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Cooperative performance of chitin whisker and rectorite fillers on chitosan films

Xiaoxia Li[a,1], Xueyong Li[b,1], Beilei Ke[a], Xiaowen Shi[a], Yumin Du[a,*]

[a] Department of Environmental Science, School of Resource and Environmental Science, Wuhan University, Wuhan 430079, China
[b] Department of Plastic Surgery, Tangdu Hospital, Fourth Military Medical University, Xi'an 710038, China

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ABSTRACT

We prepared chitosan/chitin whisker/rectorite ternary films and studied the cooperative effects of nanofibril whisker and layered organic rectorite on the properties of chitosan matrix. The ternary film exhibited higher thermal stability and smoother surface than the whisker or rectorite reinforced binary films due to the intercalation of chitosan chains into the layered rectorite and the impregnation between the clay and rod-like whisker. With increasing the ratio of mixed fillers from 0 to 229 mg/g, the break elongation first increased to a maximum value (144%) with the mixed fillers of 114.5 mg/g and then decreased. Antimicrobial assay against *Escherichia coli* and *Staphylococcus aureus* showed that the ternary films had higher antimicrobial effect than the pure or binary films because of the significant adsorption capacities of the enlarged interlayer of rectorite. Overall, the chitosan-based ternary film with chitin whisker and rectorite proves to be a suitable material for food-packaging applications.

1. Introduction

Petroleum-based packaging materials are arising increasingly serious environmental issues and food safety these years. Current research has focused on the use of bio-based compounds (Petersen et al., 1999). The potential use of polysaccharides in food packaging is of great interest owing to their specific properties. Chitosan (CS), a linear polysaccharide of randomly distributed β-(1-4)-linked D-glucosamine and N-acetyl-D-glucosamine, is a deacetylated derivative of chitin and considered as an environmentally friendly material. Chitosan offers real potential for applications in food industry due to its non-toxicity, excellent film-forming property and antimicrobial activity (No, Meyers, Prinyawiwatkul, & Xu, 2007).

Chitosan coatings have been successfully probed at an experimental level on food such as eggs, fruits, vegetables, dairy products and meats (Kim, No, & Prinyawiwatkul, 2007; Lin et al., 2011; Yingyuad et al., 2006). The antibacterial mechanism comes from its positive charges, which would interfere with the negatively charged residues of macromolecules on the microbial cell wall and cause leakage of fluids (Liu, Du, Wang, & Sun, 2004; Muzzarelli et al., 1990). However, the antibacterial activity of chitosan is more available in a coating solution than in a film matrix (Aider, 2010). For example, the chitosan and starch mixture solution showed inhibitory effect against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis*, whereas the chitosan–starch composite film was proved to be negative (Dutta, Tripathi, Mehrotra, & Dutta, 2009). In order to improve the antimicrobial activity of the films, functional substances such as minerals, organic acids and essential oils were incorporated into the chitosan matrix (Dutta et al., 2009; Kanatt, Chander, & Sharma, 2008; Pelissari, Grossmann, Yamashita, & Pineda, 2009). Rectorite is a type of layered silicate commonly used to prepare polymer matrix nanocomposite in recent years (Srodon, 1999; Wang, Zhang, Wu, Yang, & Zhang, 2005). Chitosan/rectorite composites have been reported with great antibacterial activity because of the positively charged amino groups on chitosan and the bacteria adsorption capacity of the layered organic rectorite (Wang et al., 2006). However, high amount of rectorite may cause the collapse of the composites (Ma, Lu, Liang, & Yan, 2004; Wang, Du, Luo, Lin, & Kennedy, 2007).

