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Preparation and Application of a Xylan-based Antibacterial Papermaking Additive to Protect Against *Escherichia coli* Bacteria

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A xylan-based antimicrobial additive agent was prepared and studied for use in paper products against *Escherichia coli* bacteria. The derived cationic-xylan-grafted-guanidine polymer (CX-g-PHGH) was successfully synthesized by graft copolymerization of cationic-xylan with polyhexamethylene guanidine hydrochloride (PHGH) using ceric ammonium nitrate as an initiator. The obtained CX-g-PHGH had a maximum PHGH grafting ratio of 18.4% and efficiency of 58.4% and showed good viscosity and thermal stability. Furthermore, the paper samples prepared in this work were reinforced noticeably with the addition of CX-g-PHGH, after which exhibited improved mechanical properties. Compared to the reference paper without any of the xylan derivatives, the index of tensile, tear, burst, and folding endurance of the paper were increased by up to 20.1%, 25.3%, 30.2%, and 77.8%, respectively. Moreover, the prepared CX-g-PHGH paper exhibited efficient antimicrobial barrier properties against *E. coli* bacteria, by which many applications based on the new xylan derived additive agent obtained in this work could be found, especially in field of antimicrobial paper products against *E. coli* bacteria from contaminated food.

INTRODUCTION

For a long time, the control of harmful bacterial infection has received significant attention, especially with the increasing demand for quality of living environments and working conditions in today's society (Yang *et al*. 2019). Pathogenic *Escherichia coli* are naturally occurring bacteria found in the environment, foods, and mostly in intestines of people and animals. *E. coli* is a common zoonotic bacterial pathogen and is an associated cause of a variety of intestinal and extraintestinal diseases, such as diarrhea, urinary tract infections, septicemia, neonatal meningitis, and other illnesses (Kim 2003; Conrad *et al*. 2014; Wang *et al*. 2016; Karmali 2017; Liu *et al*. 2018) The contamination of food, specifically meat, with pathogenic *E. coli* can occur during evisceration and harvesting of the meat, making it one of the most common causes of food-borne diseases (Lin *et al*. 2019; Manage *et al*. 2019). Moreover, contact of pathogenic *E. coli* to the human body *via* different kinds of residing platforms, such as food-related paper products, *e.g.* packaging paper, baking paper, napkin paper, pulp molded tableware, *etc.*, is also a common pathway that can lead to human health problems. Therefore, using antimicrobial products for food-

related applications is one of the most efficient and popular ways to reduce the risk of infection from daily eating habits.

Antimicrobial products based on various raw materials after modification have been extensively studied in the world (Shaer *et al*. 2012; Poverenov *et al*. 2013; Demetrescu *et al*. 2018; Diaz-García *et al*. 2019). Especially in recent years, antimicrobial products based on biological macromolecules from biomass including lignin, pectin, and different kinds of polysaccharides (*e.g.*, cellulose, starch, and hemicellulose) have gained increasing interest due to many superior properties of these kinds of macromolecules such as biodegradability, good biocompatibility, good bioactivity, a wide range of resources from which to make the product, and easy modification of the end product (Guan *et al*. 2008; Dutta *et al*. 2009; Kang *et al*. 2013). For instance, composites of silver-nanoparticles/bacterial-cellulose exhibited significant antibacterial activities against *E. coli* and other bacteria (Wu *et al*. 2014). Antimicrobial nanostructured films based on starch demonstrate antimicrobial activity against *Staphylococcus aureus* and other bacteria (Abreu *et al*. 2015).

Food-related cellulosic products, such as food packaging paper, baking paper, and napkin paper, are important products in daily life due to many superior properties of them such as their light weight, ease of modification and use, renewability, and biodegradability. However, cellulosic products naturally do not have any antimicrobial properties. Therefore, bacteria can multiply rapidly in the cellulosic products if proper conditions are fulfilled. Therefore, there have been many different kinds of natural and synthetic polymers or materials widely studied and used as antibacterial additive agents on these paper products (Qian *et al*. 2008, 2011a,b; Sun *et al*. 2010). The addition of antimicrobial agents, especially the agents based on biodegradable biomass on the paper products, is a promising way to efficiently and sustainably introduce antimicrobial properties to the required materials.

Xylan is the next highest naturally occurring biomolecule in the plant world after cellulose and possibly lignin. It is the most common polysaccharide in angiosperms, grasses, and cereals, where it exists in different compositions and structures (Ebringerová and Heinze 2015). The content of xylan varies in different species, but it constitutes up to 35% in sugarcane bagasse and 50% in straws (Zyl *et al*. 1988; Sun 2010). Similar to cellulose, xylan is also a polyhydroxyl carbohydrate polymer with xylose as a main sugar unit linked by glycosidic bonds and functioning as support material in the cell wall (Horton 1968). Xylan is readily available from pulp refining and cereal processing industries, and is at the same time environmentally friendly, renewable, sustainable, biodegradable, and biocompatible. Xylan has been found to have many potential applications in food, papermaking, textile, plastic industries, and biomedical applications (Hromádková *et al*. 1999; Ünlü *et al*. 2009; Li *et al*. 2011). Moreover, xylan also has great potential to be applied as an antimicrobial material after modification and has received increasing attention from the international community. Recently, the importance of xylan-based macromolecules (Schwikal *et al*. 2005; Petzold *et al*. 2006; Ren *et al*. 2007, 2008) and materials (Lindblad *et al*. 2001; Goksu *et al*. 2007; Hansen and Plackett 2008) has obtained increasing focus, including the antimicrobial modification of xylan used in the field of film packaging, food preservation, as well as in biomedical areas (Wang *et al*. 2010; Melosilveira *et al*. 2011; Pristov *et al*. 2011; Ren *et al*. 2015). There have been many ways reported to prepare xylan-based antimicrobial materials. For example, a novel food preservative was prepared by co-heating xylan with chitosan, and it exhibited excellent antimicrobial activity against *E. coli* and *Staphylococcus aureus* (Li *et al*. 2011). Chitosan-

xylan/cellulose nanowhiskers (CNW) nanocomposite films with antibacterial and antioxidant properties have successfully been prepared in which CNW was used as nanofillers (Bao *et al.* 2018). Carboxymethylated xylan was blended with agar (Ag), ammonium zirconium carbonate (AZC), and linoleic acid (LA) to produce edible films with antimicrobial activity (Queirós *et al.* 2017). Despite these reported efforts, the studies of xylan for high value-added applications are still insufficient. As one of the important subjects, the exploration of possibilities for xylan to be used as antimicrobial additive agent in paper products against *E. Coli* has been the focus of researchers.

Similarly to cellulose, xylan originally does not have antimicrobial activity and requires modification to achieve this property. Polyhexamethylene guanidine hydrochloride (PHGH) is one kind of water-soluble polycation that has antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as having low mammalian toxicity (Guan *et al.* 2008). The introduction of PHGH could endow the macromolecule with efficient antimicrobial activity (Guan *et al.* 2007; Liu *et al.* 2014), which also offers a potential pathway of endowing xylan with antimicrobial ability against *E. coli* bacteria.

