Physical and Antioxidant Properties of Edible Chitosan Ascorbate Films

Wenqiang Tan,† Fang Dong,† Jingjing Zhang,‡ Xiang Zhao,§ Qing Li,† and Zhanyong Guo*†‡§

†Key Laboratory of Coastal Biology and Bioresource Utilization, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai, Shandong 264003, People’s Republic of China
‡University of Chinese Academy of Sciences, Beijing 100049, People’s Republic of China
§College of Chemistry and Chemical Engineering, Yantai University, Yantai, Shandong 264005, People’s Republic of China

ABSTRACT: Chitosan ascorbates with different substitution degrees were synthesized on the basis of salification of chitosan and ascorbic acid at various molar ratios in water and were successfully used to prepare antioxidative films by casting for the first time. Fourier transform infrared and 1H nuclear magnetic resonance spectra recorded the structural characteristics of all chitosan ascorbates; meanwhile, physicochemical property and antioxidant activity of the produced chitosan ascorbate films were characterized, with chitosan acetate film serving as the control, and these properties were also measured for comparison. The results revealed that salification of chitosan with ascorbic acid not only improved the total color difference, chroma, opacity, capacity for blocking ultraviolet–visible light, and water solubility of chitosan-based films but also decreased water content, swelling degree, and water vapor permeability compared to chitosan acetate film. Also, as was expected, the antioxidant activity assays showed that incorporation of ascorbate into the chitosan matrix effectively enhanced the scavenging activity against the DPPH radical and reducing power. Cs2Vc8 and Cs2Vc6 especially exhibited the strongest scavenging capacities against the DPPH radical (EC50 < 0.025 mg/mL). These findings offered a suggestion that the prepared chitosan ascorbate films can be applied as novel green oxidation-resistant materials in the food packaging industry.

KEYWORDS: chitosan-based biodegradable film, ascorbic acid, physical property, antioxidant activity

1. INTRODUCTION
As a result of poor biodegradation and chemical residues in the food products, petroleum-based packaging has been creating serious environmental consequences around the world.1,2 An ever growing concern has shifted to the development in the field of biodegradable and edible films using natural polymeric ingredients for eco-friendly food packaging, for instance, chitosan, sodium alginate, cellulose, and gelatin.3,4 Among them, chitosan is a linear aminopolysaccharide, obtained from chitin by the complete or partial deacetylation under alkaline conditions.5,6 Chitosan has been considered as a promising applicable biomaterial in the area of biomedical engineering, food packaging, cosmetics, and agriculture,7,8 in view of its good biodegradability, biocompatibility, nontoxicity, and film-forming properties.

Chitosan-based bioplastics or biodegradable films have great potential as effective food packaging materials with satisfactory mechanical property and gas permeselectivity.9,10 However, real applications of chitosan-based films are considerably limited by the high water vapor permeability and the poor free radical scavenging activity.9,11 To improve the antioxidant property of native chitosan films, many natural active compounds, such as essential oils,12,13 polyphenols,14,15 and polyphenols,12,16,17 have been incorporated by physical blend into the chitosan matrix to fabricate composite films. Pastor et al. developed chitosan-based polymeric composite films containing resveratrol, and the antioxidant activity of composite films was enhanced with the rising amounts of resveratrol in the composite film.18 Talon et al. used natural polyphenols extracted from thyme to develop the antioxidant polymeric film based on chitosan for food preservation.19 In fact, the remarkable antioxidant potential of chitosan-based films encapsulating natural active antioxidant agents could be achieved via the release of these oxidation-resistant compounds. In other words, once the effective antioxidant ingredients are completely released, the antioxidant properties of polymeric composite films will be inevitably lost.20

The natural antioxidants could be chemically grafted into chitosan backbones for the purposes of the development of new approaches to exhibit effectively long-term antioxidant property.21 However, the use of organic catalysts and organic solvents and relatively complicated post-processing operations are required in most grafting techniques, which could be likely to bring potential cytotoxicity and push up the additional costs.22 Fortunately, chitosan ammonium salts can be synthesized automatically based on the electrostatic interaction of chitosan and organic acids in water without other organic reagents or multi-step purification procedures.22,23 However, two crucial prerequisites for the successful preparation of chitosan ammonium salts are the good water solubility and the acidity (pKa < 6.0) of active organic acids.

Ascorbic acid, a vital natural water-soluble vitamin, is an essential dietary factor.24 As one of the most effective potent antioxidants and biological reductants, ascorbic acid has been extensively applied in food, beverages, pharmaceutical,

Received: August 24, 2018
Revised: February 12, 2019
Accepted: February 15, 2019
Published: February 15, 2019

DOI: 10.1021/acs.jafc.8b04567
J. Agric. Food Chem. 2019, 67, 2530−2539

© 2019 American Chemical Society
cosmetic, and animal feed fields. Because humans are unable to synthesize ascorbic acid endogenously, it can only be ingested from external sources, such as citrus fruits and vegetables. However, it is impossible to retain long-term stability and very easy to become oxidized when exposed to the external circumstances for a long time. Strategies to increase the stability of ascorbic acid go through the incorporation with chitosan. Meanwhile, ascorbic acid is also regarded as a potentially suitable acid to directly dissolve chitosan in water and simultaneously form a water-soluble chitosan ascorbate. Elbarbary et al. described that chitosan ascorbate in the range of nanoparticles had stronger antioxidant property than ascorbic acid and, meanwhile, significantly decreased the concentration of malondialdehyde formed during the storage of meat. However, the research about the physical properties of chitosan ascorbate films and their scavenging activities against active free radicals should be explored for further applications in the food packaging field.

In this paper, the purpose is to prepare chitosan ascorbate films based on the salification of chitosan and ascorbic acid with different molar ratios and analyze the effect of salification of chitosan with ascorbic acid on the physical property and antioxidant activity of produced chitosan-based films. The casting technique and oven drying method were employed to obtain chitosan-based films. Then, the physical properties (mechanical behavior, optical performance, thermal property, and water vapor permeability) and antioxidant activities (DPPH radical scavenging activity and reducing power) of chitosan ascorbate films were investigated. The effect of substitution degrees of ascorbate in chitosan ascorbate films on these properties was also evaluated. Meanwhile, as the most common solvent in the dissolution of chitosan, acetic acid was also used to prepare the chitosan acetate film, serving as the control group in physicochemical experiments and antioxidant tests.

