Preparation and Characterization of Tea Polyphenols-Chitosan-based Nanoparticles and Their Application in Starch Films

Qifeng Chen,[a] Liting Wei,[a] Yanping Lai,[b,]* and Yayun Liu [a]

Green composite starch films were prepared using tea polyphenols-chitosan-based nanoparticles as active agent and nanocellulose as strength agent, which showed good mechanical and antioxidant activities and is a candidate for food packaging in this work. Here, chitosan-based nanoparticles containing tea polyphenols (CNTP) were prepared by the ionic gel method. The best utilization of tea polyphenols could be achieved when the mass ratio of tea polyphenols to sodium tripolyphosphate was 3:1, the concentration of tea polyphenols was 0.01 g/mL, and the pH of chitosan solution was 4. Uniform and stable CNTP with a particle size of approximately 200 nm was prepared. CNTP release rates were 45.9% after 6 h, showing the ability to release slowly. Using starch as the substrate, nanocellulose as the enhancer, and tea polyphenols as the antioxidant and antibacterial agent, an active film was prepared that demonstrated improved transparency, mechanical properties, and antioxidant and antibacterial activity. Its transmittance reached 83.9%, and the tensile strength was 33.4 MPa. The scavenging efficiency of 2,2-diphenyl-1-picrylhydrazyl (DPPH) by the composite film was 42%, and the OD value of the composite film was 0.9348. The results showed that CNTP can prolong the release period and activity of tea polyphenols, and the composite film with CNTP had antibacterial and antioxidant activities to prolong the shelf life of food.

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Keywords: Chitosan-based nanoparticles; Starch film; Nanocellulose; Antimicrobial/antioxidant material; Tea polyphenols

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INTRODUCTION

Nanoparticles are designed to target specific cell groups or control the release of bioactive substances because of their unique size and surface properties (Yang *et al.* 2020). Biomacromolecules, such as polysaccharides, proteins, *etc.*, can form polycationic, polyanionic, or zwitterionic polymers. Association between polyanions and polycations results in polyelectrolyte complexes (PEC) (Meka *et al.* 2017). The driving force for their formation is electrostatic in nature, but hydrogen bonding, dipole-dipole, and charge transfer also contribute to their formation. Lall *et al.* (2020) synthesized chitosan-casein nanoparticles by polyelectrolyte complex (PEC) formation for applications in drug-controlled release. In terms of drug delivery, nanoparticles have the advantages of controlling the slow release of drugs, improving drug solubility, and enhancing efficacy

(Jahangirian *et al.* 2017; Archana *et al.* 2021). In addition, nanoparticles can increase the water solubility of lipophilic drugs as well as maximize their bioavailability and tissue biological distribution in drug delivery systems (Bakry *et al.* 2016; Maresca *et al.* 2016). When simulating the sustained release behavior of nanoparticles, the release mechanism of the core material is that the water in the buffer solution passes through the wall material into the nanoparticles, and the core material dissolves and spreads to the outside of the nanoparticles through the wall material (Cuomo *et al.* 2015). The release of microencapsulated core material is a complex process that is affected by many factors, such as the properties of core material, thickness of wall material, temperature, pH, and light (Nooshkam and Varidi 2020).

Chitosan (CS) has been developed as a carrier for the delivery of various active substances because of its high biocompatibility, excellent biodegradability, low toxicity, rich availability, and low cost. Ionic gelling refers to the addition of opposite charge electrolyte solution to cationic polymer solution to form compounds by electrostatic action. For example, when chitosan is used as a wall material to prepare nanoparticles, under acidic conditions, the $-NH^{3+}$ salt in the chitosan molecular chain is electrostatically combined with anionic electrolyte sodium tripolyphosphate, thus reducing the solubility of chitosan and precipitating precipitates in the solution to form nanoparticles (Hadidi *et al.* 2020). Compared with chitosan solution, chitosan-based nanoparticles carriers can slowly and controllably deliver different types of drugs, while maintaining interaction with the mucosal surface, which promotes the absorption of related drugs, improves drug bioavailability, and causes less side effects (Mehanny *et al.* 2016).

Tea polyphenols which also can be called polyhydroxyphenols, are comprised of more than 30 kinds of phenols. The main chemical components of tea polyphenols are divided into four categories: catechins, flavonoids, anthocyanins, and phenolic acids. Tea polyphenols are natural antioxidants, and catechins play an important role in its antioxidant reaction (Zhou *et al.* 2019). Dou *et al.* (2018) prepared active edible films by incorporating tea polyphenols into gelatin and sodium alginate. Antioxidant capacity was improved by increasing the tea polyphenols content in the films. The chemical structure that plays a key role in the antioxidant activity of tea polyphenols is the ortho-dihydroxyl or trihydroxyl structure, which can chelate metal ions and prevent the production of free radicals. This structure allows electron delocalization and is highly reactive to the quenching of free radicals (Hu *et al*. 2020). In many studies, tea polyphenols have been shown to have broad-spectrum antibacterial properties (Ignasimuthu *et al.* 2019; Zhang *et al.* 2021, 2022). Ren *et al.* (2020) prepared a highly antibacterial ultrahigh molecular weight polyethylene implant by incorporating tea polyphenols for joint replacement prostheses with multifunctionality. The study confirmed that tea polyphenol could locally kill bacteria by increasing intracellular reactive oxygen species and destroying bacterial films. Antibacterial packaging films containing tea polyphenols have also been prepared, and the films have good antibacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, and *Escherichia coli* (Shao *et al.* 2020).