Though chitosan has been used in the field of food packaging industry, especially as safe food preservation and edible films, the inherent water sensitivity and relatively low mechanical properties restrict its use in a broader range of application (Sebti, Chollet, Degraeve, Noel, & Peyrol, 2007). To suit the high strength/high modulus requirements, many reinforcing fillers have been used to modify these kinds of biopolymer-based films (Chang, Jian, Yu, & Ma, 2010; de Mesquita, Donnici, & Pereira, 2010; Lu, Weng, & Zhang, 2004). Chitin is known to form microfibrillar arrangements in living organisms. Chitin whiskers have been prepared by acid hydrolysis and successfully used as reinforcing fillers in polymeric matrices due to its high aspect ratios and highly crystalline nature (Lu et al., 2004; Muzzarelli & Muzzarelli, 2005; Nair & Dufresne, 2003; Watthanaphanit, Supaphol, Tamura, Tokura, & Rujiravanit, 2010; Wongpanit et al., 2007). However, the number of

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[*] Corresponding author. Tel.: +86 27 68778501.
E-mail address: duyumin@whu.edu.cn (Y. Du).
[1] Co-first author with the same contribution to this work.

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amino groups on the chitin whiskers was less than that on chitosan (Phongying, Aiba, & Chirachanchai, 2007), and the incorporation of chitin whisker would not show improvement of antibacterial effect for the chitosan matrix.

Based on the preferable antibacterial activity of rectorite–chitosan composites and strength enhancement of chitin whisker, the chitosan/chitin whisker/rectorite ternary films were prepared in this study, in order to develop a suitable material for food-packaging applications. The cooperative effects of the fillers on the film structure and properties were also investigated in detail.

2. Experimental

2.1. Materials

Chitin was supplied by Dalian Xindie Co., Ltd. (Dalian, China) and calcium rectorite was provided by Hubei Mingliu Inc. Co., Ltd. (Wuhan, China). Chitosan with the average molecular weight of 2.1×10^5 and 80% deacetylation degree was obtained from Qingdao Yunzhou Biochemical Co., Ltd. (Qingdao, China).

2.2. Preparation of chitin whiskers and organic rectorite

Chitin whisker was prepared as previously described by Nair and Dufresne (2003) with some modifications. In brief, 1 g of chitin was treated in 100 mL of 3 N hydrochloric acid at 104 °C for 90 min. The residue was collected after centrifugation, treated with hydrochloric acid for another two times, and finally dialyzed in distilled water until it was neutral. Solid content of the as-prepared whisker suspensions was about 4.58 mg/mL. Rectorite was organic modified by cation exchange between Ca^{2+}-rectorite galleries and cetyltrimethyl ammonium bromide, according to the previous work (Wang et al., 2006).

2.3. Preparation of composite films

The ternary composite film was prepared by a casting-solvent evaporation technique. Mixed fillers with equal mass of chitin whisker and organic rectorite were first dispersed in distilled water by ultrasonic treatment for 10 min. Chitosan was dissolved in 1% (v/v) aqueous acetic acid to obtain the 3% (w/v) solution, and then was added slowly into the pretreated filler suspension and stirred to obtain a homogeneous dispersion with chitosan content of 2% (w/v). The resulting mixture was cast into a dish and placed at 50 °C for 6 h. After neutralized in a 2% NaOH (w/v) for 30 min, the obtained composite film was washed with water. Pure chitosan films, chitosan/chitin whisker and chitosan/rectorite composite films were prepared as controls. In the ternary and binary films, the ratio of total fillers to chitosan in the composites was changed from 0, 22.90, 57.25, 114.50, 171.75, and 229.00 mg/g respectively.

2.4. Characterization of composite films

Upper surface morphologies of the films were examined using a scanning electron microscopy (Model Quanta 200, FEI, Dutch). XRD experiment was performed by a D8 Advance diffractometer (Bruker, USA) with Cu target and Kα radiation ($\lambda = 0.154$ nm) at 40 kV and 40 mA. The scanning rates were 0.8 and 6°/min with scanning scopes of 0.5–6° and 5–35° respectively. The thermal behavior was monitored using a TG-DSC instrument (STA449C, Jupiter, Netzsch, Germany). About 4 mg of sample was heated in a corundum crucible from 50 to 400 °C at a heating rate of 20 °C/min under an air atmosphere.

