Preparation and physicochemical properties of antioxidant chitosan ascorbate/methylcellulose composite films

Wenqiang Tan a,b, Jingjing Zhang a,b,c, Xiang Zhao d, Qing Li a,b, Fang Dong a,b,⁎, Zhanyong Guo a,b,c,⁎⁎

a Research and Development Center for Efficient Utilization of Coastal Bioresources, Yantai Institute of Coastal Zone Research, Chinese Academy of Sciences, Yantai 264003, China
b Center for Ocean Mega-Science, Chinese Academy of Sciences, 7 Nanhai Road, Qingdao 266071, China
c University of Chinese Academy of Sciences, Beijing 100049, China
d School of Biomedical Sciences, Huaqiao University, Quanzhou 362021, China

ARTICLE INFO

Article history:
Received 25 October 2019
Received in revised form 30 November 2019
Accepted 5 December 2019
Available online 6 December 2019

Keywords:
Chitosan ascorbate
Methylcellulose
Composite film
Physicochemical property
Antioxidant activity

ABSTRACT

Polysaccharide-based biodegradable films have been considered as the promising candidates for food packaging industry instead of petroleum-based packaging materials. Here, we reported a class of edible composite films based on chitosan ascorbate and methylcellulose prepared by mixing different ratios (1:0, 4:1, 2:1, 1:1, 1:2, 1:4, and 0:1) of the biopolymers using the casting technique. Their physicochemical properties as well as the DPPH radical scavenging ability and reducing power were investigated. All physicochemical properties and antioxidant activities were significantly affected by the chitosan ascorbate/methylcellulose ratio in the matrix. The increases in tensile strength and elongation at break values, maximum decomposition temperatures, whitish index, compactness, moisture content, and a reduction in water vapor permeability were observed as the proportion of methylcellulose increased in the matrix. But the composite films containing a greater proportion of chitosan ascorbate exhibited the better barrier properties against UV–vis light and the stronger DPPH radical scavenging effect and reducing power. The chitosan ascorbate/methylcellulose composite films with interesting physicochemical properties and strong antioxidant action showed the potential value as biodegradable and edible biomaterials for food packaging.

© 2019 Published by Elsevier B.V.

1. Introduction

Nowadays, the inappropriate disposal, non-biodegradability, chemical residues in the food products of petroleum-based plastics in food packaging industries are creating serious environmental problems worldwide, especially marine pollution and soil contaminant [1,2]. Recently, in order to control the environmental pollution caused by the use of petroleum plastic packaging and to reduce consumption of fossil fuel reserve, considerable research has been conducted to develop alternative eco-friendly and non-toxic bio-based packaging materials like biodegradable and edible films using natural polymers [3,4]. Among various biopolymers, chitosan, a linear polysaccharide with unique polyctionic nature derived from the deacetylation of chitin, is regarded as one of the most attractive packaging materials due to its great film-forming property, good biodegradability, appropriate mechanical performance, nontoxicity, and easy modification [5,6]. However, the lack of sufficient antioxidant action of chitosan film seriously hampers the real applications of chitosan based films in food packaging field [7]. The inclusion of many natural active antioxidant compounds may be an effective choice to compensate for the deficiencies of these films in antioxidant activity [8,9]. Priyadarshi et al. [10] reported that chitosan films incorporated with natural apricot kernel essential oil extracted from kernels of bitter apricot in different concentrations displayed excellent antioxidant and antimicrobial properties as compared to pure chitosan films. Compared with physical blending of natural antioxidant ingredients into chitosan matrix, long-term antioxidant property could be effectively and more easily achieved using chemical bonding methods between them [11,12]. Thereinto, chitosan ascorbate, prepared quite easily based on the salification reaction of chitosan and ascorbic acid in water without multi-step purification procedures, could be an ideal film-forming biomaterial in food packaging industry due to its excellent antioxidant capacity and great capacity for blocking UV–vis light [13]. But one of the major challenges in the real applications of chitosan ascorbate film in food packaging industry is the insufficient mechanical strength which is a significant parameter required for food packaging materials to withstand external stress and maintain the integrity during their transportation and storage [10,14].
The blending of different polysaccharides has gradually become a valuable strategy to produce composite films with synergistic improved properties [15,16]. Suriyatem et al. [17] developed biodegradable rice starch–based films blended with carboxymethyl chitosan and the composite films that incorporated carboxymethyl chitosan exhibited stronger mechanical properties and thermal stability than the pure rice starch film with fragility. Saha et al. [18] prepared and screened a strong and tough composite film based on methylcellulose and pectin at a ratio of 90:10 and sodium montmorillonite was incorporated in this matrix to fabricate nanocomposite films, which could be useful in food packaging and controlled transdermal drug delivery applications.