Starch is a natural macromolecule that is present in the roots, stems, or seeds of plants. Starch is polymerized by glucose molecules, and its basic unit is α-D-glucopyranose (Pedroso and Rosa 2005). Due to the requirements of environmentally friendly and degradable packaging materials, starch has been developed because of its wide range of biological sources, low price, green safety, good film-forming properties, *etc*. However, the insufficient mechanical properties and strong hydrophilicity of starch film are a major problem in its application in active packaging film (Bhat *et al.* 2013; Lopez *et al.* 2015;

Song *et al.* 2018; Jamroz *et al.* 2018; Nordin *et al.* 2020). Therefore, starch is blended with other substances to prepare active packaging film to improve its mechanical properties.

As a natural nano-scale material, nanocellulose has a variety of properties that are different from traditional materials, including special shape and geometric size, high crystallinity, and high specific surface area. These properties result in advantages in the mechanical strength, rheological properties, and barrier properties of the composites. Bamboo has been selected because of its short growth cycle, wide range of sources, low price, and excellent aspect ratio of its nanocellulose (Chen *et al.* 2020).

In this study, the best preparation conditions of CNTP were explored to obtain nanoparticles with high loading rate and entrapment efficiency. The appropriate particle size and stable particle size were selected to fully improve the utilization efficiency of tea polyphenols. A bio-based composite packaging film with both strength and good antioxidant and antibacterial properties was prepared to ensure food quality and safety and to prolong the shelf life of food.

EXPERIMENTAL

Materials

Deionized water was produced by laboratory purification equipment. Sodium bromide (NaBr) and 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) were purchased from Shanghai Aladdin Biochemical Co., Ltd (Shanghai, China). Corn starch (amylose content: 32.5 ± 0.3%), sodium hypochlorite (NaClO), sodium hydroxide (NaOH), acetic acid (AR), sodium carbonate, and (Na_2CO_3) and glycerin ($C_3H_8O_3$) were obtained from Sinopharm Chemical Reagent Co., Ltd (Beijing, China). Chitosan (deacetylation degree ≥ 90%) was supplied by Shanghai Boao Biotechnology Co., Ltd. (Shanghai, China). Sodium tripolyphosphate (TPP) and methanol were provided by Tianjin Fuchen Chemical Reagent Factory (Tianjin, China). Tea polyphenols were purchased from Shandong Xiya Chemical Co., Ltd (Shandong, China). 2,2-Diphenyl-1-picrylhydrazyl (DPPH), gallic acid, and folin phenol were supplied by McLean Reagent Co., Ltd (Shandong, China). Bamboo pulp was a kraft pulp obtained from a pulp mill in Chongqing. All chemicals were reagent grade and used without further purification.

Preparation of Nanofibrillated Cellulose (NFC)

The bamboo pulp was oxidized according to the proportion of 1 g of absolute dry fiber (bamboo pulp) requiring 100 mL of deionized water, 0.016 g of TEMPO, 0.1 g of sodium bromide, and 10 mL of sodium hypochlorite. Deionized water, TEMPO, and sodium bromide were added into the slurry and stirred for 10 min. Sodium hypochlorite solution was added drop by drop and reacted for 4 h. The pH of the reaction slurry was maintained at 10 to 11 with sodium hydroxide solution. After 4 h, a small amount of ethanol was added to stop the reaction. The slurry was washed three times with deionized water. Finally, bamboo nanocellulose was obtained by cycling the pulp under 180 MPa for 8 times with a high-pressure homogenizer.

Preparation of CNTP

The formation process is shown in Fig. 1. A certain amount of chitosan was added to 1% acetic acid solution to form 0.5% chitosan-acetic acid solution, and the solution was adjusted to pH 4 by 0.04 g/mL NaOH. Tea polyphenols were configured with the concentration of 0.01 g/mL. Next, 2 mL of TPP solution was added to the chitosan solution and mixed for 30 min. A certain concentration of sodium tripolyphosphate solution was slowly dropped into the mixture, and the final mass ratio of sodium tripolyphosphate to chitosan was 1:3. After cross-linking for 30 min, the solution was centrifuged at 4 °C for 30 min. The supernatant was separated from the precipitate, and the concentration of tea polyphenols in the supernatant was determined by UV spectrophotometer. The precipitate was washed 3 times by centrifugation and then stored at 4 °C.