Mechanical properties including tensile strength and percentage of elongation at break were measured with a universal testing machine (CMT6503, Shenzhen SANS Test Machine Co. Ltd., China) following the ASTM Standard Test Method D 882-91. To obtain the mechanical properties of the films in wet state, the films were soaked in water for 30 min at room temperature before testing. Each film strip (10 mm × 100 mm) was mounted with the gauge length and the crosshead speed of 40 mm and 10 mm/min, respectively. The reported values were averages from five measurements.

Water uptake of the films was determined by swelling degree between the initial weight of the dry films and the equilibrium weight after water absorption. Opacity was determined according to the method of Park and Zhao (2004) by measuring the film absorbance at 600 nm using a Varian Cary 500 Scan UV-Vis spectrophotometer. Results were reported as absorbance divided by film thickness (mm) based on three samples.

2.5. Antibacterial assay of composite films

Disk-diffusion method was used to investigate the inhibitory effect of the ternary films against *E. coli* and *S. aureus*. The inhibition zone assay was conducted by inoculating a tryptose agar medium with 50 μL of diluted bacterial. Film disks (diameter = 7.5 mm), after UV sterilization, were placed on the Petri dishes and then inoculated at 37 °C for 24 h. The inhibition zones were measured with a tolerance of 1 mm. Each sample was repeated three times.

3. Results and discussion

Chitin whisker and organic rectorite were prepared as previous references (Nair & Dufresne, 2003; Sriupayo, Supaphol, Blackwell, & Rujiravanit, 2005; Wang et al., 2006); because their structures have been well characterized, it will not be discussed here. An examination of the morphology of the composite films was carried out using SEM. As shown in Fig. 1(a) and (b), smooth surfaces were obtained from both the neat chitosan and whisker-reinforced chitosan films. The phenomenon can be attributed to the fine interfacial interaction between chitin whisker and chitosan matrix, both of which are coming from the similar structures of the same source. The rectorite-reinforced binary and ternary films with the total fillers of 229 mg/g were much rougher, with an evidence of the aggregates of rectorite appearing on their surfaces, the same as the morphology of the polyvinylpyrrolidone-layered silicate nanocomposite (Koo, Ham, Choi, Kim, & Chung, 2003). Furthermore, the ternary film exhibited much smoother surface than the binary one. It is reasonable that the amount of rectorite in the ternary film was only half of that in the binary one. This surface difference can be expected to greatly influence the miscibility between fillers and the matrix.

Optical transparency is an auxiliary criterion for the miscibility of the composite materials. As shown in Fig. 2, the opacity of whisker-reinforced films slightly decreased with increasing whisker concentration, suggesting the complete incorporation of chitin whisker in the chitosan matrix. With the increase of organic rectorite in chitosan matrix, the optical transmittance increased gradually, which could be ascribed to the aggregation of clay particles. The values of opacity decreased much in the ternary composite films. It is because that the amount of rectorite was only half of that in the clay-reinforced binary films when they contained the same amounts of total fillers. The decrease of rectorite and incorporation of chitin whisker in the films improved the compatibility of the fillers and matrix, and the interaction of the fillers would change the structure and properties of chitosan matrix.

Small angle X-ray diffraction was used to measure the ordered-structural of the materials. Evidently, neither neat chitosan nor chitosan/chitin whisker films exhibited any diffraction peaks in the range of 1–6°, while the rectorite-reinforced binary and ternary films showed diffraction peaks at 2θ around 2.2° (Fig. 3). The inter-