2. EXPERIMENTAL SECTION

2.1. Material. Commercial-grade chitosan was purchased from Golden-Shell Pharmaceutical Co., Ltd. (Zhejiang, People’s Republic of China) with a deacetylation degree of 95% and an average molecular weight of 700 kDa. Glacial acetic acid, ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), glycerol, absolute ethanol, potassium ferricyanide, ferric chloride, and trichloroacetic acid were obtained from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, People’s Republic of China).

2.2. Synthesis of Chitosan Acetate and Chitosan Ascorbates. To the solution of acetic acid (1.15 mL, 20 × 10⁻³ mol) or different amounts of ascorbic acid (Vc, 0.88 g, 5 × 10⁻³ mol; 1.76 g, 10 × 10⁻³ mol; 2.64 g, 1.5 × 10⁻³ mol; 3.52 g, 20 × 10⁻³ mol; 5.28 g, 30 × 10⁻³ mol; and 7.04 g, 40 × 10⁻³ mol) in 50 mL of distilled water, chitosan (1.61 g, 10 × 10⁻³ mol of glucosamine) was added and the mixture was vigorously stirred on a magnetic stirrer at 30 °C away from light for 12 h. The remaining insoluble particle was discarded by centrifugation at 3000 rpm for 20 min. Meanwhile, the probable remaining organic acids in the supernatant were finally freeze-dried in vacuum. Here, the reaction molar ratios of chitosan/ascorbic acid were 2:1, 2:2, 2:3, 2:4, 2:6, and 2:8 for Cs₂Vc₁, Cs₂Vc₂, Cs₂Vc₃, Cs₂Vc₄, Cs₂Vc₆, and Cs₂Vc₈, respectively, and the reaction molar ratio of chitosan/glacial acetic acid was 2:4 for Cs₂Ac₄.

2.3. Structural Characterization of Chitosan Acetate and Chitosan Ascorbates. 2.3.1. Fourier Transform Infrared (FTIR) Spectroscopy. Preliminary structures of chitosan acetate and chitosan ascorbates were characterized by a Jasco-4100 FTIR spectrometer (JASCO Co., Ltd., Japan) in the wavenumber range of 4000–400 cm⁻¹ in transmission mode at a resolution of 4.0 cm⁻¹.

2.3.2. ¹H Nuclear Magnetic Resonance (NMR) Spectroscopy. ¹H NMR spectra of chitosan acetate and chitosan ascorbates were recorded on a Bruker AVIII-500 spectrometer (Bruker Tech. and Serv. Co., Ltd., Switzerland) operated at a resonance frequency of 500 MHz. ¹H NMR spectral analysis was used to calculate the substitution degrees (DS) of chitosan acetate and chitosan ascorbates.

2.4. Film Preparation. The casting technique was employed to prepare chitosan-based films. First, chitosan acetate or chitosan ascorbates (1 wt %) were dissolved in distilled water and vigorously stirred using a magnetic stir plate for 2 h to produce the film-forming solution. As a plasticizing agent, glycerol (0.3%, v/v) was added dropwise to the above solution, and the homogenous system was then achieved by vigorously stirring for 10 min. Afterward, the solution was placed in an ambient environment for at least 2 days to remove the hidden air bubbles. The thicknesses of chitosan films could be controlled by the amount of all film-forming solutions poured into the casting surface, and the surface density of dry films of 78 g/m² was provided in all formulations. The chitosan-based films were formed in a drying oven at 25 ± 0.5 °C in darkness for 3 days and then carefully shucked off from the Petri dishes. Finally, these films were stored away from light in a desiccator containing saturated Mg(NO₃)₂ solution at room temperature for at least 2 days before the next test measurements.

2.5. Film Characterization. 2.5.1. Thickness and Density. The thickness of each chitosan film was measured using a hand-held digital micrometer (Jingcheng, People’s Republic of China) at 10 randomly chosen points, and the average value was used. Film density (i.e., mass per unit volume) was determined from the quotient of the weight and the volume of the film, and this volume was estimated by the product of thickness and area of the film.

2.5.2. Mechanical Properties. Tensile strength and elongation at break of the chitosan-based films were carried out using a universal tensile tester (Instron 5848 MicroTester, U.K.). The prepared film (50 × 10 mm) was installed in the tensile grips at a strain speed of 10 mm/min. Data were collected for at least five replicates, and the average values were used for each film.

2.5.3. Surface Color. The color of film was assessed by a surface reflectance SC-80C colorimeter (Beijing Kang Guang Optical Instrument Co., Ltd., People’s Republic of China). CIE L*a*b* coordinates chroma and hue (where L*, lightness, indicated relatively changes between black and white, a* values indicated relatively changes between green and red, and b* values indicated relatively changes between blue and yellow) were obtained. Color calibration measurement was performed timely using a standard white plate. Chroma (C*), hue (h*), total color difference (ΔE), and whitness index (WI) were used to explain the changes in color and calculated according to eqs 1-5,

\[ C^* = (a^2 + b^2)^{1/2} \]  
\[ h^* = \tan^{-1}(b/a) \text{ (if } a > 0) \]  
\[ h^* = \tan^{-1}(b/a) + 180 \text{ (if } a < 0) \]  
\[ \Delta E = ((L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2)^{1/2} \]  
\[ WI = 100 - ((100 - L)^2 + a^2 + b^2)^{1/2} \]

where \( L, a, \) and \( b \) are the color parameters of chitosan films and \( L^*, a^*, \) and \( b^* \) are the color parameters of the standard white plate (\( L^* = 93.49, a^* = -0.25, \) and \( b^* = -0.09)\).

2.5.4. Light Transmittance and Opacity. Optical transmittance of the produced films was measured using a TU-1810 ultraviolet-visible (UV−vis) spectrometer (General Instrument Co., Ltd., Beijing, People’s Republic of China) in the scanning wavelength range of 200–800 nm. An empty test cuvette was used as the blank. The opacity of the chitosan films was determined at 600 nm and calculated by the following equation:

\[ \text{Opacity} = \frac{100}{T} \]
opacity \(= A_{600}/d \quad (6)\)

where \(A_{600}\) is the absorbance at 600 nm and \(d\) (mm) is the average thickness of the film.

2.5.5. X-ray Diffraction (XRD). XRD patterns were obtained on Bruker D8 Advance X-ray diffractometer (Bruker AXS GmbH, Germany) with Cu Kα radiation (\(\lambda = 1.541874 \text{ Å}\)) at a scanning rate of 6°/min in the diffraction angle (2\(\theta\)) range of 5–50°.