Therefore, in this work, a new antimicrobial additive agent based on a xylan-derivative by graft polymerization of PHGH with xylan was prepared using ceric ammonium nitrate as an initiator. The optimal conditions for xylan derivative preparation were first determined by evaluating the whole preparation process. The obtained xylan derivatives were characterized by structure, thermal stability, and rheological behavior determined by Fourier transform infrared spectroscopy (FTIR), carbon nuclear magnetic resonance spectroscopy (^{13}C NMR), thermogravimetric analyser (TGA), elemental analyser (EA), and a rheology meter. Additionally, mechanical properties are also an important aspect for the food-related paper products that need to be studied. Therefore, the antimicrobial activity against *E. coli* of the xylan derivative obtained at the optimal condition as well as its ability as a strengthening agent to improve mechanical properties of paper sheet were systematically investigated. This work found a new way to modify xylan and investigated its applications as a new antimicrobial additive agent against *E. coli* bacteria and as a mechanical enhancing agent for paper products. The obtained antimicrobial additive agent product shows great potential for application in many areas, especially for food-related paper products, such as packaging paper, baking paper, and napkin paper.

EXPERIMENTAL

Materials

Xylan (M_w 49000 g/mol, purity 85%) extracted from sugarcane bagasse was obtained from Shanghai Yuanye Bio-Technology Co., Ltd. (Shanghai, China). Pretreated waste newspaper pulp (mainly American wastepaper) was provided by a local paper company (Guangzhou Paper Group Ltd., Guangzhou, China). Ceric ammonium nitrate (CAN) (99.0%, Analytical Reagent, AR) was purchased from Tianjin Damao Chemical Reagent Factory (Tianjin, China). The 2,3-epoxypropyltrimethylammonium chloride (ETA) (95%), hexamethylene diamine (98%, AR), guanidine hydrochloride (99%, AR), dimethyl sulfoxide (≥ 99%, AR), and glycidyl methacrylate (GMA) (97%, AR) were purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China).

The acetone used in this procedure was purchased from Nanjing Chemical Reagent Co., Ltd. (Nanjing, China). Sodium hydroxide (NaOH) (95%, AR) and ethanol (99%, AR) were purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). Gram-negative bacteria (*E. coli*, ATCC 25922) were purchased from Shanghai Beinuo Bio-Technology Co., Ltd. (Shanghai, China). Chemicals used in this study were all analytical reagent grade and used without any purification. Deionized (DI) water was also used in all experiments. Polyhexamethylene guanidine hydrochloride (PHGH) was prepared by condensation polymerization of hexamethylene diamine and guanidine hydrochloride as described in a previous work (Qian *et al*. 2011b).

Preparation of cationic-xylan

Cationic xylan (CX) was prepared based on the procedure described in the authors' previous work with some minor changes (Peng *et al*. 2012). In summary, a solution of 3 g xylan in 90 mL deionized water was prepared in a 250-mL flask and treated with 0.736 g NaOH (the molar ratio of NaOH and the xylose unit in xylan was 0.8) was added for alkalization of xylan for 1 h. Afterwards, the flask was placed in a microwave reactor (400 W) (GAS-800; Beijing Xianghu Science and Technology Development Reagent Co., Ltd., Beijing, China) and 20.907 g of ETA (the molar ratio of ETA and xylose unit in xylan was 6) was added to the flask when the temperature of microwave reactor reached 70 °C. After allowing the reaction to proceed for 40 min, the precipitate was formed in 100% ethanol and fractionated by filtration with three times the volume of ethanol. The precipitate was washed by filtration again with 70% ethanol until there was no white precipitate formed in the ethanol filtrates by titration with silver nitrate. The washed precipitate was dissolved in deionized water and dialyzed with membrane of molecular weight cutoff of 3500 in DI water for 5 days until the pH of the dialyzed liquid became neutral. The CX was finally obtained after drying in a vacuum oven at 50 °C for 24 h. The degree of substitution (DS) of the prepared CX was 0.38, which was determined by an elemental analysis method (Peng *et al*. 2011).

Preparation of cationic-xylan-grafted-PHGH

Unsaturated double bonds were introduced to PHGH by reacting with GMA, where the molar ratio of amino and epoxy groups was kept at 1.0 (Guan *et al*. 2007). The reaction was performed at room temperature for 6 h. The obtained products were precipitated and washed with acetone to remove unreacted GMA. The washed products were dissolved in an appropriate amount of methanol, followed by precipitation and washed with acetone again. The same treatment was performed three times, and the final purified GMA-modified PHGH product was obtained after drying in vacuum at 25 °C for 12 h.

A solution of 0.33 g CX in 25 mL deionized water was prepared in a three-necked round flask (250 mL) under stirring and purging with nitrogen for 20 min. Then, 5 mL CAN solution was added to the flask while purging with nitrogen continuously for another 10 min. Subsequently, 10 mL GMA-modified PHGH was added to the CX solution and stirred with a slow stream of nitrogen for 4 h. The solution was then dialyzed for 3 days, and the CX-grafted-PHGH (CX-g-PHGH) was finally obtained after drying in a vacuum oven at 50 °C for 24 h. All conditions of preparing the CX-g-PHGH are listed in Table 1.

Table 1. Influence of the Synthesis Conditions on CX-g-PHGH

Sample Number	Temperature (°C)	PHGH Concentration (mol/L)	Initiator Concentration (mmol/L)	Time (h)	Graft Ratio (%)	Graft Efficiency (%)
1	60	0.039	3	4	15.54	52.54
2	60	0.039	4	4	18.45	58.45
3	60	0.039	5	4	16.9	56.69
4	60	0.039	6	4	15.93	53.35
5	60	0.020	4	4	7.27	56.65
6	60	0.078	4	4	16.01	53.21
7	60	0.117	4	4	15.26	40.73
8	60	0.156	4	4	15.03	36.58
9	50	0.039	4	4	15.66	49.60
10	70	0.039	4	4	16.82	53.28
11	80	0.039	4	4	16.24	51.44
12	60	0.039	4	2	13.56	50.26
13	60	0.039	4	3	14.18	53.47
14	60	0.039	4	5	17.21	57.56
15	60	0.039	4	6	16.52	55.48

Grafting ratio and efficiency of the as-prepared polymers were determined based on Eqs. 1 and 2, and the mean value of the results obtained from three parallel samples under each condition is reported in Table 1,

$$\text{Grafting ratio (\%)} = (W_g - W_0)/W_0 \times 100 \tag{1}$$

$$\text{Grafting efficiency (\%)} = (W_g - W_0)/W_p \times 100 \tag{2}$$

where W_0 is the weight (g) of CX, W_g is the weight (g) of CX-g-PHGH, and W_p is the weight (g) of functional PHGH.

Methods

Characterizations of the prepared products

Xylan, CX, and CX-g-PHGH were dried in an infrared drying oven before characterization for their structures and properties. Fourier transform infrared spectra was obtained with a Fourier transform spectrophotometer (Nicolet 750; ThermoFisher Scientific, Waltham, MA, USA) appended with an attenuated total reflectance (ATR) technique. A total of 32 scans were accumulated in the transmission mode with a resolution of 4 cm^{-1}. The spectrum was obtained from a range of 4000 cm^{-1} to 400 cm^{-1}.

The solution-state ^{13}C-NMR spectra were recorded on a Bruker DRX-400 spectrometer (Bruker, Karlsruhe, Germany) at 25 °C after 15000 scans. The sample (80 mg) was dissolved in 1 mL D$_2$O. The running parameters were: 30º pulse flipping angle, 9.2 μs pulse width, and a 1.36 s acquisition time with 2 s relaxation delay.