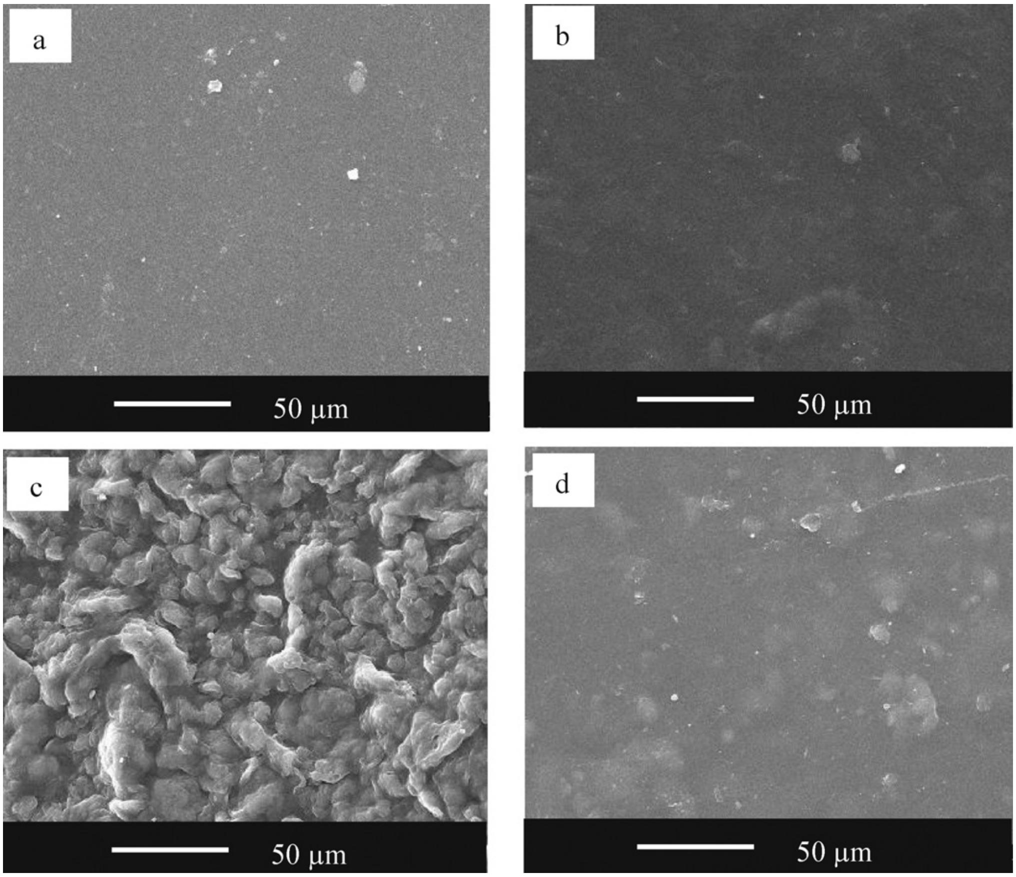

Fig. 1. SEM of (a) chitosan film, (b) chitosan/chitin whisker film, (c) chitosan/rectorite film and (d) chitosan/chitin whisker/rectorite ternary film with weight ratios of fillers to chitosan of 229 mg/g.

layer distances of rectorite in the composite films were around 3.9 nm calculated by the Bragg's equation, which were larger than that of rectorite itself ($2\theta = 3.16^\circ$, interlayer distance = 2.94 nm), suggesting chitosan chains had inserted into the layered silicates (Wang et al., 2006). Interestingly, comparing with the ternary films with rectorite of 57.25 mg/g and the binary film with total fillers of 114.5 mg/g (the amount of rectorite was also 57.25 mg/g), the interlayer distance was 3.87 nm in the binary film whereas the value increased to 3.98 nm in the ternary film (Fig. 3(c) and (f)). This observation could probably be interpreted as the effect of chitin whiskers. Usually, chitin whisker nanofibrils consist of slender rods with sharp point, and the lengths range up to several hundred nanometers while the width ranged from a few to tens of nanometer (Morin & Dufresne, 2002; Nair & Dufresne, 2003; Sriupayo et al.,

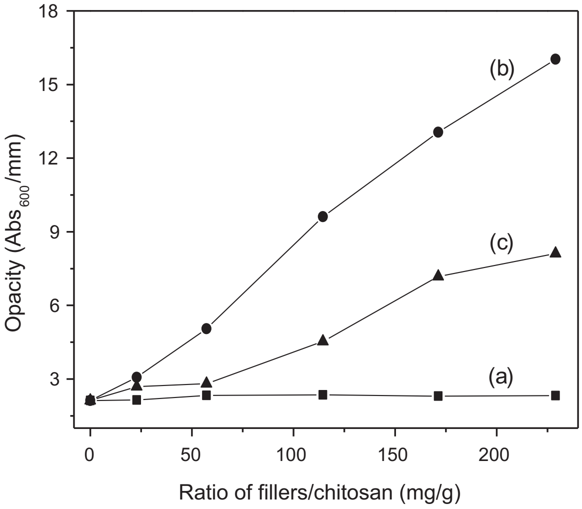

Fig. 2. Optical transparency of (a) chitosan/whisker films, (b) chitosan/rectorite films and (c) chitosan/chitin whisker/rectorite ternary films at various weight ratios of fillers to chitosan.