2.5.6. Thermal Analysis. Thermogravimetric analysis (TGA) and derivative thermogravimetry (DTG) were measured by means of a STA 449F3 (ETZSCH-Gerätebau GmbH, Germany) system at a heating rate of 10°C/min from 25 to 600°C under a nitrogen atmosphere.

2.5.7. Water Content, Swelling Degree, and Water Solubility. Each film specimen was uniformly trimmed to a rectangle (2 \(\times\) 2 cm) and weighted \((W_0)\). Afterward, the square film was dried in a hot oven at 75°C for 24 h to obtain the initial dry weight \((W_1)\). Next, the dried sample was spread on a Petri dish with 30 mL of deionized water covered with cling film, followed by storage at room environment for 24 h. Filter paper was then used to carefully remove the surface water, and the sample was weighed \((W_2)\). The residual film was desiccated at 75°C for 24 h to record the final dry mass \((W_3)\). Data were collected with three replications to calculate the water content, swelling degree, and water solubility according to eqs 7–9.

\[
\text{water content (%) } = \frac{(W_0 - W_3)}{W_0} \times 100 \quad (7)
\]

\[
\text{swelling degree (%) } = \frac{(W_2 - W_3)}{W_1} \times 100 \quad (8)
\]

\[\text{water solubility (%) } = \frac{(W_1 - W_3)}{W_1} \times 100 \quad (9)\]

2.5.8. Water Vapor Permeability (WVP). Water vapor transmission rate (WVTR) and WVP of chitosan-based films were gravimetrically determined by a modified method of ASTM E96-95. The circular test cups containing 18 mL of deionized water were sealed by test films, which were cut into pieces (4 cm diameter). The cups were then placed in an airtight desiccator containing dry silica gel and maintained at 25°C. The changes in total weight of the test cups were measured every 2 h in triplicate. The slopes of the linear portion in the curves of weight loss as a function of time were used to calculate the WVTR (g m\(^{-2}\) s\(^{-1}\)). Equation 10 was used to calculate the WVP in g m\(^{-1}\) Pa\(^{-1}\) s\(^{-1}\).

\[\text{WVP } = \frac{(\text{WVTR} \times L)}{\Delta P} \quad (10)\]

where \(L\) (m) is the average thickness of the film and \(\Delta P\) (Pa) is the difference in the partial water vapor pressure across the film.

2.6. Antioxidant Assay. The antioxidant activities of chitosan-based films were measured by a scavenging assay against the DPPH radical and a reducing power assay. First, the film sample (1%, w/v) was cut into the smallest possible pieces and then immersed into deionized water. After placement at room environment for 24 h, insoluble residual film was removed from each mixture by the centrifugation at 3000 rpm for 20 min. The supernatants were used as stock solutions to evaluate the antioxidant activity, and their concentrations were regarded as 10 mg/mL.

2.6.1. DPPH Radical Scavenging Activity Assay. The DPPH radical scavenging activities of chitosan-based films were evaluated using an earlier reported method, with some modifications. Briefly,
the extract solutions of film samples were diluted with deionized water at 0.075, 0.15, 0.30, 0.60, 1.20, 2.40, and 4.80 mg/mL. Afterward, 1.0 mL of diluted solution was thoroughly mixed with 2.0 mL of DPPH solution (180 μM, dissolved in absolute ethanol), followed by shaking for 10 s. The reaction mixture was then incubated in a dark condition for 20 min before triple-measuring the absorbance of each solution using an UV−vis spectrophotometer at 517 nm. A deionized-water-displaced sample was used as the blank. The scavenging effect against the DPPH radical was assessed using eq 11:

$$\text{scavenging effect (\%)} = \left[1 - \frac{A_1 - A_2}{A_0}\right] \times 100$$

where $A_0$, $A_1$, and $A_2$ are the absorbances of the blank group, the sample group, and the background of the sample (absolute ethanol instead of DPPH), respectively.

### 2.6.2. Reducing Power Assay.

The reducing power of chitosan-based films was estimated using a modified version of a previously reported method. The extract stock solutions were diluted with deionized water at 0.60, 1.20, 2.40, 4.80, and 9.60 mg/mL. A total of 1.0 mL of diluted solution with various concentrations was added to 1.0 mL of potassium ferricyanide solution (1%, w/v), and the mixture was reacted at 50 °C for 20 min, followed by the addition of 1.0 mL of trichloroacetic acid solution (10%, w/v). After centrifugation, a 1.5 mL aliquot of the supernatant was mixed with 1.5 mL of ferric chloride solution (0.2‰, w/v) and then incubated away from light for 10 min. The absorbance was recorded at 700 nm, and the reducing power of chitosan-based films was calculated using the following formula:

$$\text{reducing power} = A_1 - A_0$$

where $A_0$ and $A_1$ are the absorbances of the blank group and sample group, respectively.

### 2.7. Statistical Analysis.

Data were expressed as the mean ± standard deviation (SD; $n \geq 3$). The significant differences between sample means were determined using one-way analysis of variance, where the level of significance was set at $p < 0.05$. Meanwhile, multiple comparisons of means were carried out by Duncan’s test.

### 3. RESULTS AND DISCUSSION

#### 3.1. Synthesis of Chitosan Ascorbates

As shown in Figure 1, chitosan ascorbates were prepared in water based on the electrostatic interaction between the reactive amino group in the chitosan backbone and the acidic hydroxyl group at C3 ($pK_a$ of 4.17) of ascorbic acid at different reaction molar ratios ($Cs_2Vc_1$, $Cs_2Vc_2$, $Cs_2Vc_3$, $Cs_2Vc_4$, $Cs_2Vc_6$, and $Cs_2Vc_8$). Besides, chitosan acetate ($Cs_2Ac_4$) was also obtained as the control group in physical chemistry experiments and antioxidant tests.