Methylcellulose is a linear hydrophilic biopolymer prepared by introducing methyl groups to the hydroxyl residues of cellulose backbone [19]. Methylcellulose inherits the interesting properties of cellulose, such as excellent film-making capability, good biodegradability, high viscosity, nontoxicity, and strong mechanical property, but meanwhile shows high solubility in water [18,20]. Thus methylcellulose can be used as a promising biomaterial for active food packaging but limited the lack of effective antioxidant activity [21].

Since chitosan ascorbate and methylcellulose are linear hydrophilic biopolymers with good compatibility, it is reasonably hypothesized that blending chitosan ascorbate and methylcellulose to fabricate binary composite films will compensate for the deficiencies of single component films and exhibit excellent antioxidant activity and satisfactory mechanical property simultaneously compared to single component films. However, to the best of our knowledge, up to now, no study has been reported on the physicochemical properties of chitosan ascorbate/methylcellulose composite films.

In the present work, the objective is to develop antioxidant edible composite films based on chitosan ascorbate and methylcellulose through casting method for potential applications as active food packaging and to evaluate the effect of the different blending ratio of both biopolymers on the various physicochemical (including optical performance, UV-light barrier, water solubility, swelling degree, thermal property, water vapor permeability, mechanical behavior) as well as antioxidant activity (DPPH radical scavenging activity and reducing power) of the fabricated binary composite films.

2. Materials and methods

2.1. Material

The original chitosan is commercially available and was purchased from Golden-Shell Pharmaceutical Co., Ltd. (Zhejiang, China). Its viscosity-average molecular weight (Mn) of about 5.95 × 10^5 Da was determined using an Ubbelohde viscometer at room temperature and its deacetylation degree of about 95.5% was calculated based on the percentage of carbon and nitrogen from elemental analysis (C: 41.28%, N: 7.91%, C/N: 5.22) according to the calculation formula described by Tan et al. [22]. Methylcellulose with viscosity of 4 × 10^4 mPa·s and methylation degree of about 30%, potassium ferricyanide, and ferric chloride were purchased from Aladdin Biochemical Technology co. Ltd. (Shanghai, China). Ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), glycerol, absolute ethanol, and trichloroacetic acid were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Deionized water was used for all experiments. All chemicals were used without further purification.

2.2. Preparation of chitosan ascorbate

Ascorbic acid (3.52 g, 20 mmol) was dissolved in 100 mL of deionized water, then chitosan (1.61 g, 10 mmol of glucosamine) was added slowly and the reaction mixture was stirred vigorously at room temperature away from light in a 250 mL three-necked round-bottomed flask equipped with a mechanical agitator. After 12 h, a light yellow clear solution was obtained and dialyzed against deionized water with a dialysis tubing (molecular weight cutoff, MWCO: 500 Da) for 2 d in dark to eliminate the non-reacted ascorbic acid. The residual solution in dialysis bag was lyophilized to obtain chitosan ascorbate.