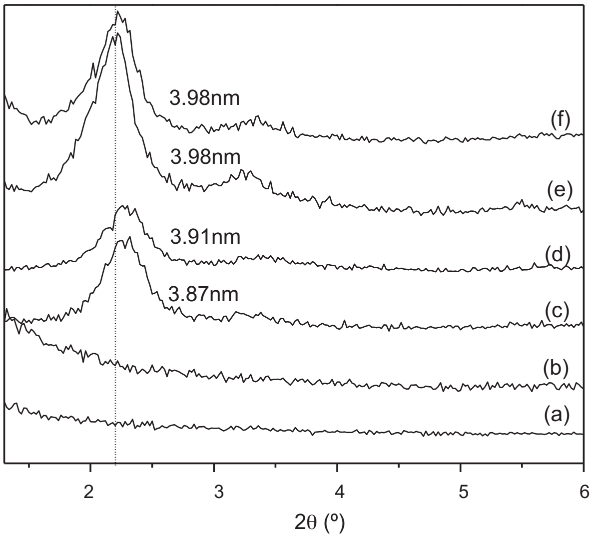

Fig. 3. SAXD patterns of (a) chitosan film, (b) chitosan/chitin whisker film, (c and d) chitosan/rectorite films with rectorite/chitosan ratios of 57.25 mg/g and 114.5 mg/g and (e and f) chitosan/chitin whisker/rectorite ternary films with fillers/chitosan ratios of 114.5 mg/g and 229 mg/g.

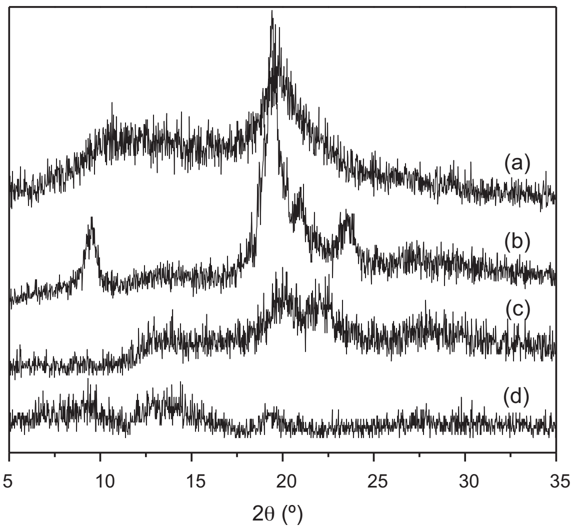

Fig. 4. WXRD patterns of (a) chitosan film, (b) chitosan/chitin whisker film, (c) chitosan/rectorite film and (d) chitosan/chitin whisker/rectorite ternary film with weight ratios of fillers to chitosan of 229 mg/g.

2005). Trace of the thin rods whiskers intercalate into the interlayers of rectorite, and this would enlarge the interlayer distance of the clays.