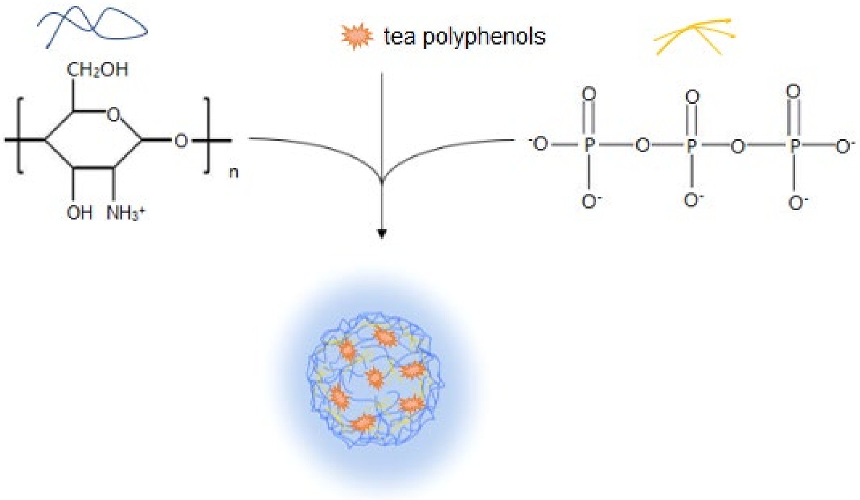

Fig. 1. Formation of chitosan-based nanoparticles

Preparation of Tea Polyphenols-Nanocellulose / Starch Composite Film

First, 5 g of starch and 1.5 g of glycerol were added to 100 mL of deionized water. The solution was heated in an oil bath at 80 °C for 30 min at the stirring rate of 300 rpm. The nano-cellulose was added to the film-forming solution with a content of 4 wt% (Chen *et al*. 2019). The film-forming solution was cast on a polytetrafluoroethylene plate, degassed in the vacuum of 0.01 MPa for 15 min to remove the bubbles in the solution, and oven-dried at 40 °C to form a film. The nanocellulose/starch composite film was completely immersed in CNTP solution for 15 min, and the composite film was dried in a constant temperature and humidity box of 40 °C and 70 % humidity for 12 h to obtain the bio-based composite active film.

Simulation Test of Sustained Release Behavior

The nanoparticles containing tea polyphenols were dispersed in a dialysis bag, placed into PBS buffer solution (0.04 mol/L) of pH 7.4, and oscillated continuously in a water bath at 120 rpm and 37 °C. At predetermined time intervals (2, 4, 6, 8, 10, 12, 24 h), 1 mL samples were taken, and the volume was replaced with fresh buffer. The concentration of tea polyphenols was measured by UV spectrophotometer at 765 nm. The efficiency of release of tea polyphenols was determined by use of Eq. 1,

$$E_r\,(\%) = P_r/P_t \times 100 \tag{1}$$

where P_r is the amount of tea polyphenols released (g) and P_t is the total amount of tea polyphenols encapsulated by chitosan-based nanoparticles (g).

Characterization

Atomic force microscopy (AFM)

The nanocellulose was diluted to 200 µg/mL and then ultrasonicated in an ice bath for 5 min. A drop of suspension was placed on the mica sheet to dry naturally. The surface morphology and morphology of the samples were analyzed by atomic force microscope (Bruker Multimode8, Karlsruhe, Germany). The images of silicon cantilevers with typical resonance frequency of 240 kHz and spring constant of 40 N/m were obtained in tapping mode at room temperature at a scanning rate of 1 Hz.

Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (Bruker VERTEX70) was used to analyze nanocellulose and CNTP. The wavelength range was 400 to 4000 cm^{-1}, and the spectral resolution was 4 cm^{-1}. A small amount of sample and KBr were thoroughly mixed and ground at a mass ratio of 1:100, and then they were pressed into a round translucent sheet with a tablet press for tests.

Transparency test

The optical transmittance of the composite film was measured by UV spectrophotometer in the wavelength range of 200 to 800 nm. The data were collected at the scanning speed of 600 nm/min and the bandwidth of 2 nm.

Test of sustained release behavior

The absorbance of free tea polyphenols in the supernatant was determined by UV spectrophotometer under the maximum absorption of 765 nm, and the concentration of free tea polyphenols in the supernatant was calculated by substituting it into the standard curve. The entrapment efficiency of tea polyphenols and the loading rate of nanoparticles were calculated using Eqs. 2 and 3,

$$EE\ (\%) = \frac{M_t - M_f}{M_t} \times 100 \tag{2}$$

$$LC\ (\%) = \frac{M_t - M_f}{M_{NP}} \times 100 \tag{3}$$

where *EE* (%) is the entrapment efficiency, *LC* (%) is the loading level, M_t is the total amount of tea polyphenols added (g), M_f is the amount of tea polyphenols in the supernatant (g), and M_{NP} is the total amount of nanoparticles produced.

Tensile tests

The film was cut into rectangular strips of 8 mm × 60 mm and placed in a constant temperature and humidity environment (23 °C, 50% relative humidity) for 48 h to balance the water content. The thickness of each strip was measured by a thickness meter (L&W251, Kista, Sweden), and the tensile strength of the composite film was evaluated with an electronic universal testing machine (INSTRON 5565, Instron, England). The test speed was 20 mm/min, and the initial gap was 20 mm. Each sample was tested three times, and the average value was recorded.

Antioxidant property test

The antioxidant property of film samples was evaluated by the DPPH (2-biphenyl-1-picrylhydrazino) free radical scavenging method. The film was cut into 5-cm discs and packed in a glass bottle containing 25 mL of deionized water. After 2, 4, 6, 8, 10, and 12 days, 3 mL of the composite film extract in the glass bottle was mixed evenly with 1 mL of DPPH methanol solution (0.1 mmol/L), which was placed in the dark for the 30 min. Then the absorbance was measured at 517 nm by ultraviolet spectrophotometer (UV3802, Korea). The percentage of DPPH radical quenching activity was determined by Eq. 4,

$$DPPH\ scavenging\ effect\ (\%) = \frac{Abs_{blank} - Abs_{sample}}{Abs_{blank}} \times 100 \qquad (4)$$

where Abs_{blank} is the absorbance value of methanol solution of DPPH at 517 nm, and Abs_{sample} is the absorbance value of sample extract at 517 nm.

