

Article

Preparation, Characterization, and Release Kinetics of *Zanthoxylum bungeanum* Leaf Polyphenol–Chitosan Films

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Abstract: This study aimed to prepare a composite film with antioxidant activity for fruit and vegetable preservation. Polyphenols were extracted from *Zanthoxylum bungeanum* leaves (ZMP), and their composition was studied. ZMP-chitosan film (ZMP/C-film) was prepared by tape casting and the film properties were evaluated. The results revealed that ZMP was mainly composed of seven components: epicatechin (3.24 mg/g), chlorogenic acid (3.59 mg/g), coumarin acid (1.40 mg/g), ferulic acid (4.36 mg/g), quercetin (7.61 mg/g), quercetin (4.52 mg/g), and kaempferol (2.51 mg/g). The opacity of the film incorporated with ZMP significantly increased by 2.28 times. Flexibility (elongation at break) increased by 72%, while the ZMP/C-films had lower tensile strength (32.15–40.3 MPa). Microstructurally, scanning electron microscopy results indicated that ZMP and Chitosan (CS) were compatible. Fourier transform infrared spectroscopy revealed the formation of a dense structure between ZMP and CS. Analysis of X-ray diffraction indicated a tendency toward an increase in the amorphous characteristic of the ZMP/C-film. The kinetic results of polyphenol release indicate that ZMP release was mainly achieved through the dissolution of CS-based polymer frameworks. The release rate and rate of ZMP in the membrane were highest in 3.0% acetic acid (*v/v*) (33.62%). The scavenging rates of DPPH and ABTS⁺ free radicals by the 1 g/dL ZMP/C-film were 0.83 and 0.82 times higher than those of 1.0 mg/mL vitamin C (Vc) under the same conditions. In addition, ZMP/C-film was used for strawberry preservation. When stored at 25 °C for 7 d, the weight loss rate and V_C content of strawberries preserved with ZMP/C-film decreased by 23.4% and increased by 14.2% compared to C-film, respectively. ZMP/C-film prolonged the shelf life of strawberries by more than 4 days.

Keywords: *Zanthoxylum bungeanum* leaf polyphenol; chitosan; slow—release film; characterization; antioxidant; preservation



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1. Introduction

Fruits and vegetables are very popular on the human dining table for their high content of biologically active components, including vitamins, phenolics, dietary fibers, and anthocyanins [1–3]. However, fresh agricultural products are highly susceptible to water loss, mechanical damage, respiratory processes, and microbial spoilage during the transportation from production plants or farms to retail stores [4,5]. Developing a green and efficient solution to extend the shelf-life period of fresh agricultural products is essential to control corruption and reduce waste.

Chitosan-based packaging materials have the characteristics of being renewable, non-toxic, and biodegradable. Applying them in the field of fruits and vegetables preservation can prevent food oxidative damage, rancidity, and microbial spoilage (e.g., Mandarins, kiwifruit, apples, and tomatoes) [2–4]. However, some physical properties such as unsound strength, water permeability, gas transmission, and antioxidants of chitosan film (C-film) have narrowed its application [6]. To further improve the physicochemical properties of C-film, various natural activity components were added into C-film to enhance its

performance, such as polyphenols, tannins, terpenoids, oils, or flavonoids. Furthermore, chitosan (CS) is used as a carrier to control the release of active substances. Hu prepared thermo-sensitive chitosan drug-loaded microspheres and nanoparticles using chitosan as a carrier and bovine serum albumin as a drug model, kinetically fitted the drug release behavior of the two carrier forms, and elucidated the diffusion mechanism [7]. Chen et al. prepared α -tocopherol encapsulated in chitosan nanoparticles by the emulsification-ionic gelation method, which could regulate the pH value to slowly release tocopherols to prolong the antioxidant effect [8].

Polyphenols are a class of active substances containing hydroxy phenolic structures. It has significant antioxidant activity, reducing oxidative damage to cells in the body [9], and is used as a natural antioxidant in food products [10]. *Zanthoxylum bungeanum* leaf is a by-product of the *Zanthoxylum bungeanum* industry, with a polyphenol content of up to 552 g/kg [9], but most of the *Zanthoxylum bungeanum* leaf is discarded, even burned or buried, causing great waste [11]. Utilizing ZMP as an antioxidant in fruits and vegetables is a good strategy. However, polyphenols are susceptible to factors such as temperature, light, oxygen, and metal ions, which affect their antioxidant activity, greatly limiting their bioavailability [8]. To overcome its volatilization and oxidation shortcomings, more and more researchers are encapsulating active substances such as polyphenols in sustained-release films to improve polyphenol utilization [12]. Gomaa et al. developed a composite alginate-chitosan film containing fucoidan and found that polyphenol release was influenced by the medium, pH, and the interaction force with the polymer matrix and other factors [13].

To improve the physical properties and antioxidant activity of C-film, the main objective of our study was to produce films by embedding *Zanthoxylum bungeanum* leaf Polyphenol (ZMP) in C-film to prepare a sustained-release film (ZMP/C-film). The physical properties, mechanical properties, structure, release performance, and antioxidant activity of the ZMP/C-film were characterized. Finally, its practical food preservation performance was also investigated using strawberry as food samples.

2. Materials and Methods

2.1. Materials

Zanthoxylum bungeanum leaf (Dahongpao) was obtained by the Institute of Agricultural Products Processing, Shanxi Academy of Agricultural Sciences, collected from pepper bases in Ruicheng County, Yuncheng City. Chitosan (molecular weight 165 kDa, low viscosity, deacetylation $\geq 95\%$) was purchased from Xi'an Big Harvest Biotechnology Co., Ltd., Xian China. Gallic acid (standard) was obtained from Beijing Century Aoke Biotechnology Co., Ltd., Beijing, China. In addition, 95% ethanol, the Folin–Ciocalteu reagent (FCR), Vitamin C, tannic acid, formic acid, acetic acid, and glycerol (analytical purity) were purchased from Shanghai Adamas Reagent Co., Ltd., Shanghai, China. 1, 1-Diphenyl-2-picrylhydrazyl radical (DPPH, analytical purity) was provided by Shanghai Huicheng Biotechnology Co., Ltd., Shanghai, China. 2,2-biazo-bis (3-ethyl-benzothiazole-6-sulfonic acid) diammonium salt (analytical purity) was provided by Hubei Zhengxingyuan Fine Chemical Co., Ltd., Hubei, China. The water used was ultrapure water.