Wide angle X-ray diffraction spectra from 5° to 35° in Fig. 4 exhibited the crystal peaks of neat chitosan and the composite films. Chitosan film displayed characteristic diffraction crystalline peaks at 2θ of 10.5° and 19.8°. These peaks still existed but the intensities weaken in the chitosan/chitin whisker film. In addition, the strong scattering peaks of α-chitin at 2θ of about 9.6° and 19.5° as well as three other weak peaks at about 21°, 23° and 26° were shown (Cho, Jang, Park, & Ko, 2000), indicating that the presence of chitin whiskers did not affect the apparent degree of the crystallinity of chitosan matrix (Sriupayo et al., 2005). For the chitosan/rectorite film, the crystal peak of chitosan near 10.5° disappeared and the peak at 20° became wider and weaker due to the disturbance of rectorite. The result was in accordance with the study of Wang et al. (2006), who confirmed that chitosan chains interacted strongly with layered clay and intercalated into the interlayer, resulting in great change of chitosan crystalline. The ternary film reinforced with both chitin whisker and rectorite showed an amorphous structure, much different from the pure or binary films. It is most likely that, due to the intercalation of slender rods whiskers into the interlayers of rectorite, the ordered multilayer morphology of rectorite was still present but the interlayer distance increased, and this may destroy the original crystallinities of chitin and rectorite. From the above analysis, it is apparent that the intercalated chitin whisker and rectorite mixture fillers in the chitosan matrix have formed strong interactions and may influence the properties of the films.

TG-DSC curves of the composite films are shown in Fig. 5. The initial endothermic peaks and weight loss up to 125 °C were attributable to the removal of moisture upon heating. The exothermic peaks at 320–340 °C were due to the decomposition of the polymers. Obviously, the binary films exhibited almost identical behaviors as chitosan film, indicating that the presence of chitin whisker or rectorite alone did not affect the thermal stability of the composite films (Sriupayo et al., 2005). The ternary composite film showed higher endothermic peak and less weight loss than that of the binary film, suggesting better thermal stability than the binary films. It can be ascribed to the interaction among the chitosan chains and the different-shape-fillers. After mixing the layered clay with the slender rods-like whisker, the ordered multilayer was still present but the interlayer distance was enlarged, as above analysis. The strong hydrogen bonds between interlayers may hinder the volatility of the pyrolysis of chitosan and limit the decomposition of chitosan. Then conclusion can be drawn that mixture fillers of rectorite and whisker could result in significant effect of the enhancement of thermal stability of the ternary composite films.

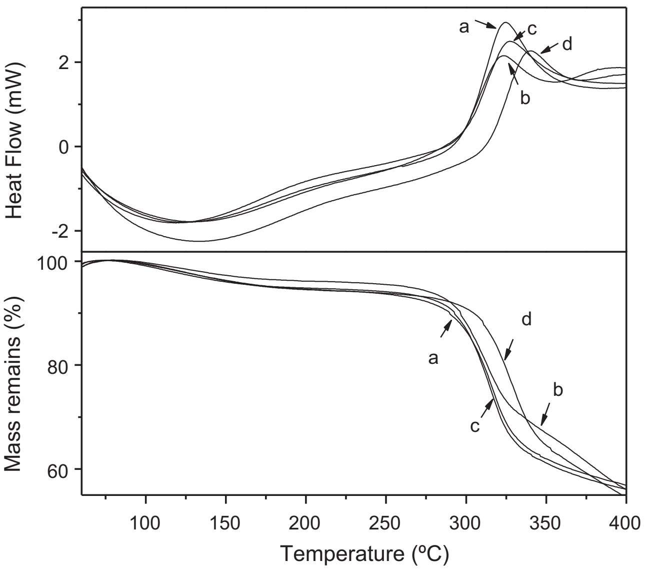

Fig. 5. TG and DSC of (a) chitosan film, (b) chitosan/chitin whisker film, (c) chitosan/rectorite film and (d) chitosan/chitin whisker/rectorite ternary film with weight ratios of fillers to chitosan of 229 mg/g.