Antibacterial property test

Escherichia coli was selected as the representative Gram-negative bacteria, and the antibacterial performance of the composite film was evaluated by agar diffusion. The composite film was cut into discs with a diameter of 1 cm, which were sterilized by UV for 30 min. A total of 108 *E. coli* suspensions with CFU/mL concentration were poured into the autoclaved solid medium at a volume ratio of 1:1000 and coated evenly. Finally, the round sample was placed in the center of the coated medium, and the samples were incubated at 37 °C. The size of the bacteriostatic zone was determined after 24 h. The nanocellulose/starch composite film and pure starch film without CNTP were evaluated in the same way.

The antibacterial rate of the composite film was determined by enzyme-labeled kinetic method. First, 20 µL of *E. coli* suspension and 160 µL of sterilized liquid medium were added to 96-well culture plate, then 20 µL of film extract was added. The nanocellulose/starch composite film extract without CNTP was used as the control. The 96-well plate was put into the enzyme labeling instrument, and the temperature was 37 °C. The OD value was determined after 24 h.

RESULTS AND DISCUSSION

Morphology Analysis of CNTP

Figure 2 shows the AFM diagram of CNTP. The chitosan-based nanoparticles were quasi-spherical, and the size distribution was uneven.

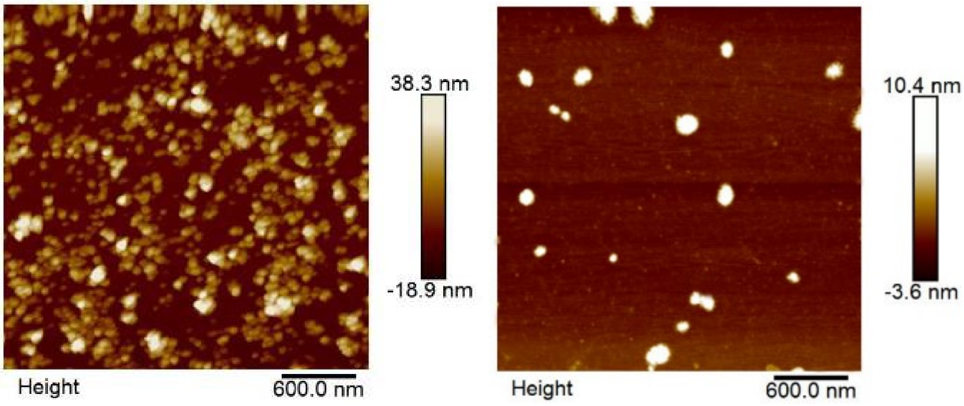

Fig. 2. AFM images of CNTP

Some particles aggregated into large groups. The size of the nanoparticles in the AFM diagram was 50 to 180 nm, which is smaller than the particle size measured by the nanoparticles analyzer. The similar finding in the size of chitosan-based nanoparticles has can be seen in the previous study (Gadkari *et al.* 2019). This result reflects shrinkage of dry samples in the AFM experiment.

Analysis of Sustained Release Behavior of CNTP

The release process of tea polyphenols and CNTP in PBS (pH 7.4) at 37 °C is shown in Table 1. Pure tea polyphenols exhibited very rapid release behavior, reaching 85.8% after 6 h, which may be due to the low molecular weight of tea polyphenols and their rapid diffusion without other forces. The tea polyphenols carried by chitosan were initially released in the first 2 h, and the extent of release reached 26.9%. The release gradually slowed, reaching 40.7% and 45.9% after 4 h and 6 h, respectively. The rapid release of tea polyphenols in the first 4 h was attributed to the release of tea polyphenols adsorbed on the surface of nanoparticles (Woranuch and Yoksan 2013). The release rate decreased thereafter. At this time, the tea polyphenols diffused out of the nanoparticles through the medium. Similar observations have been reported in the literature (Matshetshe *et al.* 2018). The results showed that the chitosan-based nano-carrier achieved the slow release of tea polyphenols and prolonged the release time of tea polyphenols.

Table 1. Release Efficiency of Tea Polyphenols from Pure Tea Polyphenols Solution (a) and CNTP (b)

Time (h)		2	4	6	8	10	12	24
Release rate (%)	a	27.88	64.36	85.76	88.12	90.30	95.89	98.66
	b	26.94	40.72	45.89	48.60	50.23	52.65	59.87

Structural and Morphological Analysis of Nanocellulose

Figure 3 shows an AFM image of bamboo nanocellulose. A partial kink was observed, indicating that the nanocellulose experienced mechanical damage. The average length of nanocellulose was 355 to 1355 nm, and the width ranged from 5 to 14 nm. Because of the tip expansion effect of AFM, the apparent size was slightly larger than the actual size. The high level of NaClO addition used in the oxidation stage can lead to significant depolymerization of cellulose chains, so some shorter nanocellulose can be observed.