2.3. Characterization of chitosan ascorbate

Chemical structures of chitosan ascorbate were characterized by Fourier transform infrared (FTIR) spectra, 1H Nuclear Magnetic Resonance (1H NMR), and 13C Nuclear Magnetic Resonance (13C NMR) spectra. FTIR spectra were obtained on a Nicolet i550 instrument (Thermo, USA) in transmission mode covering the frequency range from 4000 to 400 cm^-1 at a resolution of 4 cm^-1 with an average of 64 scans using the KBr pellet method (about 1 mg sample/99 mg KBr). 1H NMR and 13C NMR spectra were acquired on a Bruker AVIII 500 MHz spectrometer (Bruker Tech. and Serv. Co., Ltd., Switzerland) at room temperature in D2O as the solvent. Chemical shifts were given in ppm and reported relative to solvent peaks. Meanwhile, the integral areas in 1H NMR spectra were used to quantify the substitution degrees (DS) of ascorbate in chitosan ascorbate, and the value was calculated using the following formula:

\[
DS = \int Ha / \int H2
\]

where Ha is the lactone CH proton next to glycol of ascorbate moiety, H2 is the proton at position 2 in the glucosamine unit.

2.4. Preparation of films

2.4.1. Preparation of chitosan ascorbate (CA) film

Chitosan ascorbate (1.0 g) was completely dissolved in 200 mL of deionized water assisted by mechanical stirring in a three-necked round-bottomed flask. Subsequently, glycerol (0.25 mL) was added to above solution and the mixture was allowed to stir gently for 20 min at room temperature. After that, the film forming solution was subjected to centrifugation at 3000 rpm for 1 h to eliminate the hidden air bubbles created during mixing. The bubble-free solution was cast on a glass plate and dried in a convection oven set to 25 ± 0.5 °C in darkness for 3 days. Finally, the obtained CA film was conditioned at 53 ± 1% relative humidity away from light for 10 days at room temperature in a desiccator containing an oversaturated solution of Mg(NO3)2 to prior to testing.

2.4.2. Preparation of methylcellulose (MC) film

Briefly, 1.0 g powder-like methylcellulose was dispersed into 200 mL of hot deionized water with 70 °C and vigorously stirred with a mechanical agitator for 1 h at 70 °C to produce transparent solution. After slowly cooling to room temperature with constant stirring, 0.25 mL of glycerol as a plasticizer was added into the above solution to prepare the film forming solution. MC film was prepared using the film forming solution by casting method as mentioned above.

2.4.3. Preparation of chitosan ascorbate/methylcellulose (CAMC) composite films

First, chitosan ascorbate solution (0.5%, w/v) and methylcellulose solution (0.5%, w/v) were prepared according to the before-mentioned dissolution method in 2.4.1 and 2.4.2, respectively. Afterward, these two transparent solutions with various volume ratios were mixed by vigorously stirring for 0.5 h using a mechanical stirrer. The film forming solution systems were then achieved after addition of glycerol as a plasticizer and mixing them. Finally, CAMC films were prepared by the same method under the condition of CA film. By changing the volume ratios of chitosan ascorbate solution (0.5%, w/v) to methylcellulose solution (0.5%, w/v), including 4:1, 2:1, 1:1, 1:2, and 1:4, a series of CAMC films were fabricated and coded as CAMC1:4, CAMC1:2, CAMC1:1, CAMC2:1, and CAMC4:1, respectively.
2.5. Characterization of films

2.5.1. Color measurement

The surface color of the films was measured and recorded using an automatic colorimeter (SC-80C, Beijing Kang Guang Optical Instrument Co., Ltd., China) with a reference standard white plate \( L^* = 93.49, a^* = -0.25, \) and \( b^* = -0.09 \) for background calibration. CIE-Lab scale, (where \( L^* \), white to black; \( a^* \), red to green; and \( b^* \), yellow to blue), was chosen and measured in triplicate for each treatment. Chroma \( (C^*) \), hue \( (h^*) \), total color difference \( (\Delta E) \), and whiteness index \( (WI) \) were used to compare the differences of color and calculated by Eqs. (2)-(6):

\[
C^* = \left( a^2 + b^2 \right)^{1/2}
\]

\[
h^* = \tan^{-1} \left( \frac{b^*}{a^*} \right) \quad \text{(if } a^* > 0) \tag{2}
\]

\[
h^* = \tan^{-1} \left( \frac{b^*}{a^*} \right) + 180 \quad \text{(if } a^* < 0) \tag{3}
\]

\[
\Delta E = \sqrt{((L^* - L)^2) + ((a^* - a)^2) + ((b^* - b)^2)} \tag{4}
\]

\[
WI = 100 - ((100 - L)/L \times a + b) \tag{5}
\]

where \( L^*, a^*, \) and \( b^* \) are the color parameters of the films.