Elemental analysis (EA) is for the quantitative determination of specific elements of the samples. Specimens weighing approximately 3 mg to 5 mg were heated in a Vario EL elemental analyzer (Elementar, Langenselbold, Germany) under an oxygen atmosphere, and elements of C, H, and N in xylan, CX, and CX-g-PHGH were determined.

The molecular weights of xylan, CX and CX-g-PHGH were determined by GPC on a PL Aquagel-OH 60 column (300 mm × 7.5 mm; Agilent Technologies, Santa Clara,

CA, USA) and calibrated with PL pullulan polysaccharide standard (average peak molecular weights of 783, 12200, 100000, and 1600000 g/mol). A flow rate of 0.5 mL/min was maintained with ultrapure water as an eluent. Samples were dissolved in ultrapure water to reach a concentration of 0.1% before characterization.

Dynamic rheological properties of CX and CX-g-PHGH were determined using a sandwich rheometer (AR2000; TA Instruments, New Castle, DE, USA). All samples were dissolved in water with a magnetic stirrer for 30 min to form stable solutions. The solutions were then dropped on a Brookfield D VIII instrument panel (LabX, Midland, Canada). Software (Rheo 2000, Brookfield D VIII, LabX) provided by the manufacturer with the instrument was used to set up the parameter, to perform rheometer control, and to collect the data. The data for the shear rate, frequency, viscosities, storage modulus (G'), and the loss modulus (G'') of all samples were collected.

Thermal stability analysis was used to determine thermodynamic properties of xylan, CX, and CX-g-PHGH. The analysis was performed using thermogravimetric analysis (TGA) and differential thermal analysis (DTA) on a simultaneous thermalgravimetric analyzer (TGA Q500; TA Instruments, New Castle, DE, USA). Approximately 5 mg samples were heated to 700 °C from room temperature with the heating rate of 10 °C /min in a nitrogen atmosphere.

Preparation and mechanical properties tests of CX-g-PHGH paper sheets

Five sheets with grammage (weight per unit area) of approximately 55 g/m^2 were prepared based on norm GB/T 2828 (1981) (Ren *et al.* 2009a). The addition amount of CX-g-PHGH was 0.3% to 1.5% based on the oven-dried weight of waste newspaper pulp. Briefly, CX-g-PHGH and the waste newspaper pulp were homogenized under stirring for 10 min before forming paper sheets. The sheets were formed *via* a fast papermaking machine (MESSMER 255; Testing Machines, Inc., New Castle, DE, USA) and dried. The obtained sheets were cut to shape and placed in a controlled humidity room to condition at 25 °C for 24 h before mechanical tests. As a comparison, CX paper sheets were also produced *via* the same preparation process. The GB/T 22865 (2008) standard was applied for the mechanical testing of the sheets (Chen *et al.* 1990). The tests were conducted 10 times for each sample, and the mean value of the mechanical tests was reported in this work.

Antimicrobial tests of the xylan derivatives

The antimicrobial activity was tested against Gram-negative bacteria (*E. coli*, ATCC 25922). The *E. coli* bacteria were cultured and grown in Luria Bertani (LB) liquid medium (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, and at pH 7.0) for 12 h at 37 °C. The bacteria were further diluted with NaCl solution (0.85%, w/v) to obtain a concentration of approximately 10^5 CFU/mL. The diluted suspensions (0.1 mL) of *E. coli* were then distributed homogeneously onto the LB agar medium (10 g/L peptone, 5 g/L yeast extract, 10 g/L NaCl, and 15 g/L agar).

The obtained paper sheets with xylan derivatives with a diameter of 6 mm were prepared and placed in the plate culture medium that was coated with the bacterial suspension. The antimicrobial ability of the xylan derivatives was evaluated by measuring the diameter of the inhibition zones for *E. coli* on the paper sheets.

RESULTS AND DISCUSSION

Synthesis Determination of CX-g-PHGH

The Ce^{4+} in ceric ammonium nitrate has the ability to attack and convert the hydroxyl groups of CX to free radicals, which could have activity to further react with modified PHGH and form CX-g-PHGH (Raj *et al*. 2010). The synthesis procedure is proposed in Scheme I.
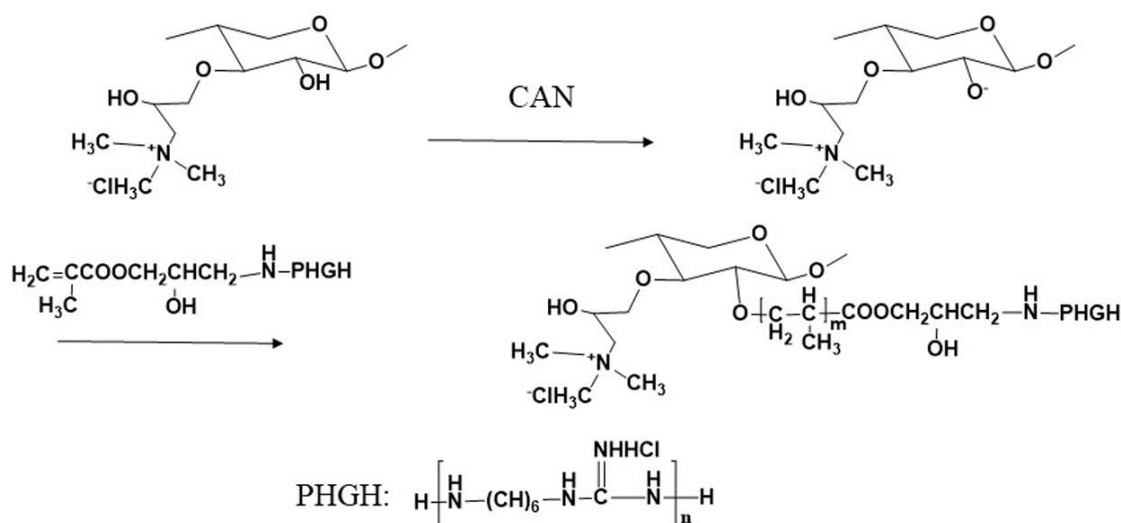
Scheme I. Copolymerization of PHGH onto CX

There are many factors in the reaction, such as the concentration of initiator, the amount of PHGH added, the reaction temperature, and the total reaction time, that could clearly affect the grafting ratio and grafting efficiency of the final products. The influences of initiator concentration on grafting ratio and grafting efficiency of the CX-g-PHGH are shown in Fig. 1a, and detailed values are shown in Table 1.

As shown, the grafting ratio and efficiency increased drastically with the increase of the initiator concentration until 4 mmol/L, with the values increasing to approximately 18.4% and 58.4% for grafting ratio and efficiency, respectively. However, when an initiator concentration higher than 4 mmol/L was applied, both the grafting ratio and efficiency decreased. This phenomenon was in accordance to previous findings by Qian *et al.* (2008), which was probably because the excess initiator started to participate in the termination step of the growing chains and subsequently initiated the homopolymerization of PHGH.

The influence of the PHGH concentration on the grafting ratio and grafting efficiency is shown in Fig. 1b. It is clear that the grafting ratio and the efficiency increased sharply up to 18.4% and 58.4%, respectively, when the PHGH concentration was lower than 0.04 mol/L. However, afterwards, the grafting ratio and efficiency decreased rapidly, which was probably caused by the self-polymerization resulting from high PHGH concentration (Guan *et al*. 2008).