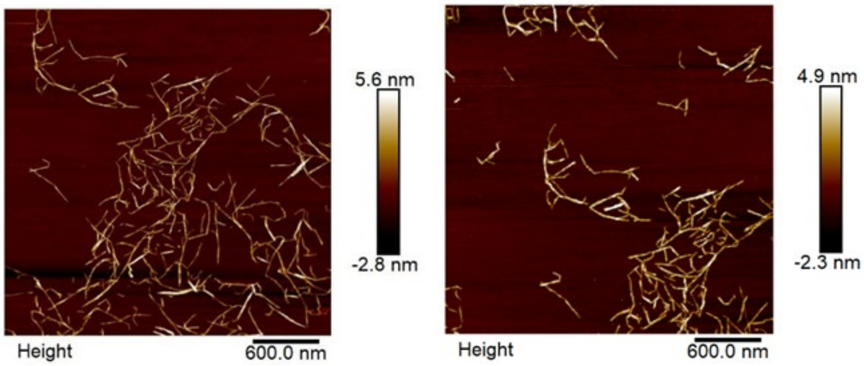

Fig. 3. AFM Image of nanocellulose prepared from bamboo pulp

Infrared Analysis of CNTP

As shown in Fig. 4, the absorption peak of 3494 cm^{-1} is the combined peak of NH_2 and OH stretching vibration in chitosan, which is sharper in the CNTP and transferred to 3450 cm^{-1}, indicating that the hydrogen bond is enhanced (Pan *et al.* 2019). The CH stretching vibration peak at 2881 cm^{-1} in chitosan flattened in CNTP. The peaks at 1667 and 1604 cm^{-1} in chitosan represent the carbonyl stretching of $CONH_2$ and the bending vibration of NH_2, respectively. These two peaks were transferred to 1646 cm^{-1} and 1554 cm^{-1} in CNTP, respectively, indicating that the amino group of chitosan was cross-linked with sodium tripolyphosphate. Its phosphate group interacted with the amino group, which was used to enhance the molecular-molecular interaction of CNTP (Liang *et al*. 2011).

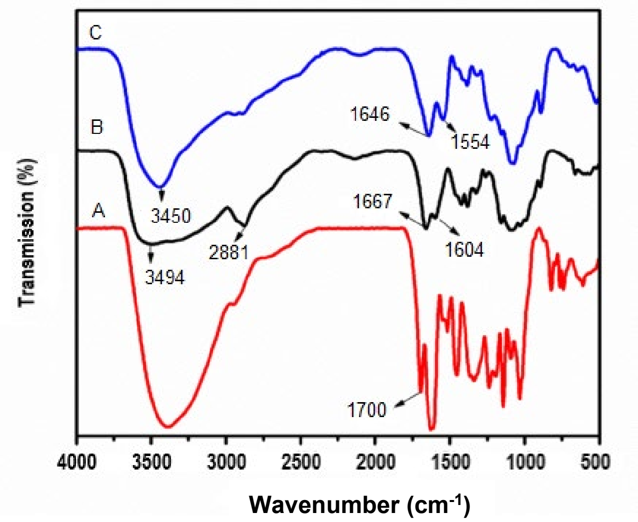

Fig. 4. Infrared spectra of tea polyphenols (A), chitosan (B) and CNTP (C)

The spike of tea polyphenols at 1700 cm^{-1} was not observed in CS-TPP nanoparticles containing tea polyphenols, indicating that the hydroxyl groups in tea polyphenols interact with the amino groups in chitosan molecules (O'Callaghan and Kerry 2016). Because of the hydrogen bond association between the amino group of chitosan and CO in tea polyphenols, the peak from 700 cm^{-1} to 850 cm^{-1} in tea polyphenols did not appear in CNTP. The results of infrared measurements confirmed that tea polyphenols were successfully encapsulated in chitosan-based nanoparticles.

Surface Morphology Analysis of Composite Film

Figure 5 shows the surface and cross-section SEM diagrams of starch/NFC/starch and NFC/starch/nanoparticles films. The surface of the pure starch film is very smooth, indicating that the starch is plasticized by glycerol. Uniformly dispersed curved filaments were observed on the surface of NFC/starch film, in which nanocellulose was wound into a reticular structure, and had good compatibility with starch. However, the surface of NFC/starch/nanoparticles film was very smooth, and no nanocellulose was observed. This was attributed to the electrostatic interaction between the positively charged amino group in chitosan and the negatively charged carboxyl group of nanocellulose, which changed the morphological distribution of nanocellulose on the surface of starch film.