2.2. Methods

2.2.1. Preparation of ZMP

A certain amount of ZMP was baked at 40 °C for 6 h, then crushed and sieved (60 mesh) to produce ZMP powder. The extract was dissolved in 60% (v/v) ethanol solution at a ratio of 1:20 (g/mL), then centrifuged at 6000 r/min for 10 min after a water bath at 40 °C for 60 min. The supernatant was concentrated to 25% volume (ZMP crude extract) and freeze-dried under vacuum to obtain ZMP. The total phenolic content of the extracted ZMP was 41.85 mg/L according to the standard curve of gallic acid (see Figure 1).

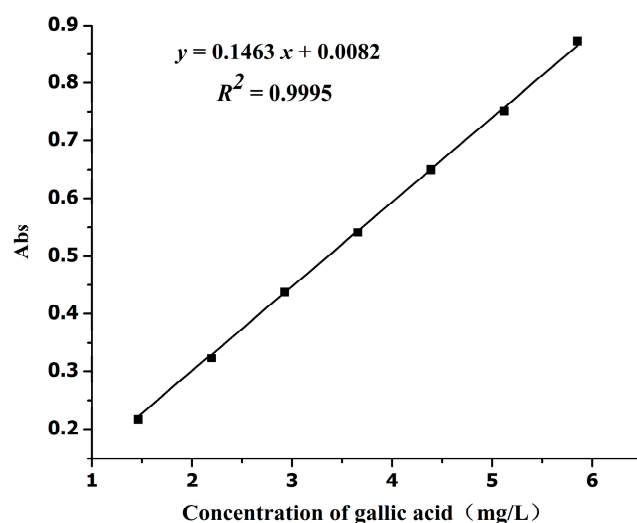


Figure 1. Standard curve of gallic acid.

2.2.2. Purification and Analysis of ZMP

For analysis of ZMP composition, ZMP crude extract was purified. The 25 mL ZMP crude extract passed down the glass column (50×1.5 cm) wet-packed with polyamide at a fixed flow rate of 1.5 BV/h. The effluent was gathered and monitored at 0.5 BV intervals. After equilibrium of the distribution system, the column was in turn eluted with ddH₂O and 75% (v/v) ethanol solutions, and the resulting solution was monitored and analyzed. The purified ZMP was eventually obtained for further composition analysis.

Analysis of the purified ZMP composition was performed by HPLC using a Symmetry C18 column ($150 \text{ mm} \times 3.9 \text{ mm}$, $5 \mu\text{m}$) at 30°C with a binary phase at a flow rate of 1.0 mL/min. The mobile phase consisted of 0.1% formic acid (A) and MeOH (B). The binary gradient elution program was set as follows: 0 min, 90% A, 10% B; 5 min, 80% A, 20% B; 10 min, 60% A, 40% B; 20 min, 50% A, 50% B; 30 min, 20% A, 80% B; 40 min, 70% A, 30% B; 50 min, 90% A, 10% B. The polyphenol standards (2 mg) (epicatechin, chlorogenic acid, catechin, vanillin, p-coumaric acid, ferulic acid, m-coumaric acid, rutin, o-coumaric acid, quercetin, quercetin, naringenin, kaempferol) were dissolved in 5 mL of MeOH. The mixed standard solutions of 1, 5, 10, 20, 40, and 60 $\mu\text{g/mL}$ were prepared by diluting the polyphenol standard solutions in MeOH. The injection volumes of both the ZMP solution and the mixed standard solution were 5 μL . The detection wavelength was 280 nm.

2.2.3. Preparation of Slow—Release Film

Slow—release film was prepared by the casting method [14]. The ZMP solutions (4 and 8% w/v) were prepared by dissolving ZMP in 25 mL of 60% (v/v) ethanol solution and stirring for 10 min. The tannic acid (TA) solution was used as a positive control and prepared by dissolving TA in 25 mL of 60% (v/v) ethanol solution and stirring for 10 min. Then, the ZMP solution and TA solution were mixed with 75 mL of 1% (v/v) acetic acid solution at 200 rpm at room temperature for 20 min, respectively. The CS solution was prepared by dissolving 2 g of CS in 100 mL of 1% (v/v) acetic acid solution and heating to 60°C while stirring for 30 min. ZMP/CS solution and TA/CS solution were prepared in ratios of 1:1 and stirred at a speed of 200 rpm at room temperature for 2 min. The ZMP/C-film, TA/C-film, and C-film were made by adding 1 g of glycerin to each of the solutions developed and stirring at 200 rpm for 10 min, at room temperature. After homogenization, filtration, and degassing, 20 mL of the mixtures were then poured into Petri dishes ($\Phi = 9$ cm) and dried at 25°C for 24 h. The films were equilibrated in an incubator at 25°C with 50% relative humidity for 48 h.

2.2.4. Performance Characterization of ZMP/C-film Thickness, Opacity, and Solubility

The film thickness was determined by selecting 10 random locations on the surface of the specimen and measuring with a micrometer (± 0.01 mm) from 0 to 25 mm. The measurement points were repeated for each test point 3 times.

The opacity of the films was measured using UV–visible spectrophotometry (Shenzhen Hongyong Precision Instrument Co., Ltd., Shenzhen, China). A prepared film of $30\text{ mm} \times 10\text{ mm}$ was fixed to the inner wall of the colorimetric dish. The absorbance of the films was measured at a wavelength of 600 nm. The colorimetric dish was used as the control group. The experiment was repeated three times. The opacity was calculated using Formula (1):

$$O = \frac{Abs_{600}}{d} \quad (1)$$

where O is opacity, cm^{-1} ; Abs_{600} is the absorption value of the film at 600 nm; d is the thickness of the film, cm.

The solubility of the films was measured using the gravimetric method. Film of $3\text{ cm} \times 3\text{ cm}$ was immersed in distilled water at $25\text{ }^{\circ}\text{C}$ for 24 h. After that, surface moisture was removed, the film was dried in an oven at $105 \pm 2\text{ }^{\circ}\text{C}$, and the film was equilibrated at room temperature for 12 h in a dryer. The mass of the film before and after drying was weighed. The experiment was repeated three times. The solubility of the film was calculated using Equation (2):

$$\text{Solubility (\%)} = \frac{m_1 - m_2}{m_1} \times 100\% \quad (2)$$

where m_1 is the starting mass of the sample (g) and m_2 is the final mass of the sample (g).

Mechanical Properties

The tensile strength and elongation at break of the films were determined according to the method of GB/T1040.3–2006 using the CMT4204 Universal Tension Machine (Mester Co., Ltd., Shenzhen, China). The maximum length and maximum tensile strength of the film at break were determined in the original scale for 100 mm at 50 mm/min stretching speed. Each treatment was to be repeated five times. Tensile strength and elongation at break are expressed in Equations (3) and (4), respectively.

$$TS = \frac{F_{max}}{bd} \times 100\% \quad (3)$$

where TS is tensile strength, MPa; F_{max} is the maximum tensile force, N; b is the sample width, cm; and d is the sample thickness, cm.

$$EB = \frac{L - L_0}{L_0} \times 100\% \quad (4)$$

where EB is the breakdown rate, %; L is the maximum length of the film break, cm; L_0 is the initial length of the film, cm.