#### 3.1.1. FTIR Analysis

As shown in Figure 2, pristine chitosan ($Cs$) displays primary spectral peaks at 3428, 2877, 1646, 1600, 1380, and 1083 cm$^{-1}$, which are assigned to $N−H$ and $O−H$ stretching vibrations, $C−H$ stretching vibration, $C=O$ stretching vibration of the residual amide bond, $N−H$ bending vibration, $C−H$ bending vibration, and $C−O$ stretching vibration, respectively. After the salt-forming reaction, the major changes in the FTIR spectra between chitosan and chitosan acetate show that the characteristic band at 1600 cm$^{-1}$ disappears but new peaks at 1558 and 1407 cm$^{-1}$ are detected, which can be attributed to the $−NH_3^+$ bending vibration and $−COO^−$ symmetrical stretching vibration resulting from the electrostatic interaction. In the spectra of chitosan ascorbates, the difference in reaction molar ratios of chitosan/ascorbic acid shows less of an effect on their FTIR spectra but the new characteristic peak is observed at 1716 cm$^{-1}$, assigned to the stretching vibration of the carbonyl group of lactone in the ascorbate molecule. Additionally, the overlapping peak of the $C=O$ stretching vibration of ascorbate...
with the $\text{C–C}$ bending vibration of chitosan is detected at about 1589 cm$^{-1}$, and the weak peaks that appear at 755 and 713 cm$^{-1}$ are assigned to the weak peaks that appear at 755 and 713 cm$^{-1}$ to the $\text{C–C}$ bending vibration of ascorbate.

3.1.2. $^1$H NMR Analysis. $^1$H NMR spectra further verified the successful preparation of chitosan acetate and chitosan ascorbates. As shown in Figure 3, the chemical shifts at $\delta$ = 3.10 and 3.50–4.00 ppm are assigned to $\text{H}_2$ and $\text{H}_3$–$\text{H}_6$ of the glucosamine monomer in chitosan.$^{36,37}$ In comparison to chitosan, a sharp peak (a in the spectrum) is observed at $\delta = 1.78$ ppm, which is attributed to methyl protons of the acetate moiety. Meanwhile, $^1$H NMR spectra of chitosan ascorbates apparently display new signals at $\delta = 4.51$ ppm (a in the spectrum), 3.99 ppm (b in the spectrum), and 3.71 ppm (c in the spectrum) that can be assigned to the lactone $\text{CH}$ proton next to glycol and the $\text{CH}$ and $\text{CH}_2$ protons of glycol groups, respectively.$^{38}$ One notable thing is that all of the chemical shifts of protons in ascorbic acid are shifted toward a high field after the salt-forming reaction with chitosan in water. Additionally, the DS values of chitosan acetate and chitosan ascorbates were evaluated on the basis of the integral areas in the $^1$H NMR spectral analysis and were calculated using the following formulas:

$$DS_1 = I_{\text{CH}_3}/(3I_{\text{H}_1})$$

(13)

$$DS_2 = I_{\text{H}_1}/I_{\text{H}_3}$$

(14)

where $DS_1$ is the DS of chitosan acetate, $DS_2$ is the DS of chitosan ascorbates, $I_{\text{CH}_3}$ is the integral of the peak of methyl protons of the acetate moiety, $I_{\text{H}_1}$ is the integral of the peak of the $\text{H}_2$ proton in glucosamine rings, and $I_{\text{H}_3}$ is the integral of the peak of the lactone $\text{CH}$ proton next to glycol of the ascorbate moiety.

The DS values of chitosan acetate and chitosan ascorbates are shown in Table 1. It is very clear that, with the increase of the reaction molar ratios of ascorbic acid to chitosan, the DS values of chitosan ascorbates increase and can reach a maximum of about 0.95 at a molar ratio of 4:1. In consideration of 95% of the deacetylation degree of pristine chitosan, it is reasonable to deduce that all amino groups of pristine chitosan have been involved in the salt-forming reaction with ascorbic acid.

### Table 1. Reaction Molar Ratio and Degree of Substitution of Chitosan Acetate and Chitosan Ascorbates

| chitosan derivative | reaction molar ratio | degree of substitution |
|--------------------|----------------------|------------------------|
| $\text{Cs}_x\text{Ac}_y$ | chitosan, 10 mM; glacial acetic acid, 20 mM | 0.60 |
| $\text{Cs}_x\text{Vc}_1$ | chitosan, 10 mM; ascorbic acid, 5 mM | 0.50 |
| $\text{Cs}_x\text{Vc}_2$ | chitosan, 10 mM; ascorbic acid, 10 mM | 0.65 |
| $\text{Cs}_x\text{Vc}_3$ | chitosan, 10 mM; ascorbic acid, 15 mM | 0.74 |
| $\text{Cs}_x\text{Vc}_4$ | chitosan, 10 mM; ascorbic acid, 20 mM | 0.80 |
| $\text{Cs}_x\text{Vc}_5$ | chitosan, 10 mM; ascorbic acid, 30 mM | 0.88 |
| $\text{Cs}_x\text{Vc}_6$ | chitosan, 10 mM; ascorbic acid, 40 mM | 0.95 |

* $\text{Cs}_x\text{Ac}_y$ chitosan acetate; $\text{Cs}_x\text{Vc}_y$ chitosan ascorbate.

3.2. Characterization of Chitosan Ascorbate Films

#### 3.2.1. Thickness, Density, and Mechanical Properties

The chitosan films were formed with the solution/solvent casting method. The thickness of the chitosan films ranges between 66 ± 6 and 71 ± 6 $\mu$m (Table 2), and this difference is not statistically significant between each film. This result indicated that the content of ascorbate had no significant influence ($p > 0.05$) on the average thickness of chitosan-based films. However, film densities decrease along with the incorporation of ascorbate compared to the chitosan acetate film, but no significant differences ($p < 0.05$) are found in densities of six chitosan ascorbate films.

Table 2 shows the effect of the category and content of organic acid salt on the tensile strength and elongation at break of chitosan-based films. First, for chitosan acetate film, the tensile strength and elongation at break values are found to be 43 ± 1 and 31 ± 6%, respectively. It can be seen that the salt-forming reaction of chitosan with ascorbic acid produced a significant effect on their mechanical properties. A significant drop ($p < 0.05$) in the tensile strength and elongation at break values of chitosan ascorbate films, resulting in tougher films, was observed in comparison to the chitosan acetate film. The mechanical property of the film could be greatly influenced by the systematic composition and intermolecular forces.$^{10,12}$ The decrease in tensile strength and elongation at break could be ascribed to the stronger destructive capacity of ascorbic acid to the crystalline structure as well as intra- and intermolecular hydrogen bonding of the chitosan matrix than that of acetic acid.$^{12}$ Moreover, the introduction of ascorbate with an increasing substitution degree from 0.50 to 0.95 decreases the tensile strength values from 27 ± 4 to 21 ± 4 MPa and elongation at break values from 16 ± 2 to 10.0 ± 4.0%. Similar findings have been reported previously by Sun et al.$^{12}$ The above result showed that an increasing content of ascorbate in the chitosan matrix resulted in an accelerating trend toward the decrease in the crystalline structure and intermolecular forces between chitosan chains.$^{10}$