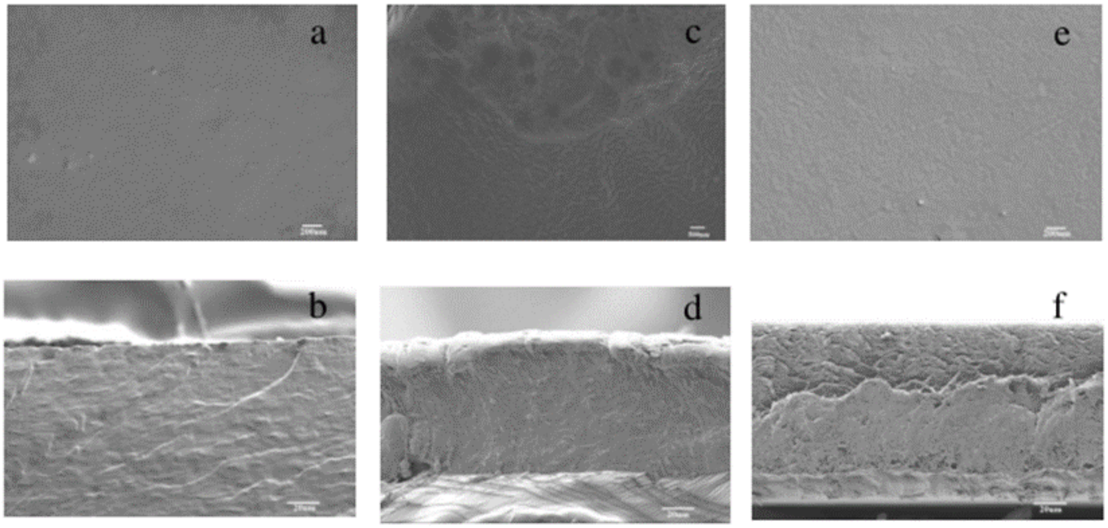

Fig. 5. SEM images of the surfaces and cross sections of starch film (a, b), NFC/starch film (c, d) and NFC/starch/ nanoparticles film (e, f)

Comparing the cross sections of the three kinds of films, the cross sections of starch films were very flat and tight. Some bumps were observed in the cross sections of NFC/starch films, but no fiber pull-out and peeling were observed, which showed that the cellulose nanofiber had good adhesion to the starch matrix. There were some pores in the cross section of NFC/starch/nanoparticles films, and the overlapping layered structure was obvious.

Transparency Analysis of Composite Film

Figure 6 shows the light transmittance of starch film, NFC/starch film, and NFC/starch film with CNTP in the wavelength range of 200 to 800 nm. The maximum light transmittance of starch film and NFC/starch film was very similar, at 57.9% and 55.0%, respectively. It was confirmed that the addition of nanocellulose did not have much effect on the transparency of starch film. The size of most nano-sized materials in a certain direction is smaller than the wavelength of visible light. When they are dispersed into the polymer matrix, there is no significant change in the transparency of the film (Zeng *et al.* 2005). After NFC/starch film was impregnated with nanoparticles solution containing tea polyphenols, the light transmittance was greatly improved, and the maximum light transmittance was 83.8%.

The transparency of the composite films prepared by impregnation was improved, which can be seen a previous study (Campa-Siqueiros *et al.* 2020). In addition, NFC/starch/ nanoparticles films showed an ultraviolet absorption peak at 260 nm, which was caused by the electron transition in the benzoyl system in tea polyphenols. This result confirmed the presence of tea polyphenols in the composite film. From the digital images of the three kinds of films (Fig. 7), it was also obvious that NFC/starch/nanoparticles films had high transparency and appeared slightly yellowish in color, which was due to the influence of tea polyphenols. The transparency results perceived by human eyes were in good agreement with the actual measurements.

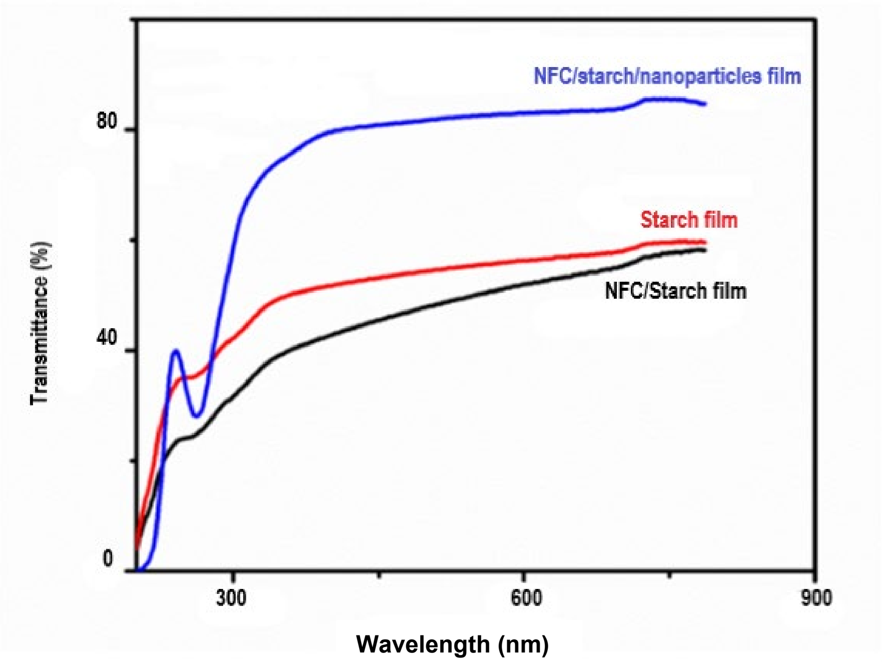

Fig. 6. UV–Vis transmittance spectra of starch, NFC/starch, and NFC/starch/ nanoparticles films

Fig. 7. Digital images of starch (a), NFC/starch(b) and NFC/starch/ nanoparticles (c) films

Analysis of Mechanical Properties of Composite Film

The tensile strength and elongation at break of pure starch film, bamboo nanocellulose/starch film, and bamboo nanocellulose/starch film loaded with CNTP were tested to evaluate their mechanical properties. The results are shown in Fig. 8.