The increase of reaction temperature may lead to multiple possible outcomes, including increasing the diffusion of CX and PHGH, facilitating redox initiator system, and enhancing the chain propagation, but likely increasing the rate of termination and homopolymerization in bulk phase (Singh *et al.* 2004; Wang and Xu 2006). Therefore, when the temperature was lower than 60 °C, there was a sharp increase for both grafting

ratio and efficiency that reached maximum values of 18.4% and 58.4%, respectively (Fig. 1c). However, the grafting ratio and efficiency decreased when the reaction temperature was higher than 60 °C, which was due to the domination of homopolymerization caused by high temperature.
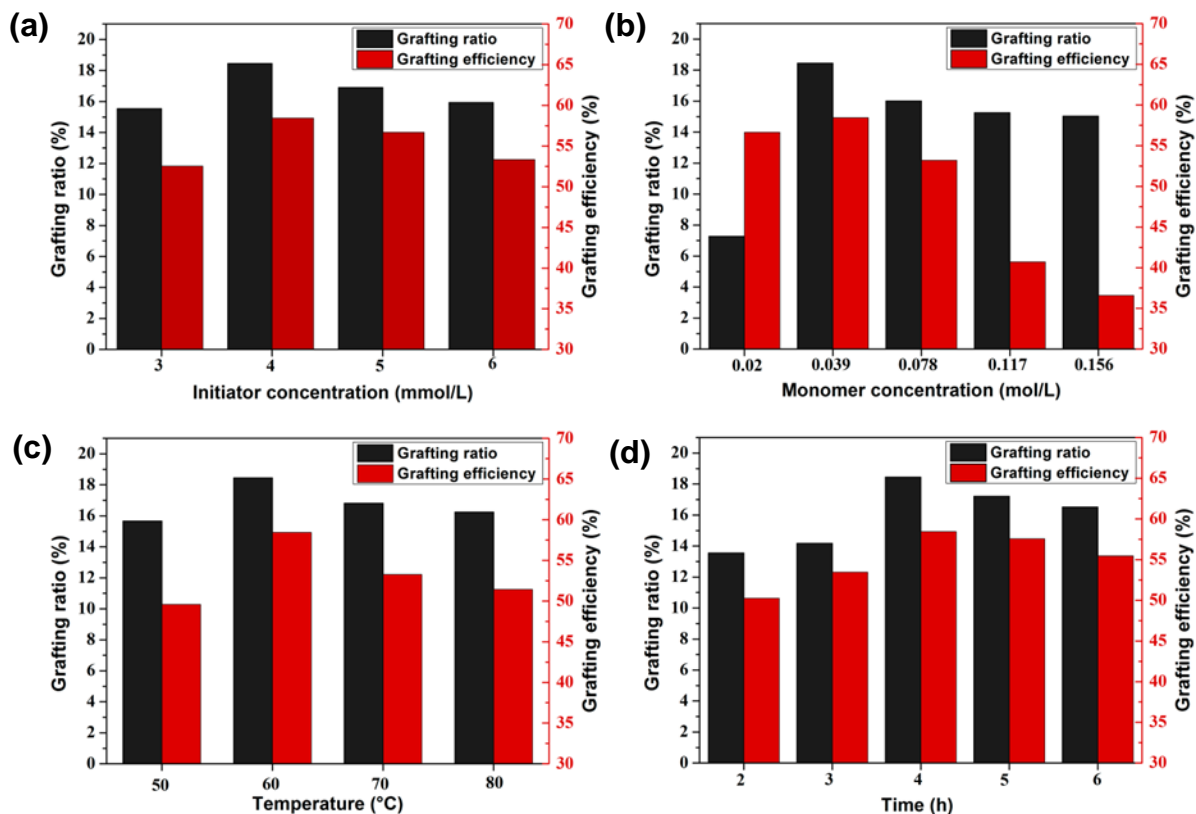

Fig. 1. Influences of conditions on grafting ratio and efficiency of CX-g-PHGH: a) initiator concentration, b) PHGH concentration, c) temperature, and d) time

Furthermore, the reaction time also had an important impact on the grafting ratio and efficiency of CX-g-PHGH, as shown in Fig. 1d. When the reaction time increased, the grafting ratio and the efficiency initially increased and then decreased. A reaction time of 4 h was determined as the optimal time for the grafting ratio and efficiency with maximum values of 18.4% and 58.4%, respectively. This was explained by the decrease of the PHGH concentration and free radicals in the system as the reaction time increased. This resulted in the leveling-off of the grafting ratio and efficiency (Xu *et al*. 2017).

It can be concluded from above that the optimal conditions for preparing CX-g-PHGH were: an initiator concentration of 4 mmol/L, a PHGH concentration of 0.039 mol/L, a reaction temperature of 60 °C, and a reaction time of 4 h. Therefore, in the following studies, the CX-g-PHGH (sample number 2, as CX-g-PHGH-1) obtained at the optimal condition with maximal grafting ratio of 18.45% was used for characterization and testing.

FTIR Spectra

To confirm the successful grafting of PHGH on CX, several analyses, including FTIR, ^{13}C-NMR, elemental analysis, and average molecular weight, were performed.

The FTIR spectra of xylan, CX, and CX-g-PHGH-1 are illustrated in Fig. 2. The signals at 3433 cm^{-1}, 2918 cm^{-1}, 1637 cm^{-1}, 1464 cm^{-1}, 1033 cm^{-1}, and 897 cm^{-1} in the

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spectrum represent the transmittance peaks of molecular bonds for xylan. The transmittance band at 2918 cm^{-1} was assigned to the C-H stretching vibration of alkane in xylan. The transmittance peak of 1044 cm^{-1} is ascribed to the C-O-C stretching of the ether groups (Peng *et al*. 2012).
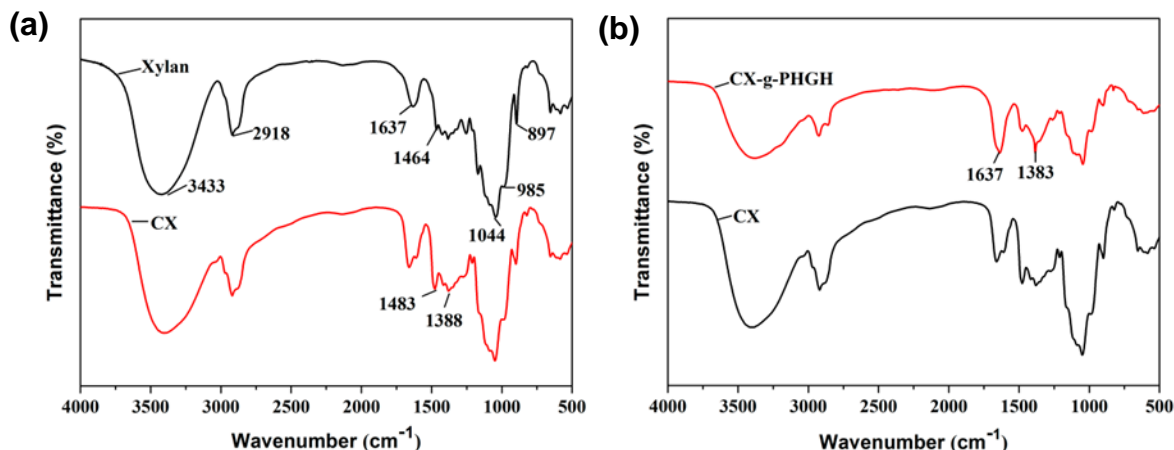

Fig. 2. FTIR spectra of xylan and CX (a) and CX and CX-g-PHGH-1 (b)

As can be seen in Fig. 2a, in the FTIR spectra of CX, an increase in the transmittance intensity of ether bond at 1044 cm^{-1} was found compared to the spectrum of xylan, indicating that more ether bonds were introduced to the xylan. The transmittance intensity around 1388 cm^{-1} increased due to the stretching vibration of C-N. The stretching vibrations of -CH$_2$ and -CH$_3$ on the quaternary ammonium group increased the intensity of the transmittance at 1483 cm^{-1}. These changes in the transmittance peaks indicate that cationic groups were successfully introduced to xylan (Kong *et al*. 2014).