Fig. 6 enumerates the mechanical properties of the composite materials. Increasing whisker content from 0 to 229 mg/g, the wet tensile strength of the composite films increased from 9 to 17 MPa, while the percentage of elongation increased from 60% to 129%. It is based on the fact that the incorporation of fibrous and rod-like chitin whisker into the chitosan matrix formed hydrogen bonds thanks to the numerous hydroxyl groups on the fiber surface. Compared to this, the tensile strength of chitosan/rectorite films decreased with the increase of the amount of rectorite, while the elongation at break was improved. For the ternary composite films, it can be noted that the percentage of elongation at break initially increased to a maximum value (i.e. 144%) at the filler content of about 114.5 mg/g but gradually decreased with further increase of the fillers, while the tensile strength decreased little when the filler/chitosan ratio was greater than or equal to 57.25 mg/g. It is believed that the mechanical properties of composite films do not only depend on the amount, the size, the shape, and the alignment of the fillers, but also on the ability of the matrix to transfer the stress to the fillers (Almgren et al., 2009). At low loading levels, the intra- and intermolecular interactions of the entanglement among chitosan chains and chitin whisker, hydrogen bonds of chitin whisker networks and the intercalation of chitin whiskers into the interlayer of rectorite resulted in the improvements of elongation break. However, the interactions were partially destroyed for the aggregation of fillers and microphase separation of rectorite with further increasing of the mixture fillers. Base on the analysis, it can be inferred that the mechanical properties of the composite materials are attributed to the geometry of the filler and the filler/filler and filler/matrix interactions (Grunert & Winter, 2002).

Water uptake dependence of the fillers on the ternary films is shown in Table 1. Among the film types investigated, the equilibrium swelling of neat chitosan film was the highest. The water uptake of the chitosan/chitin whisker film decreased from 214% to

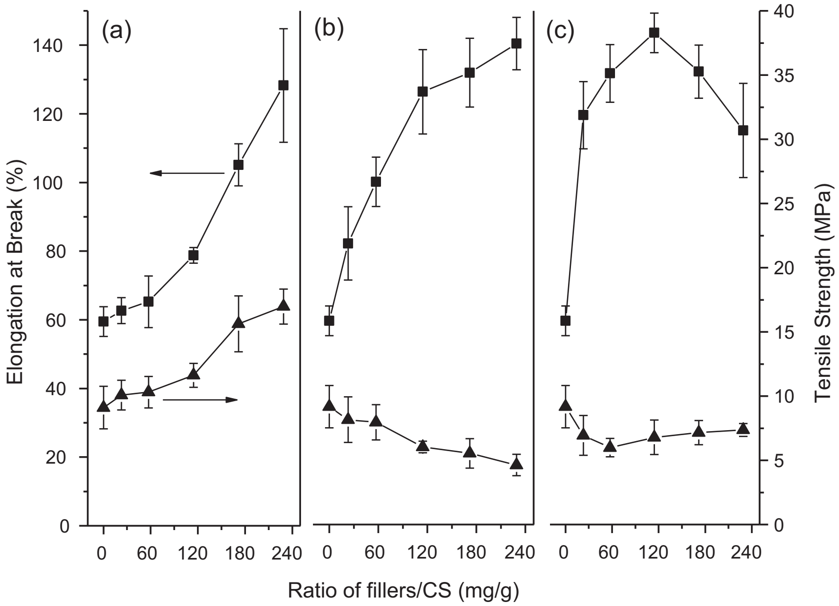

Fig. 6. Tensile strength (MPa) and elongation break (%) of (a) chitosan/chitin whisker film, (b) chitosan/rectorite film and (c) chitosan/chitin whisker/rectorite ternary film at various weight ratios of fillers to chitosan.

153% with an increase of the whisker content from 0 to 229 mg/g. The phenomena can be ascribed to the fact that highly crystalline chitin whisker was less hydrophilic than chitosan and the strong interaction between chitosan matrix and the whisker fillers (Li, Zhou & Zhang, 2009; Lu et al., 2004; Sriupayo et al., 2005). The chitosan/rectorite binary and the chitosan/whisker/rectorite ternary films also exhibited higher water resistance capacity due to the large aspect ratio of the silicate layers dispersing into the chitosan matrix and the existence of chitin whiskers. However, with the fillers content increasing, the rectorite-reinforced binary and ternary films exhibited irregular swelling. It was not surprising. As we mentioned above, high mixtures of whisker and rectorite in the chitosan matrix resulted in aggregation and microphase separation, and then supplied space for the water permeation. In view of this, the chitosan/chitin whisker/rectorite composite film with better water resistance should be obtained with the addition of proper amount of fillers.