#### 3.2.2. Surface Color, Opacity, and Light Transmittance

Significant differences between the chitosan-based films in the color parameters, including $L_*$, $a_*$, $b_*$, $C^*$, $h^*$, $AE$, WI, and opacity, are observed in Table 3. As the control group, the chitosan acetate film was colorless and transparent. However, chitosan ascorbate films had a fawn-colored appearance compared to the chitosan acetate film, and changes occurred in all of the color parameters with the incorporation of ascorbate into chitosan films. Moreover, as the substitution
degrees of ascorbate increase from 0.50 to 0.95, a decrease in L values from 59.03 ± 0.05 to 51.53 ± 0.01 and increases in b values (from 14.48 ± 0.02 to 25.57 ± 0.01) and C* values (from 14.8 ± 0.8 to 26 ± 3) indicate that the films lose clarity and become darker, yellower, but increased color saturation. Chitosan ascorbate films show higher redness as growing a values are noted when the substitution degrees of ascorbate are 0.50–0.80, while the decrease in redness is also observed from a values, which decline from 2.50 ± 0.14 to 1.99 ± 0.01 when the substitution degrees of ascorbate reach 0.80–0.95. The increasing ΔE values (from 37.52 ± 0.04 to 49.23 ± 0.01), the decreasing WI values (from 56.44 ± 0.05 to 45.17 ± 0.01), and the decreasing h* values (from 102 ± 2 to 84.1 ± 0.4) indicate that the more colored films are obtained. The transparency is an important property for the film and is generally expressed as opacity. A higher value of opacity indicated the lower transparency and vice versa. The increasing opacities of chitosan ascorbate films from 0.78 ± 0.07 to 1.31 ± 0.01 \( A_{600/\text{mm}} \) are observed in Table 3 with the growing substitution degrees of ascorbate.

UV and visible light can catalyze the oxidation process of packaged food, and too much exposure to UV–vis light can accelerate the food deterioration and nutrient loss. Thus, the capacity for blocking UV–vis light to extend the shelf life of foodstuffs is an important characteristic for food packaging materials. Figure 4 shows the light transmittance of chitosan-based films in the wavelength range of 200–800 nm. The transmittance values of the chitosan acetate film in the wavelength range of 200–400 nm are 4.29–78.02%. The introduction of ascorbate into the chitosan matrix significantly exhibits transmittance values of below 35% compared to the chitosan acetate film; this higher blocking capacity might be due to the absorption capacity of ascorbate toward UV light. In particular, the UV light transmittances of all of the chitosan ascorbate films attain almost zero in the range of 230–295 nm. Additionally, the light transmittances of chitosan ascorbate films are further reduced with the increasing content of ascorbate in the range of 380–600 nm. All of the data indicated that chitosan ascorbate films had great capacity to block UV–vis light.

### Table 3. Optical Properties of the Chitosan Acetate Film and Chitosan Ascorbate Films

| film          | \( L \)     | \( a \)  | \( b \) | \( C^* \) | \( h^* \) | \( \Delta E \) | WI   | opacity (\( A_{600/\text{mm}} \)) |
|---------------|-------------|---------|--------|---------|---------|-------------|------|----------------------------------|
| Cs\(_2\)Ac4  | 62.16 ± 0.01| -1.64 ± 0.10 | 2.18 ± 0.04 | 2.7 ± 0.2 | 127.0 ± 0.7 | 31.45 ± 0.02 | 62.06 ± 0.02 | 0.92 ± 0.02 |
| Cs\(_2\)Vc3  | 59.03 ± 0.05| -3.10 ± 0.06 | 14.48 ± 0.02 | 14.8 ± 0.8 | 102 ± 2 | 37.52 ± 0.04 | 56.44 ± 0.05 | 0.78 ± 0.07 |
| Cs\(_2\)Vc1  | 55.31 ± 0.13| 0.75 ± 0.08 | 22.49 ± 0.03 | 22.5 ± 1.1 | 88.1 ± 0.5 | 44.37 ± 0.12 | 49.96 ± 0.13 | 0.90 ± 0.06 |
| Cs\(_2\)Ac4  | 55.2 ± 0.4 | 1.78 ± 0.14 | 23.91 ± 0.05 | 24 ± 2 | 85.7 ± 0.3 | 45.2 ± 0.3 | 49.2 ± 0.3 | 0.99 ± 0.02 |
| Cs\(_2\)Vc3  | 51.72 ± 0.01| 2.50 ± 0.14 | 24.30 ± 0.02 | 24 ± 0.7 | 84.1 ± 0.4 | 48.44 ± 0.02 | 45.90 ± 0.02 | 1.27 ± 0.07 |
| Cs\(_2\)Vc1  | 51.62 ± 0.02| 2.43 ± 0.02 | 25.09 ± 0.04 | 25.2 ± 1.3 | 84.5 ± 0.6 | 48.93 ± 0.03 | 45.45 ± 0.03 | 1.28 ± 0.03 |
| Cs\(_2\)Vc4  | 51.53 ± 0.01| 1.99 ± 0.01 | 25.57 ± 0.01 | 26 ± 3 | 85.6 ± 1.2 | 49.23 ± 0.01 | 45.17 ± 0.01 | 1.31 ± 0.01 |

*Values are given as the mean ± SD. Different letters within the same column indicate significant differences among films (\( p < 0.05 \)). Cs\(_2\)Ac\(_4\), chitosan acetate; Cs\(_2\)Vc\(_3\), chitosan ascorbate.*

![Figure 4](image-url)

Figure 4. UV–vis spectra of the chitosan acetate (Cs\(_2\)Ac\(_4\)) film and chitosan ascorbate (Cs\(_2\)Vc\(_3\)) films.

![Figure 5](image-url)

Figure 5. XRD patterns of the chitosan acetate (Cs\(_2\)Ac\(_4\)) film and chitosan ascorbate (Cs\(_2\)Vc\(_3\)) films.
crystal structures of chitosan ascorbate films could be identified from XRD patterns with an increment of ascorbate contents.  