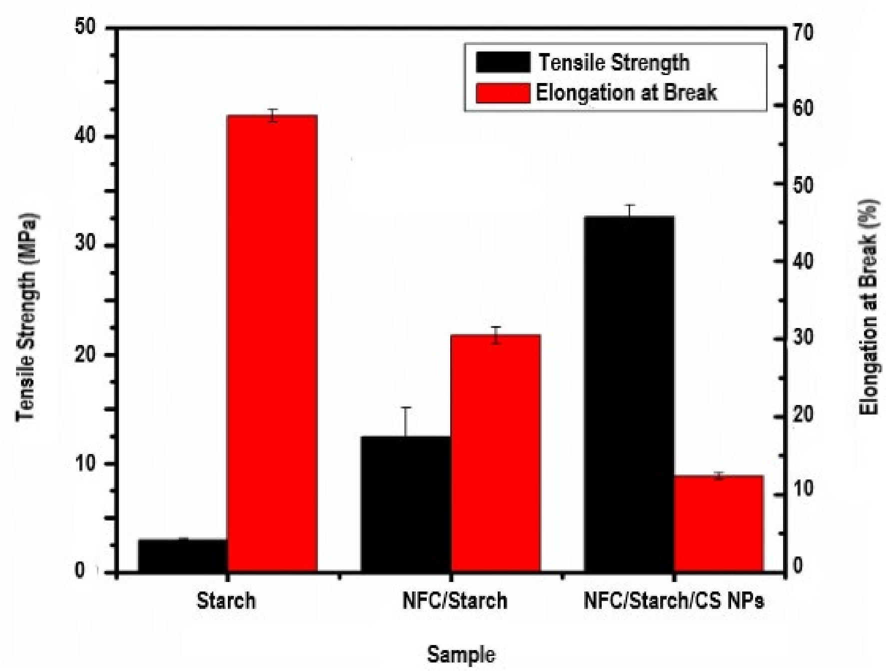

Fig. 8. Tensile strength and elongation at break of default film, NFC/starch film, and NFC/starch/CS NPs film

The tensile strength of starch film with bamboo nanocellulose was approximately 4 times that of pure starch film, indicating that bamboo nanocellulose can enhance the mechanical properties of starch film. The tensile strength of bamboo nanocellulose/starch film loaded with CNTP was the highest, at approximately 2.5 times higher than that of bamboo nanocellulose/ starch film. Similar behaviors have already been observed (Chen *et al.* 2020; Hou and Yan 2021). This result may be due to the destruction of the crystalline region of starch during gelatinization, resulting in more OH-exposed, binding to the NH^+ of chitosan particles in a hydrogen bond (Huang *et al.* 2006; de Mesquita *et al.* 2010).

After the addition of CNTP, the flexibility and ductility of the composite film were seriously weakened, and the elongation at break of the composite film was only approximately 1/6 of that of the pure starch film. This was consistent with the change trend of the elongation at break of the films after adding chitosan nanoparticles to starch films reported by Shapi'i *et al.* (2022). This phenomenon was attributed to the intermolecular forces generated by the adsorption of chitosan-based nanoparticles on the surface of starch films. This resulted in a decrease in the free volume of the starch matrix and a decrease in the elongation at break of the films.

Analysis of Antioxidant Performance of Composite Film

The antioxidant activity of the composite film was evaluated by DPPH radical scavenging test. DPPH exists stably in methanol solution and has characteristic absorption at 515 nm. Antioxidants react with DPPH to fade it from purple to yellow, resulting in a change in absorbance (Enayat and Banerjee 2009). As shown in Fig. 9, the scavenging rate of DPPH by the composite film was approximately 42%.

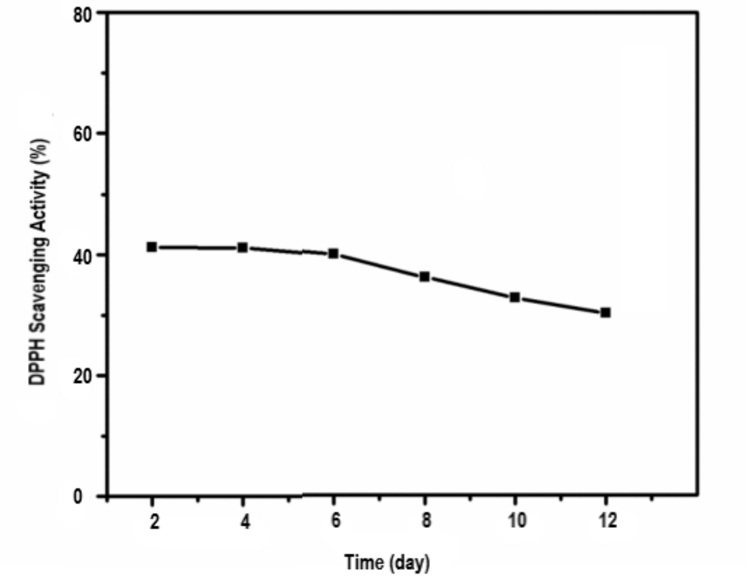

Fig. 9. Free radical scavenging efficiency of bamboo nanocellulose / starch film loaded with CNTP

Compared with the results of previous study (He *et al.* 2020), the scavenging rate of DPPH by composite film was lower, because tea polyphenols were wrapped by chitosan-based nanoparticles. Tea polyphenols contained several aromatic rings with hydroxyl groups, which can form stable compounds with free radicals to achieve the purpose of scavenging free radicals (Yen *et al.* 2008). With the increase of experimental time, the free radical scavenging efficiency of the composite films decreased, but the decrease was not significant. The reason may be the consumption of anti-oxidation active substances in the process of free radical scavenging, which also shows that the use of chitosan-based nanoparticles as carriers can improve the stability and activity of tea polyphenols and prolong the shelf life of food.