Structural Characterization

The microstructural morphology of the films was observed using a Zeiss MERLIN Compact scanning electron microscope (Carl Zeiss Co., Ltd., Oberkochen, Germany). A $5\text{ mm} \times 5\text{ mm}$ sample of the film was taken and fixed on a copper stage after vacuum gold spraying. The surface and cross-section of the sample were scanned using an electron beam with an accelerating voltage of 5 kV for electron microscopic observation.

Infrared spectra of the films were recorded using a Nicolet 670 Fourier Transform Infrared spectrometer (Nicolet Co., Ltd., WI, USA). Each sample was scanned 64 times at a resolution of 4 cm^{-1} in a wavenumber range of 4000 to 650 cm^{-1} .

X-ray diffraction spectra of the films were scanned with a D/max 2550 X-ray powder diffractometer (Rigaku Co., Ltd., Tokyo, Japan). The sample diffractograms were observed at a voltage of 40 kV, a current of 100 mA, a scan range of 10° to 80° , and a scan rate of $5^\circ/\text{min}$.

2.2.5. Kinetic Analysis of Polyphenol Release from ZMP/C-Film

The release of polyphenols from ZMP/C-film (1 g/dL) was investigated in three food simulants: water, 3% (v/v) acetic acid, and 10 % (v/v) ethanol. The film samples were cut into $1\text{ cm} \times 1\text{ cm}$ pieces. After weighing and placing in separate volumetric flasks containing 20 mL of the simulated solution, polyphenols of ZMP/C-film were released under dark conditions at 25°C for 48 h. At different times, 0.3 mL of the above release solution (replenished promptly with an equal amount of mock solution to the volumetric flask) was taken and mixed with 0.3 mL of FCR. After standing for 5 min, 0.6 mL of Na_2CO_3 (10%, v/v) was transferred and fixed to 3 mL to display color for 2 h. Absorbance was measured at 760 nm. The content of total polyphenol was calculated by the 1.3.1 gallic acid standard curve, expressed as milligram equivalents of gallic acid per gram of sample (mg GAE/g film). The experiment was repeated three times and the cumulative release rate of polyphenols was calculated according to Equation (5).

$$\text{The cumulative release rate (\%)} = \frac{M_t}{M_0} \times 100 \quad (5)$$

where M_t is the cumulative release of polyphenols at time t , μg ; and M_0 is the total polyphenol load, μg .

2.2.6. Mathematical Modeling of Polyphenol Release

To further understand the kinetic mechanism of polyphenol release from ZMP/C-film, the total phenol release data were fitted to a kinetic model with reference to previous studies [15]. The best fit of the release profile to different kinetic models was used to determine the polyphenol release mechanism:

The zero-order kinetic model is applicable to swelling-controlled ingredient release systems, where the release process is controlled by the relaxation of the polymer chains. The rate of release of the component is constant and independent of concentration, as shown in Equation (6):

$$\frac{M_t}{M_\infty} = k_0 t \quad (6)$$

The first-order kinetic model, where the rate of release of a component depends on its concentration, is given in Equation (7):

$$\ln\left(1 - \frac{M_t}{M_\infty}\right) = -k_1 t \quad (7)$$

The Higuchi model with component release controlled by Fickian diffusion is shown in Equation (8):

$$\frac{M_t}{M_\infty} = k_h t^{\frac{1}{2}} \quad (8)$$

The Korsmeyer–Peppas model, where component release is controlled by diffusion in concert with polymer degradation dissolution and relaxation behavior, is shown in Equation (9):

$$\frac{M_t}{M_\infty} = k t^n \quad (9)$$

where M_t is the cumulative release of polyphenols at time t , mg; M_∞ is the total amount of polyphenols in the composite film, mg; M_t/M_∞ is the cumulative percentage release of polyphenols at time t , %; n is the release index; k_0 , k_1 , k_h , and k are rate constants.

2.2.7. ZMP/C-Film Antioxidant Capacity

Antioxidant properties were evaluated with 1,1-Diphenyl-2-picrylhydrazyl radicals (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). All samples were assayed 6 times in parallel. An amount of 40 μL of the 1 g/dL ZMP/C-film sample solution with different release times (0 h, 4 h, 8 h, 12 h, 16 h, 20 h, 24 h) from Section 2.2.5 was mixed with 100 μL of MeOH and 60 μL of 0.15 mmol/L 1,1-Diphenyl-2-picrylhydrazyl radical (DPPH). The mixture was reacted in a dark room at 30 °C for 30 min. The absorbance at 517 nm was measured by a UV—Vis spectrophotometer (Shenzhen Hongyong Precision Instrument Co., Ltd., Shenzhen, China). The antioxidant capacity was calculated according to the following formula:

$$\text{Free radical scavenging rate}_{\text{DPPH}} (\%) = \frac{A_0 - A_t}{A_0} \times 100 \quad (10)$$

where A_0 is the absorbance at 517 nm of a mixture of 60 μL of DPPH solution and 100 μL of MeOH; A_t is the absorbance at 517 nm of a mixture of 40 μL of sample solution, 60 μL of DPPH solution, and 100 μL of MeOH.

An amount of 30 μL of the sample solution was mixed with 300 μL of ABTS⁺ assay solution (composed of 7 mmol/L 2,2-linked nitrogen-two (3-ethyl-benzene-6-sulfate) dual ammonium salt and 140 mmol/L overcurrent potassium liquid, with a volume ratio of 60:1). The absorbance was measured at 734 nm after 30 s of vortex shaking. The scavenging rate of ABTS⁺ was calculated according to

$$\text{Free radical scavenging rate}_{\text{ABTS}^+} (\%) = \frac{A_0 - A_t}{A_0} \times 100 \quad (11)$$

where A_0 is the absorbance at 743 nm of a 300 μL ABTS⁺ assay solution; A_t is the absorbance at 743 nm of a mixture of 30 μL sample solution and 300 μL ABTS⁺ assay solution.

2.2.8. Practical Application of Film on Fresh Strawberry Fruit

Fresh strawberries were collected from the Shanxi Yuci Farm. The fruits with consistent color, size, weight, and no apparent damage were selected. The samples were divided into three groups, namely a blank control group (CK), C-film group, and ZMP/C-film group. The samples were weighed at 25 °C. Except for the CK, each treatment group consisted of eight strawberries placed in polystyrene containers. A 20 cm $\times$ 20 cm ZMP/C-film or C-film was used to cover the container mouth for strawberry preservation. Unpackaged and packaged samples were analyzed at room temperature for weight loss rate, vitamin C content (Vc), and appearance at 0, 1, 2, 3, 4, 5, 6, and 7 d during storage. Each group of fresh-keeping tests were repeated three times.