2.5.2. Light barrier properties and opacity

The permeability of the films to UV–visible was measured using a UV–visible spectrometer (Lambda 265, PerkinElmer). Spectra were recorded at room temperature in steps of 1 nm at wavelengths from 200 to 800 nm with an empty test cuvette used as the blank. The absorbance of the films was recorded by means of a UV–visible spectrometer (T6, General Instrument Co. Ltd., China) at 600 nm and the opacity \( (OP) \) was calculated using Eqs. (7):

\[
OP = \frac{Abs600}{x}
\]

where \( Abs600 \) is the absorbance at 600 nm and \( x \) is the average thickness of film (mm). This test was carried out in triplicate for each sample.

2.5.3. Thermal analysis

The thermal stability of the prepared films was analyzed using a thermogravimetric analyzer (Mettler 5MP, Mettler-Toledo, Switzerland). Each film sample (about 10 mg) was cut into small pieces and heated at a constant rate of 10 °C·min\(^{-1}\) in the temperature range of 25 °C to 600 °C under continuous nitrogen flow of 20 mL·min\(^{-1}\) to obtain thermogravimetric analysis (TGA) and derivative thermogravimetric (DTG) data.

2.5.4. Water content, swelling degree, and water solubility

Film portions of 2 cm \( \times \) 2 cm were cut and weighed \( (W_0) \) in pre-weighed glass Petri dishes. Subsequently, the film specimens were subjected to drying at 75 °C for 24 h in a hot oven to obtain the initial constant dry weights \( (W_1) \). The dried films were then immersed into 30 mL of deionized water for 24 h at room temperature. The wet weights of the films were recorded \( (W_2) \) immediately after pulling the films and gently blotting the surface water with filter paper. Finally, the final dry weights of the residual films were accurately weighed \( (W_3) \) again after drying at 75 °C for 24 h in a hot oven. Measurements were determined in triplicate for each film and water content, swelling degree, and water solubility of the films were calculated using Eqs. (8)-(10), respectively:

\[
\text{Water content (\%)} = \frac{(W_0 - W_1)}{W_0} \times 100 \tag{8}
\]

\[
\text{Swelling degree (\%)} = \frac{(W_2 - W_1)}{W_1} \times 100 \tag{9}
\]

\[
\text{Water solubility (\%)} = \frac{(W_1 - W_3)}{W_1} \times 100 \tag{10}
\]

2.5.5. Water vapor permeability

The water vapor transmission rate (WVTR) and water vapor permeability (WVP) were measured gravimetrically by the dry cup method of ASTM E96-95 with a few modifications [23]. Films with diameter of about 4 cm were sealed on top of the circular test cups containing 18 mL of distilled water. The cups were weighed to get initial mass and stored in a closed desiccator filled with anhydrous silica gel at 25 °C. The difference of partial pressure between both sides of the films generated a driving force for the transport of water vapor through the film, which led to a decrease in the weight of the test cups. Every 12 h, the weight losses of cups were recorded using an analytical balance to the nearest 0.0001 g in duplicate and plotted as a function of time. The slope of each line of weight loss vs. time was calculated by linear regression \((R^2 > 0.99)\). Each measurements was performed in triplicate, WVTR \((\text{g/m}^2\cdot\text{s})\) and WVP \((\text{g} \cdot \text{m}^{-2} \cdot \text{Pa}^{-1} \cdot \text{s})\) of the films were calculated using Eqs. (11) and (12), respectively:

\[
\text{WVTR} = \frac{\text{Slope}}{A}
\]

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

where \( L \) is the mean thickness of film (\( \text{m} \)), \( A \) is the permeation area of the films (\( \text{m}^2 \)), and \( \Delta P \) is the partial water vapor pressure difference (Pa) between the inner and outer film surfaces at 25 °C.