In Fig. 2b, compared with the spectrum of CX, an increase in the intensities of transmittance at 1383 cm^{-1} and 1637 cm^{-1} was seen in the FTIR spectra of CX-g-PHGH, corresponding to the stretching vibration transmittance peaks of C-N and C=N, respectively, which confirmed the successful introduction of PHGH to CX (Guan *et al*. 2007).

^{13}C-NMR Spectra

The molecular structures of xylan, CX, and CX-g-PHGH-1 were further characterized by ^{13}C-NMR (Fig. 3). In the spectrum of xylan (Fig. 3a), the main (1→4)-linked β-D-xylp units were characterized by the signals at δ of 102 ppm, 77.9 ppm, 76.4 ppm, 75.9 ppm, and 63.1 ppm, which are attributed to C-1, C-4, C-3, C-2, and C-5 of β-D-xylopyranosyl units, respectively (Kong *et al*. 2014).

Compared to the ^{13}C-NMR spectrum of xylan, the spectrum of the CX (Fig. 3b) had great changes in both intensity and position of the strong signals. The most intense signal appeared at 54.1 ppm, which is assigned to carbons of the quaternary ammonium moiety, and the signal at 68.1 ppm is attributed to the -CH$_2$- (Katsura *et al*. 1992). This demonstrated that the cationic groups were introduced successfully onto the xylan. In the ^{13}C-NMR spectrum of CX-g-PHGH-1 (Fig. 3c), some new peaks appeared in comparison with CX. The peaks at 28.0 ppm, 41.1 ppm, and 168 ppm are attributed to the signal peaks of -CH$_2$-, C-N, and the ester group carbon (Tanodekaew and Channasanon 2006), respectively, which indicated that PHGH was grafted onto CX successfully.
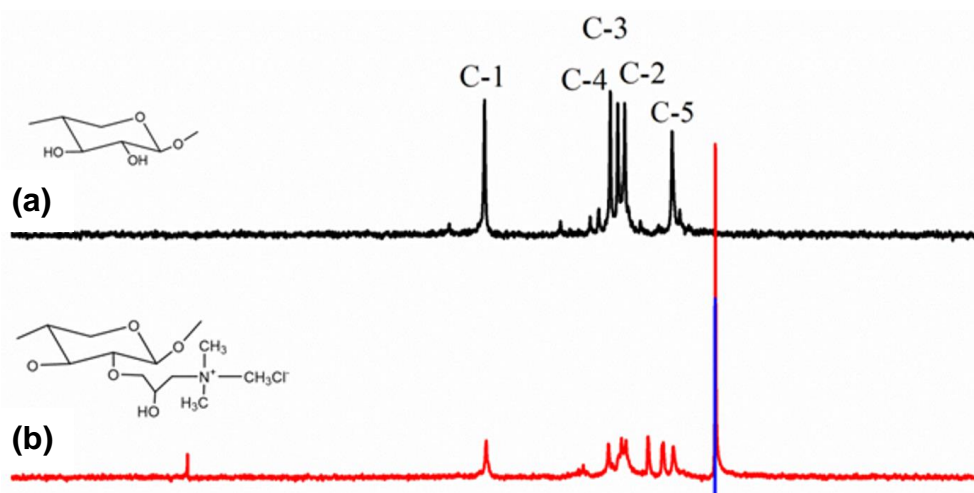
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Fig. 3. ^{13}C-NMR spectra of a) xylan, b) CX, and c) CX-g-PHGH-1

Elemental Analysis

The element analysis of xylan, CX, and CX-g-PHGH-1 are shown in Table 2. The contents C, H, and N in xylan were 40.36%, 6.91%, and 0.00%, respectively, while they became 40.36%, 7.43%, and 2.16% after the cationic modification of xylan, indicating that cationic groups were grafted onto xylan.

Table 2. Elemental Analysis of Xylan, CX, and CX-g-PHGH-1

Sample	C (%)	H (%)	N (%)
Xylan	40.36	6.91	0
CX	40.36	7.43	2.16
CX-g-PHGH	40.88	7.79	9.46

Furthermore, the contents of C, H, and N in CX-g-PHGH-1 were 40.88%, 7.79%, and 9.46%, respectively. The increase in the N content confirmed the presence of nitrogenous compounds (PHGH) in CX-g-PHGH-1.

Average Molecular Weight

Average molecular weight is also a promising factor that can confirm the successful conversion of xylan into its derivatives. The molecular weight and molecular weight distribution of xylan, CX, and CX-g-PHGH-1 are shown in Table 3.

Table 3. Molecular Weight and Molecular Weight Distribution of Xylan, CX, and CX-g-PHGH-1

Sample	M_w (g/mol)	M_w/M_m
xylan	49000	4.59
CX	37500	1.99
CX-g-PHGH-1	1477129	1.15

As shown, the molecular weight of CX was lower than that of xylan, which was probably caused by the degradation of xylan during chemical reaction under alkaline

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conditions. As expected, the molecular weight of CX-g-PHGH-1 was higher, approximately 30 to 40 times than that of xylan and CX, which indicated the successful copolymerization of xylan and CX with PHGH. In addition, the CX and CX-g-PHGH-1 had a relatively low index of polydispersity (1.14 to 1.99) in comparison to xylan (4.59), which indicated that the molecular chain length distribution of CX and CX-g-PHGH-1 was more uniform than that of xylan.

Rheological Properties

Properties of xylan, CX, and CX-g-PHGH-1, as well as their influence on paper products were tested to fulfill the requirements to be used as an additive agent for antimicrobial paper products.