3.2.4. Thermal Analysis. TGA and DTG variation trends of pristine chitosan powder, chitosan acetate film, and chitosan ascorbate films are presented in Figure 6. Chitosan powder displays two thermal decomposition stages: the first stage (≤100 °C) with about 6 wt % weight loss mainly goes through the loss of residual water, and the second stage (200−400 °C) with the remaining 36 wt % solid residue corresponds to the decomposition of the chitosan backbone.39,42 However, for all of the chitosan films, degradations are recorded in three steps. The first mass loss at 25−100 °C with about 6 wt % degradation could also be ascribed to the evaporation of bound or free moisture. The second step occurs at about 100−225 °C with about 25 wt % degradation and can be assigned to the decomposition of glycerol.16 The onset temperature of the main mass loss is also observed at nearly 225 °C, accompanied by a massive weight loss (40 wt %). The thermal degradation of chitosan backbones mainly happens in this step, and the temperatures at a maximum decomposition rate of chitosan films are observed at 271−288 °C, which are below that of pristine chitosan at 296 °C. Given the above, the much lower degradation temperature and bigger weight loss in the decomposition process of the chitosan backbone displayed poor thermal stability of chitosan-based films compared to pristine chitosan. This reduced thermal stability might be related to the lower crystallinity of chitosan films. However, it was found that the increment of ascorbate contents did not exert a substantial influence on the thermal stability of chitosan ascorbate films.

3.2.5. Water Content, Water Solubility, Swelling Degree, and Water Vapor Permeability. The chitosan acetate film as the control group shows the highest moisture content and swelling degree but the lowest solubility in water. Trends toward the decreases on the moisture content and swelling degree and an increase on the water solubility are detected in Table 4 for chitosan ascorbate films. This result might be ascribed to the salt-forming reaction and hydrogen-binding interaction between chitosan and ascorbic acid molecules, which could cause the lack of free hydrophilic groups, such as amino and hydroxyl groups, in chitosan backbones to bind with water by forming hydrogen bonds as a result of the competitive binding effect.15,40 Meanwhile, the hydrophilic property of the ascorbate moiety in chitosan backbones and the disruption of the intra- or intermolecular hydrogen bond networks might take responsibility for the higher solubility in water of chitosan ascorbate films. A similar result was found for chitosan−protocatechuic acid (PA) composite films.13 Besides, these obvious variation tendencies can be further strengthened with an increment on the substitution degrees of ascorbate groups.

For edible films, the blocking of water exchange between packaged foodstuffs and the external environment can efficiently extend their shelf life.17,43 Table 4 shows the influence of the salt-forming reaction on the WVP values of chitosan-based films. The WVP values of chitosan ascorbate films are significantly lower (p < 0.05) than that of the chitosan acetate film. However, no significant differences (p > 0.05) in WVP values are detected with the increase in the ascorbate content. Past research has demonstrated that the permeation of...
water vapor for chitosan-based films involved two processes, including adsorption and diffusion steps. Moisture could be easily adsorbed into the polymeric matrix by free hydrophilic groups of chitosan backbones, followed by the improved diffusion step. The electrovalent bond and hydrogen bond in the chitosan ascorbate molecule greatly occupied a large amount of hydrophilic groups of chitosan, which ultimately led to the decrease on water affinity of chitosan ascorbate films.

### 3.3. Antioxidant Activity

Antioxidant activities of chitosan films were measured using the scavenging activity assay on the DPPH radical and reducing power assay. Meanwhile, the EC$_{50}$ value in milligrams per milliliter (namely, the mass concentration of antioxidants produced a 50% scavenging effect against active free radicals) against the DPPH radical (shown in Table 4) is also calculated as an indicator of the antioxidant capacity of chitosan-based films. Naturally, the lower the EC$_{50}$ values, the stronger the scavenging abilities against free radicals of the compounds.

As illustrated in Figure 7, the chitosan acetate film shows poor capacity for scavenging the DPPH radical, with an EC$_{50}$ value far exceeding 1.60 mg/mL and a negligible reducing power within the tested dosage. However, chitosan ascorbate films exhibit a powerful DPPH radical scavenging capacity and reducing power in a dose-dependent manner in the range of the selected concentration. Continuous enhancements in the scavenging activities against the DPPH radical are detected for Cs$_2$VC$_1$, Cs$_2$VC$_2$, Cs$_2$VC$_3$, Cs$_2$VC$_4$, Cs$_2$VC$_6$, and Cs$_2$VC$_8$, with scavenging values of 63.73 ± 0.01, 2.87 ± 0.12, 3.45 ± 0.18, 3.74 ± 0.17, 3.85 ± 0.18, and 3.86 ± 0.14, respectively. This result demonstrated that, with the increment of the ascorbate content, the antioxidant activities of chitosan ascorbate films were further enhanced. This wonderful effect might mainly obtain the benefit of the function of ascorbate, which can be available to act as a one-electron donor to potentially damage active oxidizing radicals by a reversible and thermodynamically favorable oxidation. Meanwhile, the ascorbyl radical can readily generate in this one-electron oxidation of ascorbate (Figure 8). Two molecules of the ascorbyl radical can also undergo a pH-dependent disproportionation reaction, resulting in one molecule of ascorbate and the two-electron oxidized form, dehydroascorbate.

In conclusion, for the first time, chitosan ascorbates were synthesized in water without any organic reagent based on the salt-forming reaction of chitosan and ascorbic acid at different reaction molar ratios to prepare chitosan-based antioxidative films for food preservation. Besides, the chitosan acetate film was also obtained, with use as the control group, in physicochemical experiments and antioxidant tests. As a result, chitosan ascorbate films showed improved surface color, reduced light transmittance, lower water content and swelling degree, decreased water vapor permeability, and higher water solubility, whereas reduced mechanical properties and lower crystallinity and thermal stability than the chitosan acetate film. Those changes in physical property were due to the stronger destructive capacity of ascorbic acid to the crystalline structure of the chitosan matrix as well as intra- and intermolecular hydrogen bonding between chitosan chains. In vitro antioxidant activity studies demonstrated that the incorporation of ascorbate could greatly enhance the scavenging capacity against the DPPH radical and reducing power of the chitosan matrix. Besides, the antioxidant activities of chitosan ascorbate films were further enhanced with the increase of the ascorbate content. Current results converge to promise the potential of chitosan ascorbate films as a suitable candidate in the field of food packaging.
The authors declare no competing financial interest.

**REFERENCES**

1. Galvis-Sánchez, A. C.; Sousa, A. M. M.; Hilliou, L.; Gonçalves, M. P.; Souza, H. K. S. Thermo-compression molding of chitosan with a deep eutectic mixture for biofilms development. *Green Chem.* 2016, 18, 1571–1580.