After every 24 h, the strawberries were used to measure the weight loss rate and photographed. The weight loss rate was determined by the weighing method and calculated based on Equation (12):

$$\text{The weight loss rate} (\%) = \frac{m_0 - m_t}{m_0} \times 100 \quad (12)$$

where m_0 is the weight at 0 days when the strawberry is stored, g; m_t is the weight at different time periods for strawberries, g.

The contents of Vc were measured with a titrimetric method, based on the reduction of 2,6-dichlorophenolindophenol. Briefly, tissue (10 g) was dissolved in 10 mL of 0.02 g/mL oxalic acid solution and then centrifuged at 10,000 $\times$ g and 4 °C for 10 min. A total of 5 mL of supernatant was titrated to a permanent pink color by 0.1% 2,6-dichlorophenolindophenol titration. Vc concentration was calculated according to the titration volume of 2,6-dichlorophenolindophenol and expressed as milligram per kg of fresh weight.

2.3. Data Analysis

Analysis of variance (ANOVA) was performed using SPSS 25.0 software with $p < 0.05$ as the significance level and plotted using Origin 9.1 software.

3. Results and Analysis

3.1. ZMP Composition Analysis

The purified pepper leaf polyphenols (ZMPs) were extracted and analyzed by HPLC. The results of the chromatographic curves of the purified ZMP and 13 standards are shown in Figure 2. The chromatogram showed good separation of the components. Seven components were detected in ZMP: epicatechin, chlorogenic acid, p-coumaric acid, ferulic acid, quercetin, quercetin, and kaempferol, in the order of 3.24 mg/g, 3.59 mg/g, 1.40 mg/g, 4.36 mg/g, 7.61 mg/g, 4.52 mg/g, and 2.51 mg/g, respectively. Guo et al. used HPLC to analyze 10 polyphenolic compounds in *Zanthoxylum schinifolium* Sieb. The results showed that the contents of epicatechin, p-coumaric acid, ferulic acid, quercetin, and kaempferol in the polyphenolic extract of *Zanthoxylum schinifolium* Sieb. were 3.03 µg/mg, 0.98 µg/mg, 0.89 µg/mg, 5.43 µg/mg, and 1.48 µg/mg, respectively [16]. In addition, it can also be seen from Figure 2B that there were some other components in ZMP with higher contents, which are to be further identified.

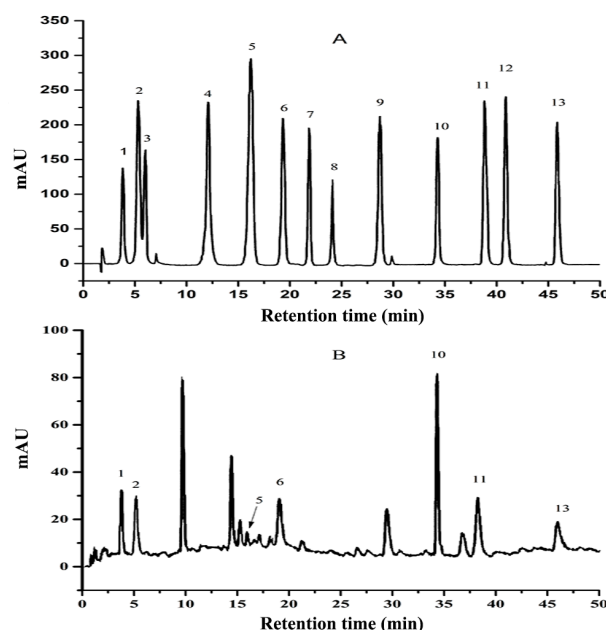


Figure 2. Thirteen polyphenol standards and ZMP compound chromatograms ((A): chromatogram of polyphenol standards; (B): chromatogram of ZMP samples). 1. epicatechin; 2. chlorogenic acid; 3. catechin; 4. vanillin; 5. p-coumaric acid; 6. ferulic acid; 7. m-coumaric acid; 8. rutin; 9. o-coumaric acid; 10. quercetin; 11. quercetin; 12. naringenin; 13. kaempferol.

3.2. Physical Properties of ZMP/C-Film

3.2.1. Thickness, Opacity, Solubility, Tensile Strength, and Elongation at Break of the Film

The thicknesses of the C-film, TA/C-film, and ZMP/C-films were compared. As shown in Table 1, TA/C-film and ZMP/C-film showed smaller thicknesses compared to C-film. The addition of TA or ZMP resulted in a decrease in C-film thickness. This is consistent with reports of starch-chitosan-based films containing thyme extract [17] and grape seed polyphenols/chitosan films [18]. Generally, polyphenols and chitosan have opposite charges in acidic media. The molecular chain of ZMP/C-film becomes tighter due to mixing, resulting in a decrease in film thickness [18].

Table 1. Thickness, light transmittance, solubility, tensile strength, and elongation at break of *Zanthoxylum bungeanum* leaf polyphenol/chitosan composite films.

Sample	Thickness (μm)	Opacity (mm ^{−1})	Solubility (%)	Tensile Strength (MPa)	Elongation at Break (%)
C-film	43.95 ± 0.11 ^a	3.81 ± 0.07 ^c	17.62 ± 0.21 ^b	47.66 ± 5.05 ^a	21.11 ± 2.59 ^b
TA/C-film	41.47 ± 0.10 ^b	11.92 ± 0.04 ^b	22.65 ± 0.39 ^a	43.14 ± 3.21 ^a	30.15 ± 1.28 ^c
0.5%ZMP/C-film	41.19 ± 0.18 ^b	12.07 ± 0.11 ^{ab}	21.98 ± 0.27 ^a	40.30 ± 2.19 ^b	32.64 ± 1.90 ^a
1.0%ZMP/C-film	39.07 ± 0.23 ^c	12.53 ± 0.06 ^a	22.44 ± 0.13 ^a	32.15 ± 1.30 ^c	36.37 ± 2.35 ^a

Note: C-film—comparison of chitosan films; TA/C-film—tannic acid/chitosan films; 0.5% ZMP/C-film—composite film containing 0.5 g/dL *Zanthoxylum bungeanum* leaf polyphenol; 1.0% ZMP/C-film—composite film containing 0.5 g/dL *Zanthoxylum bungeanum* leaf polyphenol. Different lowercase letters (a–c) in superscript after the data in the table indicate significant differences ($p < 0.05$) for data in the same column.

