages coated with different materials. The total bacteria on sausages coated with CS-2 film were fewer than the sausages coated with control groups during the 10 days storage period. It indicated that the chitosan grafted on the composites killed the bacteria on the sausage. It is obvious that the total number of bacteria on sausage coated with CS-2 film on the tenth day was similar to the sausage coated with PE wrap on the fourth day. It can be inferred that the CS-2 film could extend shelf-life of at least for 6 days compared with PE wrap. The results could be confirmed by the TVB-N value. The TVB-N value, an indicator of spoilage, was mainly composed of ammonia salt by microbial actions and decomposition. The TVB-N value of all the samples increased gradually during the storage period. Changes of TVB-N in sausage during storage were showed in Fig. 5b. It was obvious that the TVB-N value of sausages which were coated with CS-2 film were lower than the sausages coated with RC film or PE wrap, and the results were similar to the total bacteria number of sausages. The CS-2 film showed an excellent antimicrobial property on the sausage model compared to common commercial packaging products PE wraps.

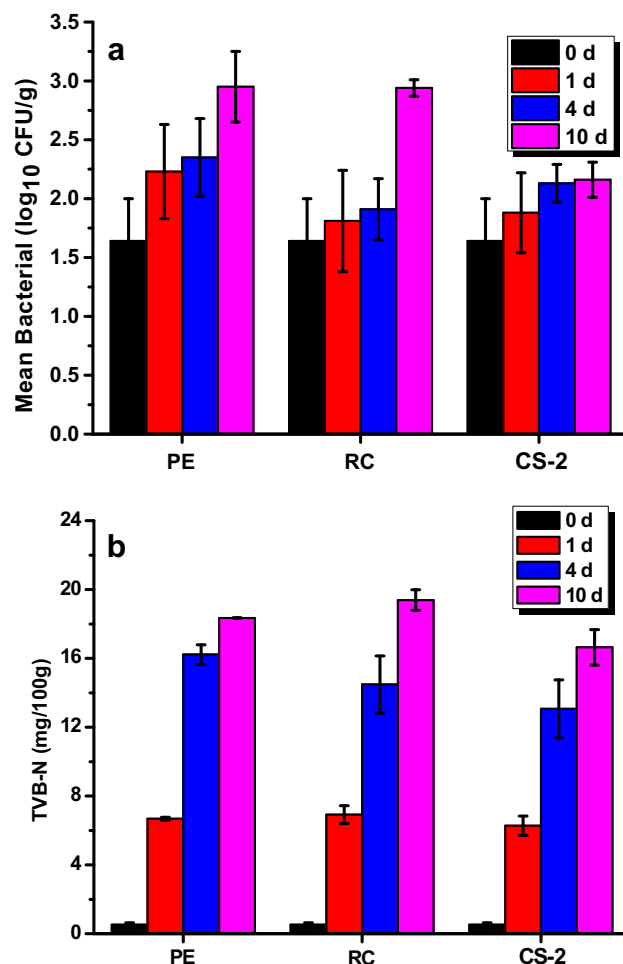


Fig. 5. Total number of bacteria (a) and total volatile basic nitrogen (TVB-N) (b) of model control group (PE wrap), control group (RC film) and test group (CS-2) in sausage during 10 days storage period.

4. Conclusions

In summary, we presented a facile way to obtain highly transparent antimicrobial films by grafting of chitosan onto the oxidized cellulose matrix. The chemical process changed the microstructure of the RC matrix, but resulted in little destroy in the mechanical performance and greatly improved the barrier property against oxygen of the composite films after grafting chitosan. The composites could kill more than 99% bacteria attached on them and were able to extend shelf-life of sausage at least for 6 days. The obtained composite films had high transparent and excellent antimicrobial properties, it integrated the merits of the cellulose and chitosan.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2015.10.127>.

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