2. Wang, H.; Qian, J.; Ding, F. Emerging chitosan-based films for food packaging applications. *J. Agric. Food Chem.* 2018, 66, 395–413.

3. Liu, K.; Lin, X.; Chen, L.; Huang, L.; Cao, S.; Wang, H. Preparation of microb fibrillated cellulose/chitosan–benzalkonium chloride bioemulsion for enhancing antibacterial and strength of sodium alginate films. *J. Agric. Food Chem.* 2013, 61, 6562–6567.

4. Liu, J.; Meng, C.-g.; Liu, S.; Kan, J.; Jin, C.-h. Preparation and characterization of protocatechuic acid grafted chitosan films with antioxidant activity. *Food Hydrocolloids* 2017, 63, 457–466.

5. Nunes, C.; Maricato, E.; Cunha, A.; Rocha, M. A. M.; Santos, S.; Ferreira, P.; Silva, M. A.; Rodrigues, A.; Amado, O.; Coimbra, J.; Silva, D.; Moreira, A.; Mendo, S.; Lopes da Silva, J. A.; Pereira, E.; Rocha, S. M.; Coimbra, M. A. Chitosan–genipin film, a sustainable methodology for wine preservation. *Green Chem.* 2016, 18, 5331–5341.

6. Pérez-Córdoba, L. J.; Norton, I. T.; Batchelor, H. K.; Gkatzionis, K.; Spyropoulos, F.; Sobral, P. J. A. Physico-chemical, antimicrobial and antioxidant properties of gelatin–chitosan based films loaded with nanoemulsions encapsulating active compounds. *Food Hydrocolloids* 2018, 79, 544–559.

7. Marin, L.; Stoica, I.; Mares, M.; Dinu, V.; Simionescu, B. C.; Barboiu, M. Antifungal vanillin–imino-chitosan biodynameric films. *Int. J. Biol. Macromol.* 2018, 107, 350–359.

8. Mauritio-Sánchez, R. A.; Salazar, R.; Luna-Bárcenas, J. G.; Mendoza-Galván, A. FTIR spectroscopy studies on the spontaneous neutralization of chitosan acetate films by moisture conditioning. *Vib. Spectrosc.* 2018, 94, 1–6.

9. Rui, L.; Xie, M.; Hu, B.; Zhou, L.; Yin, D.; Zeng, X. A comparative study on chitosan/gelatin composite films with conjugated or incorporated gallic acid. *Carbohydr. Polym.* 2017, 173, 473–481.

10. Liang, J.; Yan, H.; Zhang, J.; Dai, W.; Gao, X.; Zhou, Y.; Wan, X.; Puligundla, P. Preparation and characterization of antioxidant edible chitosan films incorporated with epigallocatechin gallate nanocapsules. *Carbohydr. Polym.* 2017, 171, 300–306.

11. Aguirre-Loredo, R. Y.; Rodríguez-Hernandez, A. I.; Morales-Sánchez, E.; Gomez-Aldapa, C. A.; Velazquez, G. Effect of equilibrium moisture content on barrier, mechanical and thermal properties of chitosan films. *Food Chem.* 2016, 196, 560–566.

12. Sun, L.; Sun, J.; Chen, L.; Niu, P.; Yang, X.; Guo, Y. Preparation and characterization of chitosan film incorporated with thinned young apple polyphenols as an active packaging material. *Carbohydr. Polym.* 2017, 163, 81–91.

13. Liu, J.; Liu, S.; Wu, Q.; Gu, Y.; Kan, J.; Jin, C. Effect of protocatechuic acid incorporation on the physical, mechanical, structural and antioxidant properties of chitosan film. *Food Hydrocolloids* 2017, 73, 90–100.

14. Sun, X.; Sui, S.; Ference, C.; Zhang, Y.; Sun, S.; Zhou, N.; Zhu, W.; Zhou, K. Antimicrobial and mechanical properties of β-cyclodextrin inclusion with essential oils in chitosan films. *J. Agric. Food Chem.* 2014, 62, 8914–8918.

15. Souza, V. G. L.; Fernando, A. L.; Pires, J. R. A.; Rodrigues, P. F.; Lopes, A. A. S.; Fernandes, F. M. B. Physical properties of chitosan films incorporated with natural antioxidants. *Ind. Crops Prod.* 2017, 107, 565–572.

16. Kaya, M.; Khadem, S.; Cakmak, Y. S.; Mutjab, M.; Ihk, S.; Akyuz, L.; Salabertia, A. M.; Labidi, J.; Abdulqadir, A. H.; Deligöz, E. Antioxidative and antimicrobial edible chitosan films blended with stem, leaf and seed extracts of *Pistacia terebinthus* for active food packaging. *RSC Adv.* 2018, 8, 3941–3950.

17. Kalaycioglu, Z.; Torlak, E.; Akin-Evingur, G.; Ozen, I.; Erim, F. B. Antimicrobial and physical properties of chitosan films incorporated with turmeric extract. *Int. J. Biol. Macromol.* 2017, 101, 882–888.

18. Pastor, C.; Sánchez-González, L.; Chiralt, A.; Cháfer, M.; González-Martínez, C. Physical and antioxidant properties of chitosan and methylcellulose based films containing resveratrol. *Food Hydrocolloids* 2013, 30, 272–280.

19. Talón, E.; Tríkovic, K. T.; Nedovic, V. A.; Bugarski, B. M.; Vargas, M.; Chiralt, A.; González-Martínez, C. Antioxidant edible films based on chitosan and starch containing polyphenols from thyme extracts. *Carbohydr. Polym.* 2017, 157, 1153–1161.

20. Ge, L.; Zhu, M.; Li, X.; Xu, Y.; Ma, X.; Shi, R.; Li, D.; Mu, C. Development of active rosmaninic acid–gelatin biodegradable films with antioxidant and long-term antibacterial activities. *Food Hydrocolloids* 2018, 83, 308–316.

21. Hu, Q.; Luo, Y. Polyphenol–chitosan conjugates: Synthesis, characterization, and applications. *Carbohydr. Polym.* 2016, 151, 624–639.

22. Tan, W.; Li, Q.; Dong, F.; Wei, L.; Guo, Z. Synthesis, characterization, and antifungal property of chitosan ammonium salts with halogens. *Int. J. Biol. Macromol.* 2016, 92, 291–298.

23. Ren, J.; Liu, J.; Li, R.; Dong, F.; Guo, Z. Antifungal properties of chitosan salts in laboratory media. *J. Appl. Polym. Sci.* 2012, 124, 2501–2507.