2.5.6. Thickness and density

The film thickness was measured using a digital micrometer (Jingcheng, China) with 0.001 mm precision at 10 random locations in each film, and the mean value was used in subsequent calculations for the volume of the film. Then, the film density (that is mass per unit volume) was calculated from the ratio of weight to volume of the film.

2.5.7. Mechanical properties

A universal tensile tester (Instron 5848 MicroTester, UK) was used to determine the mechanical properties of the films, including tensile strength (TS) and elongation at break (EB). The rectangular strips of films (5 cm \( \times \) 1 cm) were mounted in the tensile grips with a load cell of 100 N working at a cross-head speed of 1 cm/min until breakage at room temperature. Average TS (MPa) and EB (%) were determined from the resulting stress–strain curves. All the mechanical measurements were collected in triplicate and the average values were used for each film.

2.6. Antioxidant assay

The film samples of 1 g were cut into small pieces and immersed in 100 mL of distilled water to obtain the extract after sufficient dissolution at the room environment for 24 h, and the extracts as stock solutions were utilized in the DPPH radical scavenging assay and ferric-reducing antioxidant power assay. The concentrations of these extracts were considered as 10 mg/mL.

2.6.1. DPPH radical scavenging assay

Antioxidant activity of the chitosan based films was firstly expressed as the capacity to scavenge the DPPH radical according to a previous method [22]. A deep purple solution of DPPH (0.18 mM) was prepared by completely dissolving it in absolute ethanol. Meanwhile, the extract solutions of the film samples were diluted to the concentration of 0.30, 0.60, 1.20, 2.40, and 4.80 mg/mL with deionized water. Then, 1 mL of the diluted solution with different concentrations was transferred into a 4 mL of tube containing 2 mL of DPPH ethanol solution. The mixture was shaken mildly and incubated for 20 min at room temperature protected from light. The absorbance at 517 nm of each solution was measured in triplicate to determine the residual amount of DPPH using a UV–visible spectrophotometer. And equal volume (1 mL) of deionized water was used as blank to displace sample. The
DPPH radical scavenging activity was calculated by Eq. (13):

\[ \text{Scavenging effect (\%) = } |1 - (A1 - A2)/A0| \times 100 \]  

(13)

where \( A_0 \), \( A_1 \), and \( A_2 \) are the absorbances of DPPH in the blank group, the sample group, and the sample's background (absolute ethanol instead of DPPH solution), respectively. The concentration required to obtain a 50% antioxidant effect (EC50) was calculated by linear regression.

2.6.2. Ferric-reducing antioxidant power assay

The ferric-reducing antioxidant power of chitosan based films was measured as described previously by W. Tan, Zhang, Mi, et al. [24]. The extract solutions of the film samples were diluted to the concentration of 0.60, 1.20, 2.40, 4.80, and 9.60 mg/mL with deionized water. 1 mL of diluted solution with various concentrations was mixed with 1 mL of potassium ferricyanide solution (1%, \( w/v \)) and reacted at 50 °C for 20 min. Then 1 mL trichloroacetic acid solution (10%, \( w/v \)) was added to stop the reaction. After centrifugation at 3000 rpm for 5 min, a 1.5 mL aliquot of the supernatant was mixed with 1.2 mL of distilled water and 0.3 mL of ferric chloride solution (0.1%, \( w/v \)). The mixture was shaken vigorously and allowed to incubate away from light at room temperature for 10 min and the absorbance was measured at 700 nm on UV-visible spectrophotometer. All experiments were carried out in triplicate. The reducing power could be assessed as follows:

\[ \text{Reducing power} = A1 - A0 \]  

(14)

where \( A_0 \) was the absorbance of the blank and \( A_1 \) was the absorbance of the sample solution.