Light blocking is also an important characteristic for packaging film [19]. As shown in Table 1, the opacity of chitosan films containing TA and ZMP was 2.28 times that of the C-film. This indicated that the ZMP composite film has a lower light transmission and can effectively block visible light. High-blocking light helps ZMP/C-film to protect the packaged food from light-induced oxidative deterioration.

The films were immersed in distilled water for 24 h. The appearance of the C-film was maintained with integrity, while the shape of the film with the addition of TA and ZMP changed the degree of dissolution somewhat from 17.62% to 22.65% (Table 1). The water resistance of C-film may be related to the molecular weight of CS and the degree of colloidal acetylation. CS has lower solubility when the molecular weight is greater than 72.12 kDa (Chang et al., 2015). CS is essentially insoluble in water (pH = 7) when the degree of deacetylation is >85% [20]. The addition of TA and ZMP may affect the hydrophilicity of phenolic hydroxyl groups in the structure, thereby increasing the solubility of the composite film. This will limit the use of film in food packaging.

The mechanical properties of the film are an important parameter as a packaging material. The effects of the tensile strength (TS) and the elongation at break (EB) of the films were compared. As shown in Table 1, flexibility (elongation at break) increased by 72%, while the ZMP-containing films had lower tensile strength (32.15–40.3 MPa). Gao et al. also observed that the addition of tea polyphenols to CS resulted in a decrease in tensile strength and an increase in elongation at break of the film [21]. However, Xue et al. found that chitosan films loaded with propolis flavonoids not only had higher tensile strength, but also had some flexibility [22]. The mechanical behavior of composite films is related to the type of polymer matrix, the content of polyphenolic compounds, and their interactions [17]. The molecular interactions between polyphenols and CS are mainly through electrostatic interactions, and hydrogen and ester bonds, which in turn affect the interactions of the polymer chains, forming more flexible structural domains between the chains [23]. Therefore, the tensile strength of the film with TA or ZMP is reduced, while the flexibility is increased.

3.2.2. Scanning Electron Microscopy (SEM)

To further investigate the microstructure of the composite film, SEM was used to scan the surface and cross-sectional microscopic morphology of the composite film (Figure 3). The surface scan showed that the C-film surface microstructure was continuous and homogeneous without any pores and cracks (Figure 3a). TA/C-film and ZMP/C-film were smoother and more regular than the surface micro-structures of C-film. This indicated that the matrix of TA or ZMP and the CS matrix have good compatibility. But, there are some evenly scattered white dots and raised small blocks (Figure 3c,e,g), and similar results were observed by Gao et al. in electron micrographs of the functional film of tea polyphenol chitosan [21].

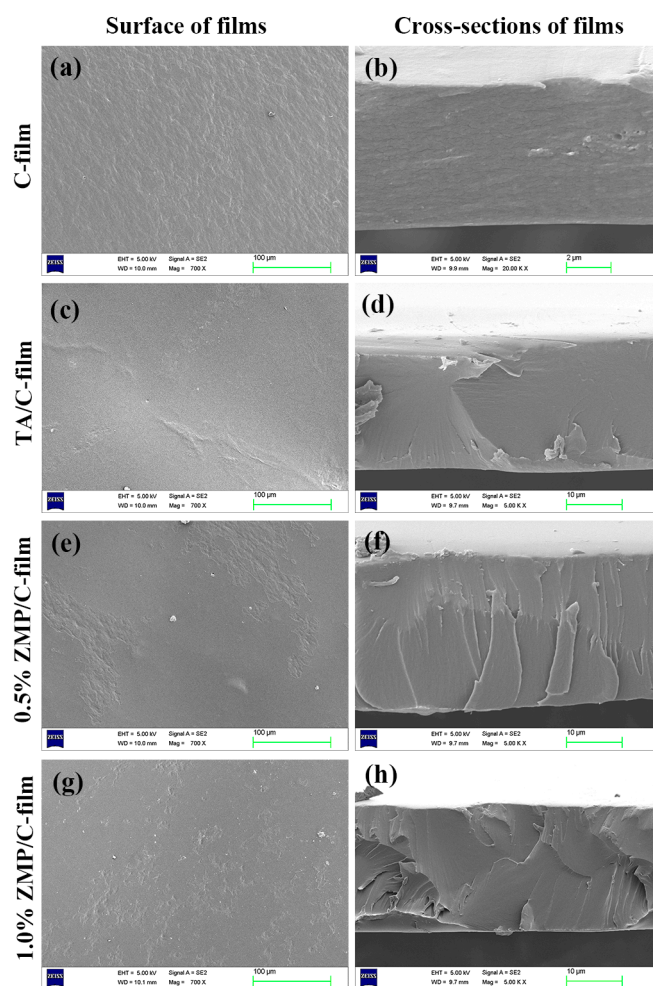


Figure 3. Microscopic images of the surface and cross-section of the C-film, TA/C-film, and ZMP/C-film: surface morphology of C-film (a), cross-section morphology of C-film (b), surface morphology of TA/C-film (c), cross-section morphology of TA/C-film (d), surface morphology of 0.5% ZMP/C-film (e), cross-section morphology of 0.5% ZMP/C-film (f), surface morphology of 1.0% ZMP/C-film (g), cross-section morphology of 1.0% ZMP/C-film (h).

Compared to the loose network structure of C-film, the cross-sectional structure of TA/C-film and ZMP/C-film had a denser network structure (Figure 3b,d,f,h). Xue et al. found that the incorporation of phenolic compounds into chitosan film can form a more rigid structure by increasing the interaction and compatibility level of the components, making the film structure more compact [22]. This further indicates that phenols can form a denser and more compact structure with CS polymers, but the enhancement of the rigid structure can also reduce the TS of the film. This is basically consistent with the research results of the mechanical properties of the membrane.