The tests of rheological behavior for xylan, CX, and CX-g-PHGH-1 would provide better insight into the physico-chemical properties of the polymers and, consequently, to discover their potential applications, *e.g.*, as a coating additive agent to paper products. The rheological behaviors of xylan, CX, and CX-g-PHGH-1 are shown in Figs. 4 and 5.
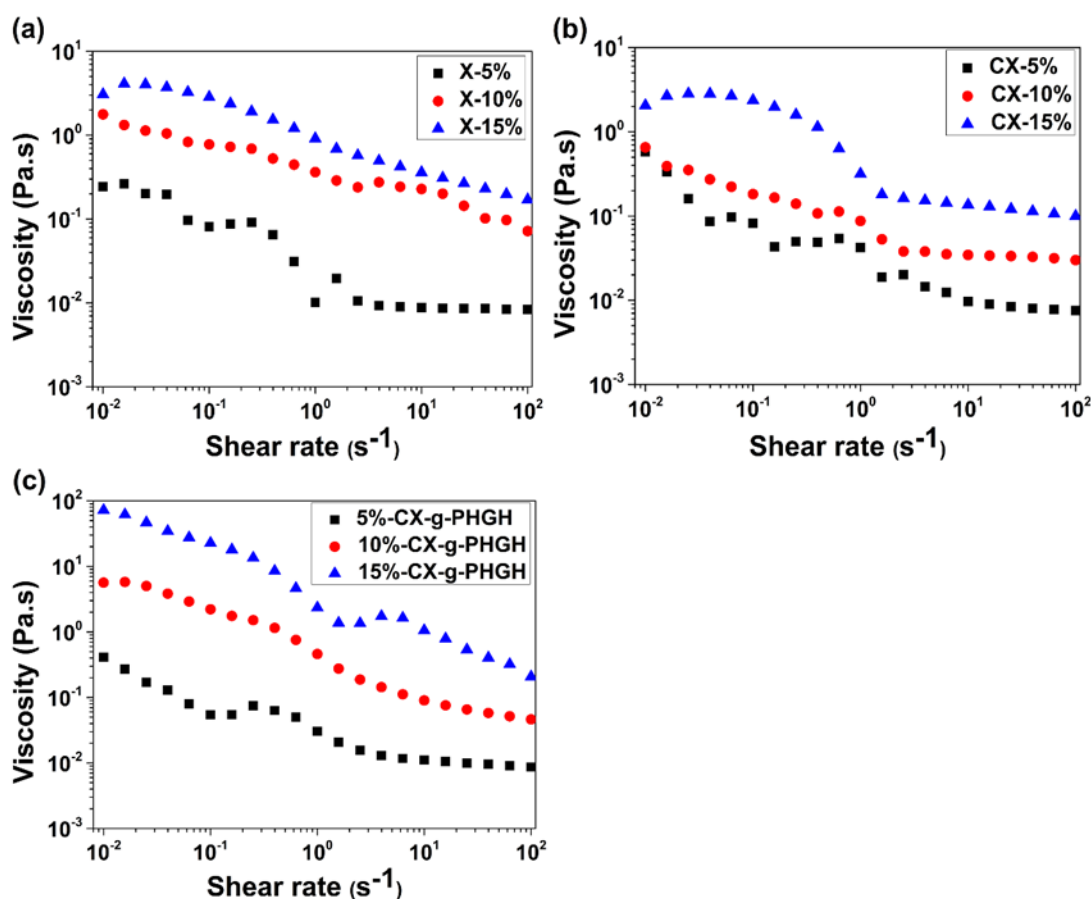

Fig. 4. Shear rate dependence of viscosity for (a) xylan, (b) CX, and (c) CX-g-PHGH-1 at different concentrations

As shown in Fig. 4, the viscosities of xylan, CX, and CX-g-PHGH-1 all decreased with the increase of shear rate. In other words, they exhibited pseudoplastic or shear-thinning behavior of these solutions in the range of shear rates tested, which was due to the damage to the network structure of xylan and its derivatives (Peng *et al.* 2011).

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Accordingly, with a certain additive speed, the xylan, CX, and CX-g-PHGH-1 would be easily applied as a coating agent on the surface of materials such as paper products. Furthermore, the viscosity of the CX-g-PHGH-1 solution was higher than that of the xylan and CX solutions across the whole shear rates range when the concentrations were 5% and 10% as well as for shear rates higher than 10 s^{-1} when the concentration was 15%. This could suggest that CX-g-PHGH-1 solution had stronger intermolecular interactions than the other two solutions (Eutamene *et al*. 2010).
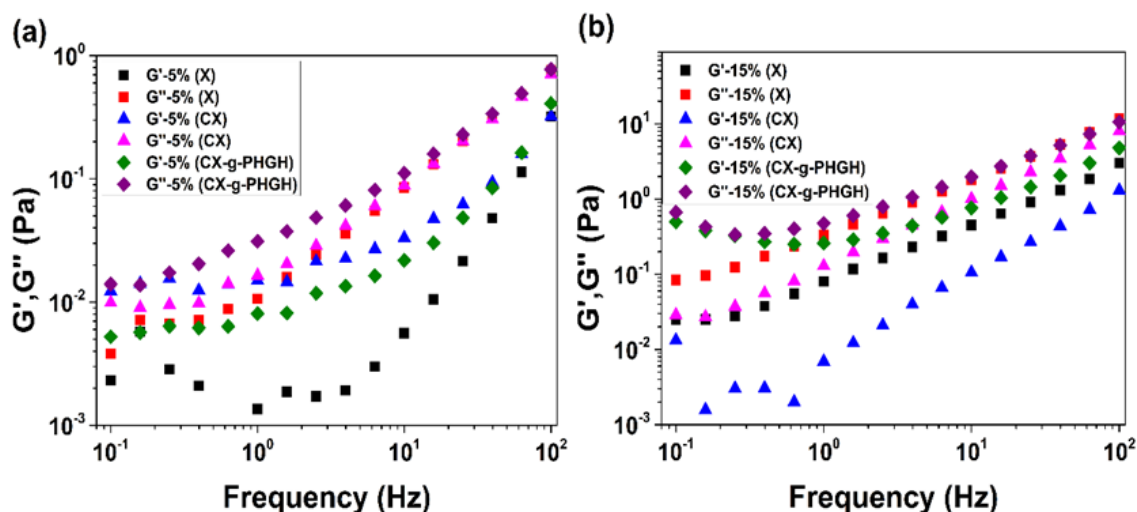

Fig. 5. Frequency dependent modulus of the solutions of xylan (X), CX, and CX-g-PHGH-1 (a) at 5% concentration and (b) at 15% concentration

       In Fig. 5, the rheological properties (storage modulus *G*' and loss modulus *G*") of xylan, CX, and CX-g-PHGH-1 are illustrated. When the concentration was 5%, the storage modulus in the whole frequency region of the xylan and CX-g-PHGH-1 solutions was lower than the loss modulus, demonstrating a viscous behavior. For the CX solution with a concentration of 5% in the range of 10^{-1} to 10^{0} Hz, the storage modulus was higher than the loss modulus, showing stronger elastic properties due to stronger molecular entanglement of CX than that of xylan and CX-g-PHGH-1 (Peng *et al*. 2011). When the concentration of the solution was 15%, all storage moduli of the xylan, CX, and CX-g-PHGH-1 in the whole frequency region were lower than those of the loss moduli, demonstrating viscous behavior. In addition, when the concentration of the solution was 15%, the storage and loss modulus of CX-g-PHGH-1 were higher than the modulus of xylan and CX, indicating a greater viscosity behavior for CX-g-PHGH-1 than xylan and CX.

       These results can be explained by the influence of molecular weight and functional groups of the macromolecule chains (Peng *et al*. 2011). Higher molecular weight and the guanidine group caused more chain segments needed for the movement of the viscous flow, thus increasing the frictional resistance and producing a greater viscous behavior. In contrast, a lower molecular weight as well as the cationic groups on the polymer chain may reduce or prevent the associative interactions among themselves in solution, and thus could change the dynamic shear rheological properties of CX (Yoo *et al*. 2005). The studies above not only further confirmed the successful grafting of PHGH to xylan, but they also

indicated that the xylan derivative obtained in this work is a promising candidate as a coating additive agent that can be applied to paper products.