2.7. Statistical analysis

Data were reported as mean values with standard deviation. A comparison of sample means was performed using one-way analysis of variance (ANOVA) by Duncan's multiple range test. The difference between means was considered to be statistically significant at \( p < 0.05 \).

3. Results and discussion

3.1. Preparation and characterization of chitosan ascorbate

Fig. 1(a) depicts a scheme of developing a one-step process to achieve chitosan ascorbate. The electrostatic interaction as the driving force could facilitate the mutual attraction and ion-combination between alkaline amino group of chitosan molecule and acidic hydroxyl group at C-3 (pKa 4.17) of ascorbic acid, chitosan ascorbate was thus prepared efficiently in water without other organic reagents.

Structures of pristine chitosan and chitosan ascorbate were confirmed by FTIR, \(^1\)H NMR, and \(^{13}\)C NMR. Chitosan shows main infrared absorption peaks located at 3428 cm\(^{-1}\) (the \( \text{NH}^+ \) bending vibration), 1646 cm\(^{-1}\) (the C=O stretching vibration of residual amide bond), 1600 cm\(^{-1}\) (the C–N bending vibration), 1380 cm\(^{-1}\) (the C–N bending vibration), and 1083 cm\(^{-1}\) (the C–O stretching vibration) [24]. The protons of the chitosan show characteristic signals at 3.10 ppm, and 4.00 ppm due to the H2 and H3-H6 protons of the glucosamine unit, respectively [25]. The carbon signals of the chitosan backbone are observed at 177.4 ppm (Cf in the spectrum), 175.1 and 113.3 ppm (\( \text{C} = \text{O} \) and \( \text{C}_2 \) in the spectrum), 78.4 ppm (C1 in the spectrum), along with glycol carbon resonances at 69.6 ppm (Cb in the spectrum) and 62.6 ppm (Cc in the spectrum) in the \(^{13}\)C NMR spectrum of CA (Fig. 1(d)) [28], confirms further introduction of the ascorbate into chitosan molecule by the salt-forming reaction.

3.2. Characterization of films

3.2.1. Optical properties

Color measurement of the films was carried out to determine changes in color after blending chitosan ascorbate and methylcellulose, and the data are shown in Table 1. On the surface, methylcellulose film was almost colorless and transparent, while chitosan ascorbate film had a fawn-colored and transparent appearance. An obvious change in color of the composite films with increasing lightness (\( L^* \) value) and decreases in redness (\( a^* \) value) and yellowness (\( b^* \) value) is observed as the proportion of methylcellulose increases in the matrix. As a result, hue (\( H^* \)) values (79.5 ± 0.3 to 177.5 ± 0.6) and whiteness index (\( W^* \)) values (41.1 ± 0.15 to 60.20 ± 0.03) of the composite films remarkably increase, while chroma (\( C^* \)) values (29.0 ± 0.3 to 2.21 ± 0.06) and total color difference (\( \Delta E \)) values (53.39 ± 0.17 to 33.29 ± 0.02) conversely decrease when the methylcellulose content increases, indicating a more light elegant-like appearance due to the nature and concentration of methylcellulose in the composite films.

The oxidation process of packaged food products, mainly caused by the exposure to ultraviolet and visible light, can bring a series of negative effects such as the deterioration in odor, flavor, color and nutrient losses of food [29]. Hence, it is a desirable strategy to develop active packaging materials with good UV–vis light barrier property to extend the shelf life of food. The UV–vis light transmittances of pure chitosan ascorbate and methylcellulose films together with the composite films prepared by mixing chitosan ascorbate and methylcellulose at different proportions are shown in Fig. 2. As is apparent, a sharp rise in transmittance at about 60% in the wavelength range of 280~800 nm are observed, indicating the lower blocking capacity of methylcellulose ascorbate content could be promising barriers to effectively protect food sensitive to light-induced oxidation reaction.