24. Patel, P.; Parmar, K.; Patel, D.; Kumar, S.; Trivedi, M.; Das, M. Inhibition of amyloid fibril formation of lysozyme by ascorbic acid and a probable mechanism of action. *Int. J. Biol. Macromol.* 2018, 114, 666–678.

25. Arumugam, N.; Kim, J. Quantum dots attached to graphene oxide for sensitive detection of ascorbic acid in aqueous solutions. *Mater. Sci. Eng., C* 2018, 92, 720–725.

26. Sha, R.; Badhulika, S. Facile green synthesis of reduced graphene oxide/tin oxide composite for highly selective and ultrasensitive detection of ascorbic acid. *J. Electroanal. Chem.* 2018, 816, 30–37.

27. Meng, H.; Yang, D.; Tu, Y.; Yan, J. Turn-on fluorescence detection of ascorbic acid with gold nanolcusters. *Talanta* 2017, 165, 346–350.

28. Haša, J.; Charfeddine, B.; Smach, M. A.; Limem, K.; Majdoub, H.; Sonia, R. Synthesis, characterization, antioxidant and antibacterial properties of chitosan ascorbate. *Int. J. Pharm.* *Biol. Sci.* 2014, 4, 1072–1081.

29. Rossi, S.; Marciello, M.; Sandri, G.; Bonferoni, M. C.; Ferrari, F.; Caramella, C. Chitosan ascorbate: A chitosan salt with improved penetration enhancement properties. *Pharm. Dev. Technol.* 2008, 13, 513–521.

30. ElBarbari, A. M.; El-Sawy, M. N.; Hegazy, E. A. Antioxidative properties of irradiated chitosan/vitamin C complex and their use as food additive for lipid storage. *J. Appl. Polym. Sci.* 2015, 132, 42105–42112.

31. Jouki, M.; Tabatabaei Yazdi, F.; Mortazavi, S. A.; Koocheki, A. Physical, barrier and antioxidant properties of a novel plasticized...
edible film from quince seed mucilage. Int. J. Biol. Macromol. 2013, 62, 500–507.

(32) ASTM International. ASTM E96-95. Standard Test Methods for Water Vapor Transmission of Materials; ASTM International: West Conshohocken, PA, 1995.

(33) Gennadios, A.; Weller, C. L.; Gooding, C. H. Measurement of water vapor permeability of highly permeable, hydrophilic edible films. J. Food Eng. 1994, 21, 395–409.

(34) Tan, W.; Li, Q.; Li, W.; Dong, F.; Guo, Z. Synthesis and antioxidant property of novel 1,2,3-triazole-linked starch derivatives via ‘click chemistry’. Int. J. Biol. Macromol. 2016, 82, 404–410.

(35) Tan, W.; Zhang, J.; Zhao, X.; Dong, F.; Li, Q.; Guo, Z. Synthesis and antioxidant action of chitosan derivatives with amino-containing groups via azide–alkyne click reaction and N-methylation. Carbohydr. Polym. 2018, 199, 583–592.

(36) Liu, X.; Xia, W.; Jiang, Q.; Yu, P.; Yue, L. Chitosan oligosaccharide-N-chlorokojic acid mannnich base polymer as a potential antibacterial material. Carbohydr. Polym. 2018, 182, 225–234.

(37) Rui, L.; Xie, M.; Hu, B.; Zhou, L.; Saeeduddin, M.; Zeng, X. Enhanced solubility and antioxidant activity of chlorogenic acid–chitosan conjugates due to the conjugation of chitosan with chlorogenic acid. Carbohydr. Polym. 2017, 170, 206–216.

(38) Zhang, J.; Tan, W.; Wang, G.; Yin, X.; Li, Q.; Dong, F.; Guo, Z. Synthesis, characterization, and the antioxidant activity of N,N,N-trimethyl chitosan salts. Int. J. Biol. Macromol. 2018, 118, 9–14.

(39) Bilbao-Sainz, C.; Chiou, B. S.; Williams, T.; Wood, D.; Du, W.; Xu, S.; Ban, Z.; Rodov, V.; Poverenov, E.; Vinokur, Y.; McHugh, T. Vitamin D-fortified chitosan films from mushroom waste. Carbohydr. Polym. 2017, 167, 97–104.

(40) Riaz, A.; Lei, S.; Akhtar, H. M. S.; Wan, P.; Chen, D.; Jabbar, S.; Abid, M.; Hashim, M. M.; Zeng, X. Preparation and characterization of chitosan-based antimicrobial active food packaging film incorporated with apple peel polyphenols. Int. J. Biol. Macromol. 2018, 114, 547–555.

(41) Moghadas, B.; Dashtimoghadam, E.; Mirzadeh, H.; Seidi, F.; Hasani-Sadrabadi, M. M. Novel chitosan-based nanobiohybrid membranes for wound dressing applications. RSC Adv. 2016, 6, 7701–7711.

(42) Bao, Y.; Zhang, H.; Luan, Q.; Zheng, M.; Tang, H.; Huang, F. Fabrication of cellulose nanowhiskers reinforced chitosan–xylan nanocomposite films with antibacterial and antioxidant activities. Carbohydr. Polym. 2018, 184, 66–73.

(43) Ren, L.; Yan, X.; Zhou, J.; Tong, J.; Su, X. Influence of chitosan concentration on mechanical and barrier properties of corn starch/chitosan films. Int. J. Biol. Macromol. 2017, 105, 1636–1643.

(44) Liu, J.; Liu, S.; Chen, Y.; Zhang, L.; Kan, J.; Jin, C. Physical, mechanical and antioxidant properties of chitosan films grafted with different hydroxybenzoic acids. Food Hydrocolloids 2017, 71, 176–186.

(45) Ma, Y.; Xin, L.; Tan, H.; Fan, M.; Li, J.; Jia, Y.; Ling, Z.; Chen, Y.; Hu, X. Chitosan membrane dressings toughened by glycerol to load antibacterial drugs for wound healing. Mater. Sci. Eng., C 2017, 81, 522–531.

(46) Du, J.; Cullen, J. J.; Buettner, G. R. Ascorbic acid: Chemistry, biology and the treatment of cancer. Biochim. Biophys. Acta, Rev. Cancer 2012, 1826, 443–457.

(47) Harrison, F. E.; May, J. M. Vitamin C function in the brain: Vital role of the ascorbate transporter SVCT2. Free Radical Biol. Med. 2009, 46, 719–730.