3.2.3. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectra of the C-film, TA/C-film, and ZMP/C-film samples are shown in Figure 4. FTIR spectra of C-film exhibited typical characteristic bands of amino polysaccharides, with a broad band in the characteristic region of $3262\text{--}3383\text{ cm}^{-1}$ due to —OH stretching vibrations with —NH symmetric stretching vibrations. The peak near $2926\text{--}2878\text{ cm}^{-1}$ is the vibrational absorption of CH. The peaks for the amide I band ($1636\text{--}1771\text{ cm}^{-1}$), amide II band ($1548\text{--}1600\text{ cm}^{-1}$), and amide III band ($1366\text{--}1404\text{ cm}^{-1}$) can be assigned to C=O stretching, N-H bending, and C-N bending, respectively [17]. In the fingerprint region, the peaks at 1249 cm^{-1} and 1152 cm^{-1} are associated with the symmetric and asymmetric stretching of C-O-C, respectively. The bands at $1024\text{--}1074\text{ cm}^{-1}$ are caused

by stretching of the C-O skeleton. When polyphenols (ZMP, TA) were added, the spectra of the composite films were like those of chitosan and no new characteristic absorption bands were found. However, Talón et al. previously reported that the intensity of the spectral absorption bands changed in composite films prepared from thyme polyphenols and chitosan [17]. Zhu et al. reported that the drift and broadening of the absorption peaks of the tannin/chitosan composite film spectra were due to the altered chemical environment by the formation of hydrogen bonds between tannin-chitosan, chitosan molecules, and chitosan amino groups [24]. In this study, the intensity of the absorption peaks at 1548 cm^{-1} , 1404 cm^{-1} , and 1024 cm^{-1} were enhanced in the composite film. The absorption peaks at 3262 cm^{-1} and 1548 cm^{-1} were shifted toward the high wave direction. These results indicated that the addition of TA or ZMP was not enough to cause significant changes in the structural matrix of CS, but the FTIR absorption peak intensity changes and the mechanical properties data of the composite film reveal the cross-linking effect between CS and TA or ZMP molecules. The results confirmed that ZMP was successfully encapsulated by CS to form a dense structure.

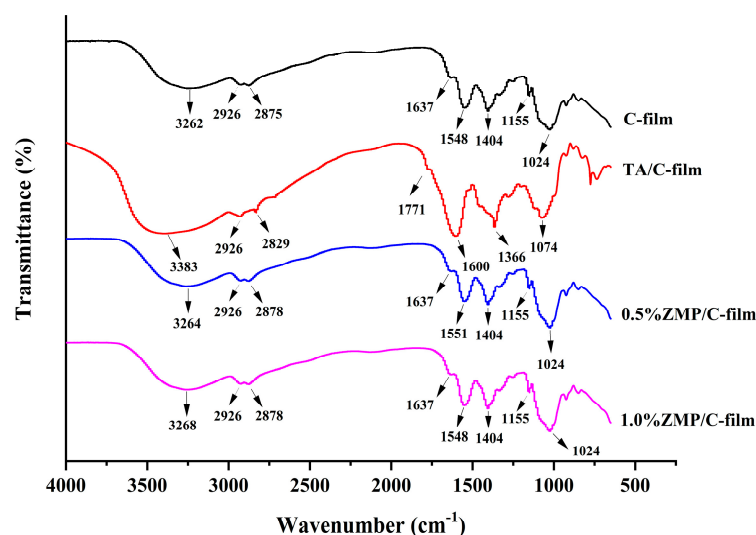


Figure 4. FTIR spectra of C-film, TA/C-film, and ZMP/C-film. Note: C-film—comparison of chitosan films; TA/C-film—tannic acid/chitosan films; 0.5% ZMP/C-film—composite film containing 0.5 g/dL *Zanthoxylum bungeanum* leaf polyphenol; 1.0% ZMP/C-film—composite film containing 0.5 g/dL *Zanthoxylum bungeanum* leaf polyphenol.

3.2.4. X-ray Diffraction Spectroscopy

The results of the X-ray diffraction spectrum are illustrated in Figure 5. The diffraction peak of C-film at the Prague angle (2θ) of 11.6° reflected the hydrophobic crystal structure of the chitosan film [25]. The characteristic diffraction peak of C-film was found at $2\theta = 22.1^\circ$, which reflected the amorphous structure of C-film [26]. The addition of ZMP or TA to CS resulted in a gradual flattening of the two diffraction peaks and a decrease in diffraction intensity, especially the characteristic peak at 22.1° . ZMP or TA induced the transformation of the C-film into an amorphous structure, resulting in a reduction in the crystallinity of the composite film. Due to the competitive effect of hydrogen bonds between chitosan and polyphenols, as well as the interaction between polyphenols and CS, the formation of intramolecular or intermolecular hydrogen bonds in CS may be hindered, leading to the further formation of amorphous complexes in the structure and a decrease in crystallinity. Similar results were observed in polyphenolic composite films of CS with black seeds [22] and apples [25]. The results of this change in the crystalline structure of the composite film at the microscopic level were consistent with the results of the change in its mechanical properties at the macroscopic level.

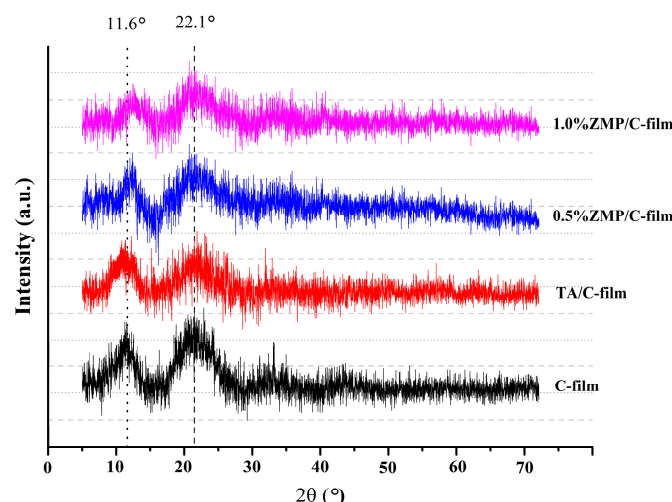


Figure 5. XRD patterns of C-film, TA/C-film, and ZMP/C-film. Note: C-film—comparison of chitosan films; TA/C-film—tannic acid/chitosan films; 0.5% ZMP/C-film—composite film containing 0.5 g/dL *Zanthoxylum bungeanum* leaf polyphenol; 1.0% ZMP/C-film—composite film containing 0.5 g/dL *Zanthoxylum bungeanum* leaf polyphenol.

3.3. Kinetics of Polyphenol Release

The release of polyphenols from ZMP/C-film was assessed in three different food simulation systems in water, 3.0% acetic acid (*v/v*), and 10% ethanol (*v/v*). As shown in Figure 6, the time for polyphenol release to reach equilibrium in water, 3.0% acetic acid (*v/v*), and 10% ethanol systems was 720 min, 540 min, and 1440 min, respectively. The release of ZMP in 3% acetic acid was higher than in water and 10% ethanol. A similar release pattern was found by Gomaa et al. examining polyphenol release from alginate-chitosan film [13]. The release of polyphenols from the polymer matrix was affected by the nature of the polymer (swelling, molecular weight distribution, density, size, etc.) and the polyphenols (molecular size, shape, density, polarity, solubility, etc.), and their interaction [13,17]. In this study, the solubility of CS in acidic media led to the dissociation of hydrogen bonds between amino and phenolic groups, promoting the entry of acetic acid solvent into the membrane matrix, causing the swelling of the polymer skeleton, an increase in free volume, and a widening of the cross-linked network structure of the polymer. This facilitated the diffusion of indefinite polyphenols in the CS polymer matrix into the entire polymer network space, further releasing into the external solution system, thereby increasing the release rate of ZMP. In addition, the polyphenol release rate depends on the ZMP distribution coefficient between the organic and aqueous phases. ZMP is slightly soluble in water, and it has a larger distribution coefficient in the 10% acetic acid volume compared to aqueous simulants. The release of polyphenols from ZMP/C-film is influenced by the polarity and pH of the environmental system.