Inhibitory effect of the composite films against *E. coli* and *S. aureus* was measured based on zone surrounding circular film strips and are shown in Fig. 7. Neat chitosan and chitosan/chitin whisker films did not show any inhibitory effect, whereas all of the rectorite-reinforced binary and ternary films exhibited significant inhibition. Furthermore, the zone diameters of the ternary film were higher than that of the rectorite-reinforced binary films with the same content of rectorite. It has been generally accepted that the positive charge on the C-2 of the glucosamine monomer of chitosan could interact vigorously with the phosphoryl groups of cell membranes and then kill the bacteria (Liu et al., 2004); but when the NH_3^+ groups were neutralized, the antibacterial ability become weak. Thus the chitin whisker-reinforced chitosan films having no antibacterial activity is expected. The layered rectorite silicate could absorb and inhibit the proliferation of bacteria on account of its significant adsorption capacities, and improved the antibacterial activity of chitosan films (Lemke et al., 2001). Furthermore, the impregnation of whisker enlarged the interlayer distance of clay and may improve the antibacterial behavior. That is why the ternary films exhibited stronger antibacterial effect compared with the same content of fillers of the binary films.

Table 1
Water uptake of (a) chitosan/chitin whisker films, (b) chitosan/rectorite films and (c) chitosan/chitin whisker/rectorite ternary films at various weight ratios of fillers to chitosan.

Ratio of filler/CS (mg/g)	Degree of swelling (%)		
	CS/whisker film	CS/rectorite film	CS/whisker/rectorite film
0	214.4 ± 16.7	214.4 ± 16.7	214.4 ± 16.7
22.90	185.4 ± 2.2	173.7 ± 13.9	177.5 ± 24.6
57.25	175.0 ± 10.2	171.5 ± 24.6	167.5 ± 26.0
114.50	169.2 ± 17.1	187.9 ± 24.2	164.8 ± 24.6
171.75	153.3 ± 10.5	189.3 ± 21.2	174.0 ± 23.5
229.00	144.1 ± 7.4	191.0 ± 21.8	168.0 ± 21.0

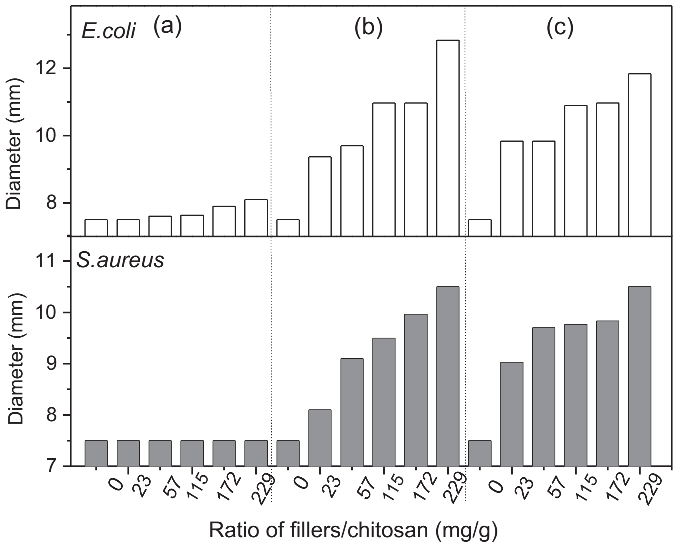

Fig. 7. Antimicrobial activities against *E. coli* and *S. aureus* of (a) chitosan/chitin whisker films, (b) chitosan/rectorite films and (c) chitosan/chitin whisker/rectorite ternary films at various weight ratios of fillers to chitosan.

4. Conclusions

Simultaneous addition of the layered rectorite and rods like chitin whisker into the chitosan matrix resulted in enhanced thermal stability and excellent antibacterial activity in comparison with the chitosan/chitin whisker or chitosan/rectorite binary films due to the cooperative interaction of the mixture fillers and chitosan. Moreover, the ternary films with the fillers only half of that in the binary ones displayed excellent tensile strength due to the interaction of the fillers and the matrix. Therefore, the chitosan/chitin whisker/rectorite ternary films could have great applications in the field of food packaging industry because of their thermal stability, high mechanical properties, water resistance and antibacterial activity.

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