Analysis of Antibacterial Performance of Composite Film

The agar diffusion experiment results are shown in Fig. 10. No bacteriostatic zone was observed around the three samples. A small transparent area could be seen under the NFC/starch/nanoparticles film, indicating that starch and nanocellulose had no antibacterial effect on *E. coli*, but the composite film containing CNTP inhibited the bacteria through contact. NFC/ starch/nanoparticle films have non-diffused antibacterial properties compared with films directly added with tea polyphenols (Chen *et al.* 2022).

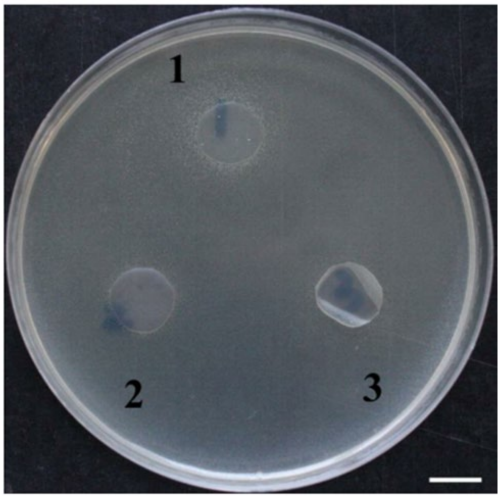

Fig. 10. Inhibition zone test of (1) starch film, (2) NFC/starch film, and (3) NFC/starch/nanoparticles film in agar

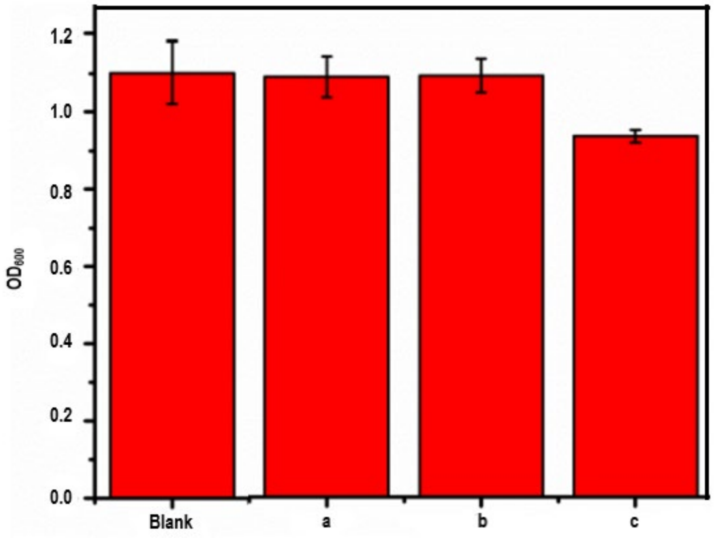

Fig. 11. OD value of culture solution (blank) without film and with starch film (a), NFC/starch film (b), and NFC/starch/nanoparticles film (c)

The turbidity of the culture medium for bacterial growth is proportional to the bacterial concentration. The antibacterial effect of the material to be tested can be quantified by testing the OD value of each culture medium on the 96-well plate. The test results are shown in Fig. 11. The OD value of the culture medium without composite film (blank sample) was 1.1009, and that of the culture medium with starch film and NFC/starch film were 1.0879 and 1.0912, respectively, which was not much different from that of the blank sample. The OD value of the culture medium of the NFC/starch/nanoparticles film was 0.9348, which was slightly lower than that of other films without CNTP. This result confirmed that the NFC/starch/nanoparticles films had a certain bactericidal effect on *E. coli*, but the bactericidal effect was not obvious. This may be due to the fact that the outer film of Gram-negative bacteria is mainly composed of negatively charged lipopolysaccharides that resist the damage of tea polyphenols (Shaheen *et al*. 2016).

CONCLUSIONS

1. Nanocellulose/starch film was placed in chitosan-based nanoparticles containing tea polyphenols (CNTP) suspension to prepare degradable composite active packaging film. The optimized nanoparticles can prolong the release period of tea polyphenols, and the drug release rate was only 45.9% after 6 h.
2. The tensile strength of the nanofibrillated cellulose (NFC)/starch/nanoparticles film increased to 30.3 MPa, which was approximately 10 times that of the starch film. The maximum light transmittance of composite films was as high as 83.8%.
3. The NFC/starch/nanoparticles film showed good antioxidant activity, and the scavenging extent of DPPH was 42%. Finally, the composite film showed good antibacterial properties.
4. The environmentally friendly composite film prepared in this study can be further applied to food packaging to prolong the shelf life of food.

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