To better explain the release mechanism of ZMP in ZMP/C-film, release data have been fitted to different kinetic equations (zero-order kinetic model, first-order kinetic model, Higuchi model, Korsmeyer–Peppas model) to study the mechanism (Figure 7). The Korsmeyer–Peppas model was a better fit ($R^2 > 0.93$). The Korsmeyer–Peppas model can determine the release mechanism based on the exponent n . When $n < 0.45$, it is mainly a Ficks diffusion mechanism. When $0.45 < n < 0.89$, it is mainly a synergistic effect of diffusion and dissolved polymer relaxation. When $n > 0.89$, it is mainly a skeletal dissolution mechanism [27]. Based on the linear fit data in Figure 7d and Equation (9), it was calculated that the n -values were all greater than 0.89. It could be inferred that the main mechanism of ZMP in ZMP/C-film was the dissolution of the CS-based polymer framework.

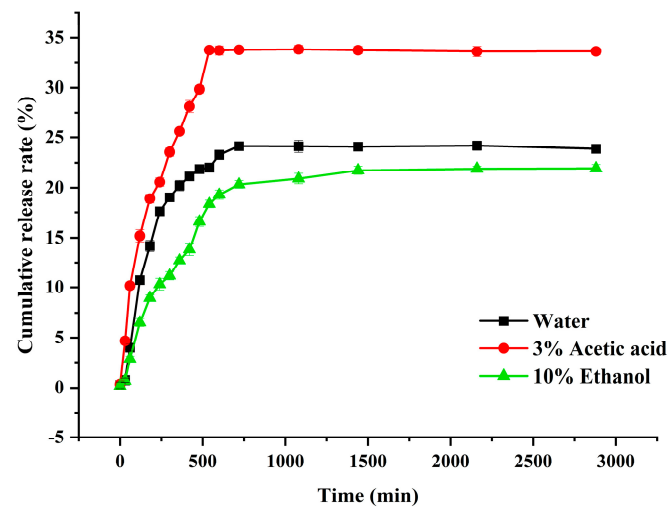


Figure 6. Release profiles of ZMP in three different food simulation systems.

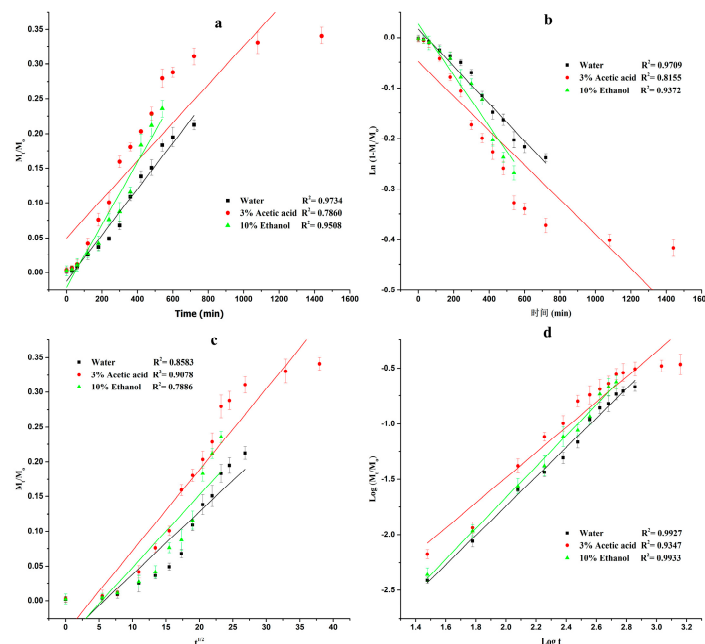


Figure 7. ZMP release data fitted to various kinetic models. Note: (a) zero-order kinetic model fit; (b) first-order kinetic model fit; (c) Higuchi model fit; (d) Korsmeyer–Peppas model fit.

3.4. Anti-Oxidant Capacity

The antioxidant capacity of the ZMP/C-film release solution was determined by DPPH and ABTS⁺ radical scavenging rates. As shown in Figure 8, the antioxidant activity of ZMP/C-film was positively correlated with the release time, and the scavenging effect of the ZMP/C-film was significantly different ($p < 0.05$) for three food simulation systems. ZMP/C-film showed the strongest antioxidant capacity in 3% acetic acid system. The scavenging rates of DPPH and ABTS⁺ radicals were 73.25% and 81.06%, respectively, which were 0.83 and 0.82 times higher than that of 1 mg/mL Vc under the same conditions. The rate of 10% ethanol solution was lowest. This supports the research results on the release rate of ZMP. Kadam et al. also found that the free radical scavenging activity of *Nigella sativa* seedcake polyphenol-chitosan films in an aqueous environment was higher than that of alcoholic food simulants [28]. The antioxidant effect of ZMP/C-film varied with different factors (e.g., polarity, pH). In practical applications, the release of ZMP can be effectively controlled by these factors, thereby achieving the desired antioxidant effect.