Thermal Stability Analysis

The thermal stability analysis of the samples could facilitate the application of xylan derivative in more scopes. The typical TGA/DTA curves of xylan, CX, and CX-g-PHGH-1 are displayed in Fig. 6. There was a substantial weight loss starting at 200 °C that corresponded to the evaporation of water from the samples.
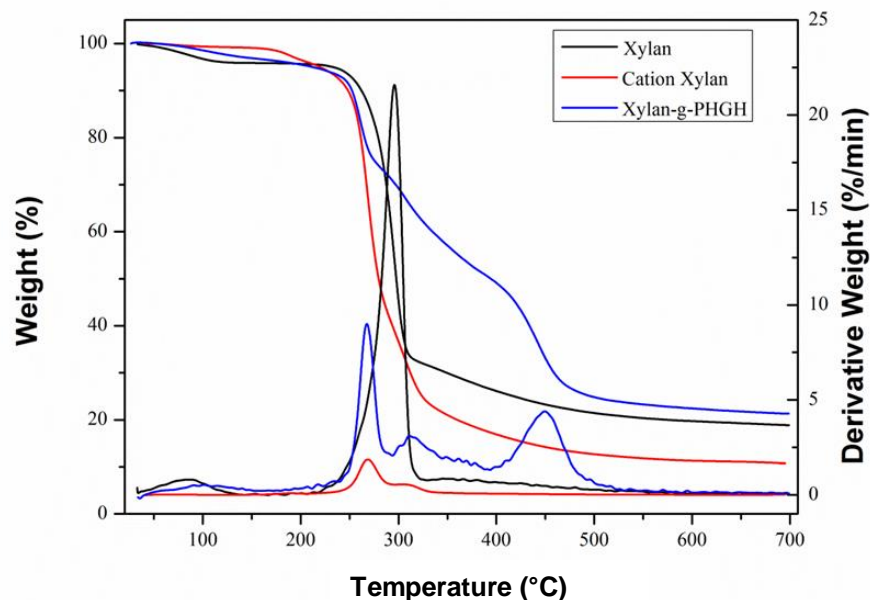

Fig. 6. The TGA/DTA curves of xylan, CX, and CX-g-PHGH-1

The substantial weight loss of xylan occurred at 250 °C to 320 °C, which could be attributed to backbone scission and following fragmentation of the xylan (Tanodekaew and Channasanon 2006). The CX exhibited a similar thermal stability pattern to that of xylan, but its decomposition temperature was lower, indicating that CX was more unstable than xylan. This is because the hydrogen bonds and molecular structure are destroyed to a certain extent after cationization, and the grafted cationic groups are not stable (Peng *et al*. 2012; Xu *et al*. 2017). Similar to the TGA curves of xylan and CX, there was also a slight loss below 200 °C for the CX-g-PHGH-1 that was attributed to surface water evaporation. A substantial weight loss of CX-g-PHGH-1 was found in the range of 230 °C to 320 °C, which was due to the thermal decomposition of the xylan. A substantial weight loss of CX-g-PHGH-1 also occurred in the range of 320 °C to 470 °C, which was attributed to the thermal decomposition of PHGH. Above 470 °C, the polymer began to carbonize and the weight of solid matter became constant (Maschio *et al*. 1992). In summary, the derivatization of xylan with PHGH endowed the new xylan derivative with enough thermal stability to allow the material to be used under high temperature conditions, such as the production of paper and container products, baking, and the packaging of hot food.

Influence of Xylan Derivatives Addition on Mechanical Properties of Paper Sheets

The mechanical properties of the paper products are important for applications in packaging, baking, and cleaning. The influences of xylan, CX, and CX-g-PHGH-1 addition on the mechanical properties of the paper sheets are shown in Tables 4, 5, and 6.

Table 4. Mechanical Properties of Paper Sheets After Addition of Xylan

Amount of Xylan (wt%)	Tear Index (mN·m^2/g)	Burst Index (kPa·m^2/g)	Tensile Index (Nm/g)	Fold Endurance (Times)
0	6.36 ± 0.41	2.02 ± 0.11	35.67 ± 0.92	9 ± 0.4
0.3	6.39 ± 0.50	2.10 ± 0.14	36.83 ± 0.73	9 ± 0.5
0.5	6.42 ± 0.45	2.18 ± 0.15	37.26 ± 0.56	10 ± 0.5
1.0	6.45 ± 0.62	2.33 ± 0.15	39.17 ± 0.62	12 ± 0.5
1.5	6.40 ± 0.55	2.24 ± 0.16	38.23 ± 0.72	11 ± 0.5

Note: Grammage of each sheet was approximately 55 g/m^2, the stirring time of pulp after addition of xylan was 10 min

As shown in Table 4, the addition of xylan had little effect on the mechanical properties of the paper sheets tested. When the amount of xylan was 1.0 wt% in the paper sheet, compared to the mechanical properties of the reference paper without xylan, the tensile index, tear index, and burst index increased 9.81%, 1.42%, and 15.35%, respectively. Meanwhile, there was also a minor increase for the folding endurance after addition of xylan.

The addition of CX also improved the mechanical properties of the paper sheets but to a larger extent than addition of xylan as shown in Table 5. This was probably because the cationic groups in the CX could absorb the anionic hydroxyl groups (-OH) on the fibers, which could improve the combined interactive forces among fibers, thereby increasing the mechanical properties of sheets (Ren *et al*. 2009b). Compared to the reference paper sheet, when the amount of CX was 1.0 wt%, the tensile index, tear index, and burst index increased 13.5%, 12.0%, and 24.3%, respectively, and folding endurance increased 55.6%. The mechanical properties of the paper sheets became lower when the addition of CX was over 1.0 wt%. This was probably caused by excessive addition of cationic group in CX that prevented hydrogen bonding between cellulose fibers, which correspondingly resulted in the decrease of the paper strength (Liu *et al*. 2014).

Table 5. Mechanical Properties of Paper Samples After Addition of CX

Amount of Cationic-xylan (wt%)	Tear Index (mN·m^2/g)	Burst Index (kPa·m^2/g)	Tensile Index (Nm/g)	Fold Endurance (Times)
0	6.36 ± 0.43	2.02 ± 0.10	35.67 ± 0.89	9 ± 0.4
0.3%	6.44 ± 0.55	2.26 ± 0.13	37.45 ± 0.82	9 ± 0.4
0.5%	6.65 ± 0.34	2.38 ± 0.13	38.89 ± 0.71	10 ± 0.5
1.0%	7.12 ± 0.52	2.51 ± 0.21	40.48 ± 0.74	14 ± 0.4
1.5%	6.89 ± 0.39	2.31 ± 0.15	39.06 ± 0.85	12 ± 0.5

Note: Grammage of each paper was approximately 55 g/m^2, the stirring time of pulp after addition of CX was10 min

When CX-g-PHGH was added to the paper sheets, there were obvious improvements to the mechanical properties of the paper sheets that were affected not only by the amount of addition but also the grafting ratios of PHGH (Table 6).