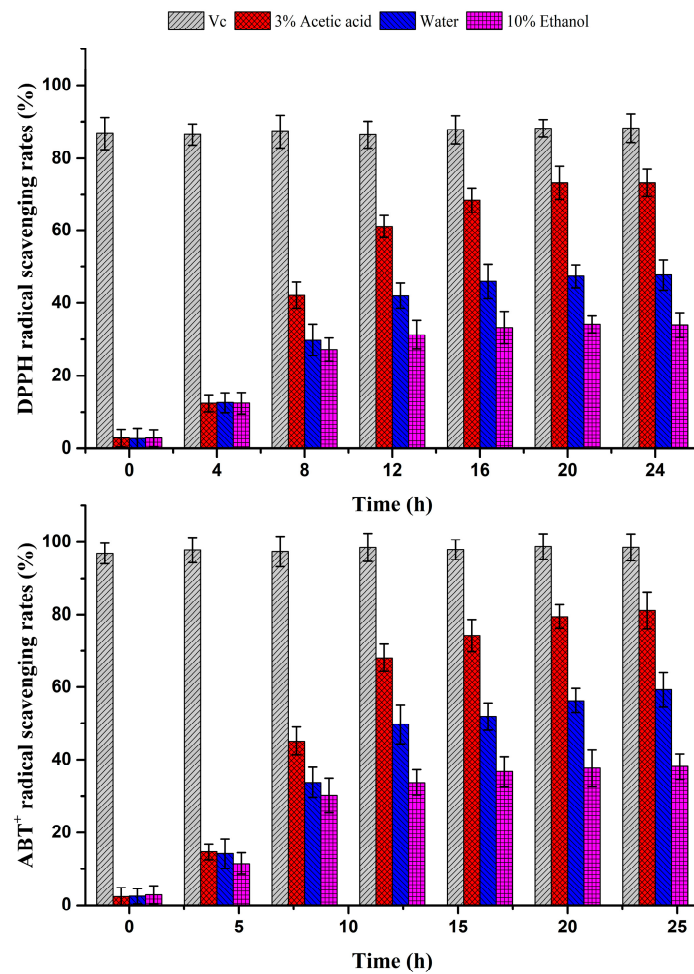


Figure 8. Release profile of antioxidant activity of 1.0% ZMP/C-film (m/v, g/mL) in different food simulation systems. Note: 1.0% ZMP/C-film—composite film containing 0.5 g/dL *Zanthoxylum bungeanum* leaf polyphenol.

3.5. Strawberry Preservation

The appearances of different packages of strawberries stored at 25 °C are shown in Figure 9A. The appearances of fruits in all treatment groups remained essentially unchanged with 0–3 days of storage. After storage for 4 days, the strawberries of CK appeared to have dark patches, visible lesions, and deterioration. Nonetheless, most of the strawberries covered in ZMP/C-film kept their fresh appearance and showed no signs of degeneration.

The weights and Vc contents of the treatment groups were significantly different in storage time ($p < 0.05$). After storage for 7 days, the weight loss rate of strawberries covered with ZMP/C-film (4.85%) was significantly lower than 15.47% in the CK and 6.33% in the C-film (Figure 9B). The results showed that adding ZMP to C-film prolonged the storage time of strawberries and inhibited water loss. This may be related to not only the dense structural properties of ZMP/C-film (see the SEM result), but also to the antioxidant and release properties of ZMP.

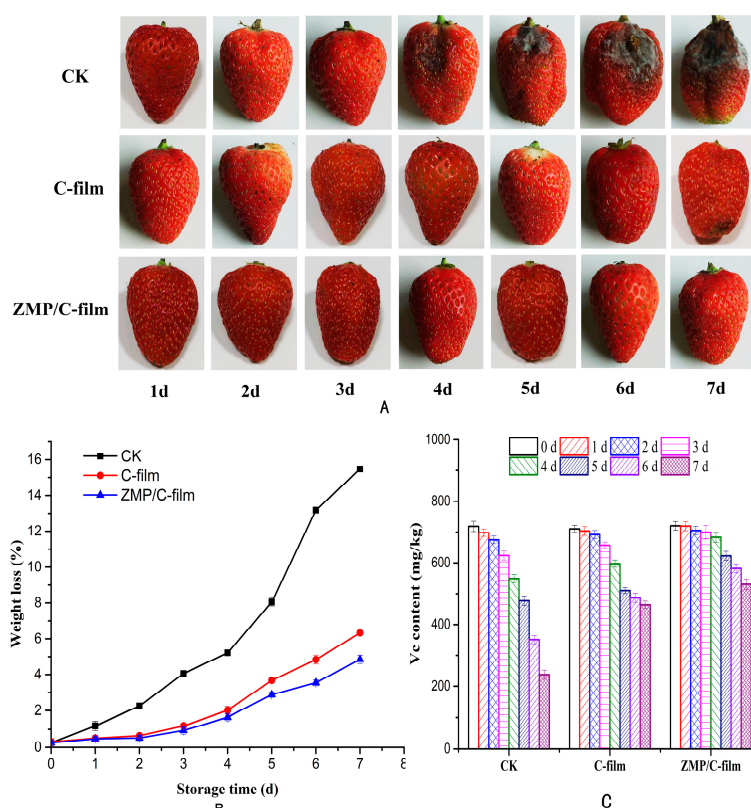


Figure 9. Effect of blank control group (CK), C-film, and ZMP/C-film treatment groups on strawberry appearance (A), moisture (B), and Vc (C) during storage at 25 °C and 50% relative humidity. Note: CK—blank control group of unsigned film; C-film—use of chitosan film to keeping-strawberry-fresh treatment group; ZMP/C-film—use of *Zanthoxylum bungeanum* leaf/chitosan films to keeping-strawberry-fresh treatment group.

The Vc content is one of the important indicators for judging the freshness of fruits and vegetables. As shown in Figure 9C, the Vc content of uncoated strawberries significantly declined along with the storage period. However, the rate of decrease in Vc content was much slower in strawberries covered with ZMP/C-film compared with other treated groups. The initial Vc content of strawberry fruit was 712.77 mg/kg. After 7 days of storage at 25 °C, the Vc contents of strawberry fruits covered with the films, C-film and ZMP/C-film, were maintained at the levels of 464.22 mg/kg and 530.84 mg/kg, respectively, whereas that of the control sample was 237.44 mg/kg. Although Vc contents of the C-film-treated group could be inhibited to a certain extent during the storage period, the use of ZMP/C-film was much more effective in reducing the Vc loss and prolonging the storage life of strawberry fruit. The weight loss rate and Vc content of strawberries covered with ZMP/C-film decreased by 23.4% and increased by 14.2% compared to C-film, respectively. ZMP/C-film prolonged the shelf life of strawberries by more than 4 days at 25 °C.

4. Conclusions

In our present study, a novel *Zanthoxylum bungeanum* leaf polyphenols/chitosan film (ZMP/C-film) with higher barrier and physicochemical properties was successfully prepared and applied to the preservation of strawberries during storage at 25 °C. Characterization results showed a strong interaction of ZMP and the chitosan substrate. The results in terms of the light transmittance and elongation at break showed that the films had excellent properties that can meet the needs and demands of the fruit preservation market. Additionally, when the ZMP content increased, the solubility of composite film increased while the tensile strength decreased. In addition, the investigation of the ZMP release properties in three food simulation systems of water, 3.0% acetic acid, and 10% ethanol,

as well as the antioxidant results for DPPH and ABTS⁺ free radicals, also showed good results. ZMP added to CS improved strawberry preservation performance, significantly reducing the loss rate of weight and Vc, and prolonging the shelf life by 4 days. These results demonstrated that the ZMP/C-film had good fruit fresh keeping and preservation effects and a great potential for the field of food packaging.

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Conflicts of Interest: We declare that we do not have any commercial or associated interests that represent conflicts of interest in connection with this work.

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