Table 6. Mechanical Properties of Paper Samples After Addition of CX-g-PHGH

Grafting Ratio (%)	Amount of CX-g-PHGH (wt%)	Tear Index (mN·m^2/g)	Burst Index (kPa·m^2/g)	Tensile Index (Nm/g)	Fold Endurance (Times)
0	0	6.36 ± 0.44	2.02 ± 0.12	35.67 ± 0.90	9 ± 0.4
14.27	1	7.35 ± 0.31	2.48 ± 0.13	40.96 ± 0.75	12 ± 0.5
15.54	1	7.84 ± 0.59	2.59 ± 0.22	41.97 ± 0.82	15 ± 0.5
16.90	1	7.73 ± 0.53	2.60 ± 0.22	42.00 ± 0.75	15 ± 0.6
18.45	1	7.97 ± 0.50	2.63 ± 0.16	42.83 ± 0.95	16 ± 0.5
18.45	0.3	6.71 ± 0.52	2.22 ± 0.18	40.35 ± 0.93	13 ± 0.5
18.45	0.5	7.35 ± 0.31	2.57 ± 0.12	40.97 ± 0.85	14 ± 0.5
18.45	1.5	7.26 ± 0.33	2.55 ± 0.15	41.23 ± 0.81	12 ± 0.4

Note: Grammage of each paper was approximately 55 g/m^2, the stirring time of pulp after addition of CX-g-PHGH-1 was 10 min.

As shown in Table 6, the higher grafting ratio of CX-g-PHGH resulted in higher mechanical properties of the paper sheet. When CX-g-PHGH-1 (with the highest grafting ratio of 18.45%, obtained at optimal conditions) was added, the index of tensile, tear, burst, and folding endurance increased 20.1%, 25.3%, 30.2%, and 77.8%, respectively, compared with the reference paper sheet. It is also well known that paper strength is dependent on the fiber strength as well as the hydrogen bonding force among fibers. The PHGH contains guanidine groups with a positive charge that allows the PHGH to adsorb onto the anionic groups on the fibers (Guan *et al.* 2008). Therefore, the improvements of mechanical properties could be explained by the following: when the CX-g-PHGH was added into the paper pulp, the CX-g-PHGH filled or adhered to the space between the fibers, which resulted in the increase of the bonding point and bonding area between fibers and correspondingly improved the mechanical properties of the sheets (Lima *et al.* 2003). In addition, the guanidine and cationic groups of CX-g-PHGH adsorbed onto the negatively charged surfaces of the fibers, which resulted in the increase in fiber retention, the improvement in interfiber bonding, and, correspondingly, improved the mechanical properties of the paper sheets (Ren *et al.* 2009b). The mechanical properties of the paper samples saw improvement as the amount of CX-g-PHGH-1 added increased. However, the mechanical properties of paper began to decrease when the amount of added CX-g-PHGH-1 increased over 1.0 wt%. This can be explained by the excessive NH$_2$ brought from CX-g-PHGH-1 that prevented hydrogen bonding between the cellulose fibers, which resulted in a decrease of the paper strength (Liu *et al.* 2014).

Antimicrobial Test of the Paper Sheets with Addition of CX and CX-g-PHGH

In this work, the antimicrobial activity of the paper sheets with addition of the xylan derivatives was evaluated against *E. coli* bacteria by an inhibition zone method as shown in Fig. 7. As a comparison, the antimicrobial activity tests for the CX-g-PHGH (sample number 1 in Table 1, shown below as CX-g-PHGH-2) obtained with a lower initiator concentration and a lower relative grafting ratio of 15.5% was also studied.

The inhibition zone method is the most used method for antimicrobial activity testing of materials. Through using this method, the growth of bacteria was inhibited in the formation of transparent circles by the diffusion of antimicrobial agents in agar plates. The antimicrobial ability of the agents was evaluated by the size of the inhibition circle. Guanidino groups of guanidine polymers adsorbed the anions on the cell surface of *E. Coli* bacteria by electrostatic action, which then proceeded to destroy the normal metabolism

and surface structure of the living bacterial cells, sequentially inhibiting the growth of the bacteria effectively (Qian *et al*. 2008).
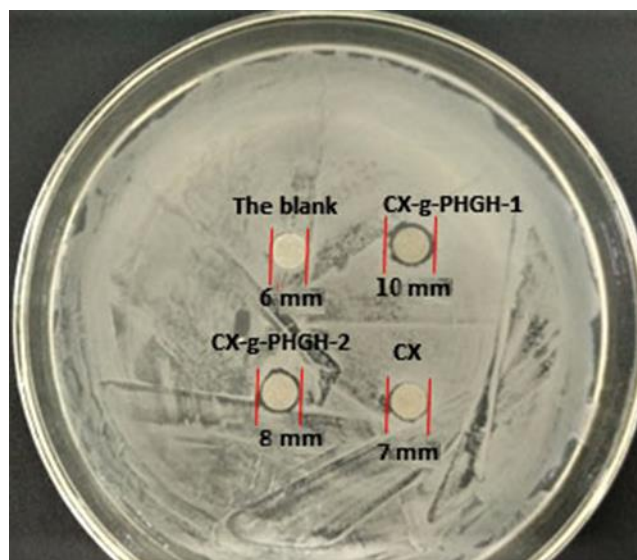

Fig. 7. The antimicrobial activity of the paper samples with the addition of CX, CX-g-PHGH-1, and CX-g-PHGH-2 against *E. coli* bacteria

Addition of the CX and CX-g-PHGH endowed the paper sheet with antimicrobial properties. The CX paper sample showed weaker antimicrobial ability than the CX-g-PHGH samples, exemplified by its smaller inhibition zone diameter of 7 mm. The diameter of the inhibition zone for the paper of CX-g-PHGH-1 (grafting ratio 18.45%) and CX-g-PHGH-2 (grafting ratio 15.54%) against *E. coli* enlarged from 6 mm to 10 and 8 mm respectively, which suggested that the CX-g-PHGH paper had good antimicrobial activity against *E. coli* and the antimicrobial activity was improved by the increase of PHGH contents grafted on the CX.

CONCLUSIONS

1. In the present study, a novel xylan-based antimicrobial additive agent was successfully prepared and applied in cellulosic paper sheets with improved the mechanical properties and antimicrobial activity against *E. Coli* bacteria. The derived xylan, cationic xylan-grafted-PHGH (CX-g-PHGH), was successfully synthesized by graft copolymerization of cationic xylan (CX) with guanidine polymer (PHGH) using ceric ammonium nitrate (CAN) as an initiator.

2. The optimal reaction parameters to efficiently obtain the CX-g-PHGH were 4 h at 60 °C with PHGH concentration of 0.039 mol/L and an initiator concentration of 4 mmol/L. At these conditions, the maximum grafting ratio and efficiency of 18.4% and 58.4% was reached. Furthermore, the mechanical properties of the paper sheets with added CX-g-PHGH had improvements. Compared to the mechanical properties of a paper sheet without any added xylan derivatives, the addition of CX-g-PHGH improved the mechanical properties of the sheet by up to 20.1% (tensile index), 25.3% (tear index),

30.2% (burst index), and 77.8% (fold endurance). Meanwhile, the paper sheet with addition of CX-g-PHGH exhibited improved viscosity and thermal stability as well as good antimicrobial activity against *E. Coli* bacteria, which was inherited from the antimicrobial activity of the guanidine in CX-g-PHGH.

3. The present work found a new way of synthesizing xylan derivatives and used it as an antimicrobial additive agent against *E. coli* bacteria in paper products. The obtained paper product demonstrated highly improved mechanical strength and antimicrobial properties as well as biodegradability and renewable properties that would find great potential in food-related areas, *e.g.*, packaging, baking, and napkin paper, as well as in other areas, such as pharmaceuticals and cosmetics, which expanded the applications of xylan in more highly value-added areas.

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