For foods susceptible to light exposure, opacity of packaging materials is also an important property to safeguard the nutritional quality of food [30]. A higher value of opacity means a less transparency. As presented in Table 2, pure chitosan ascorbate and methylcellulose films show a relatively clear and transparent appearance with opacity values 4.51 ppm (the lactone CH proton next to glycol, \( \text{Ha} \) in the spectrum), 3.99 ppm (the CH proton of glycol groups, \( \text{Hb} \) in the spectrum), 3.71 ppm (the CH2 protons of glycol groups, \( \text{Hc} \) in the spectrum) in the \(^1\)H NMR spectrum of CA (Fig. 1(c)) [27], which indicate the presence of a ascorbate fragment in the side chains of chitosan. DS of ascorbate is calculated to be 0.80 using \(^1\)H NMR integration by Eq. (1). Additionally, the emergence of characteristic resonances for lactone carbons at 177.4 ppm (Cf in the spectrum), 175.1 and 113.3 ppm (\( \text{Cd} \) and \( \text{Ce} \) in the spectrum), 78.4 ppm (C1 in the spectrum), along with glycol carbon resonances at 69.6 ppm (Cb in the spectrum) and 62.6 ppm (Cc in the spectrum) in the \(^{13}\)C NMR spectrum of CA (Fig. 1(d)) [28], confirms further introduction of the ascorbate into chitosan molecule by the salt-forming reaction.
of no >1.50 Å₆₀₀/mm. However, a significant tendency to be more opaque for the composite films is observed once the mixture of both is finished, and the maximal value of opacity even reaches 5.5 ± 0.7 Å₆₀₀/mm at a chitosan ascorbate/methylcellulose ratio of 4:1. As a whole, this increase in opacity of the composite films was probably due to a certain chemical binding effect of chitosan ascorbate and methylcellulose in the matrix. Meanwhile, there is a gradual increase of the transparency as the content of methylcellulose in the mixture increases.

Table 1

<table>
<thead>
<tr>
<th>Film</th>
<th>L</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>h</th>
<th>ΔE</th>
<th>WI</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>48.74 ± 0.13°</td>
<td>5.30 ± 0.19°</td>
<td>28.5 ± 0.3°</td>
<td>29.0 ± 0.3°</td>
<td>79.5 ± 0.3°</td>
<td>53.39 ± 0.17°</td>
<td>41.11 ± 0.15°</td>
</tr>
<tr>
<td>CA₄MC</td>
<td>53.3 ± 0.3°</td>
<td>4.32 ± 0.15°</td>
<td>26.71 ± 0.22°</td>
<td>27.05 ± 0.20°</td>
<td>80.8 ± 0.4°</td>
<td>48.49 ± 0.21°</td>
<td>46.06 ± 0.23°</td>
</tr>
<tr>
<td>CA₅MC</td>
<td>54.98 ± 0.13°</td>
<td>2.83 ± 0.21°</td>
<td>23.5 ± 0.3°</td>
<td>23.63 ± 0.24°</td>
<td>83.7 ± 0.5°</td>
<td>45.84 ± 0.25°</td>
<td>49.16 ± 0.24°</td>
</tr>
<tr>
<td>CA₅MC</td>
<td>55.9 ± 0.3°</td>
<td>1.01 ± 0.05°</td>
<td>19.67 ± 0.13°</td>
<td>19.70 ± 0.13°</td>
<td>87.07 ± 0.17°</td>
<td>42.49 ± 0.21°</td>
<td>51.70 ± 0.22°</td>
</tr>
<tr>
<td>CA₅MC</td>
<td>57.3 ± 0.4°</td>
<td>0.65 ± 0.13°</td>
<td>18.75 ± 0.19°</td>
<td>18.76 ± 0.19°</td>
<td>88.0 ± 0.4°</td>
<td>40.8 ± 0.4°</td>
<td>53.4 ± 0.4°</td>
</tr>
<tr>
<td>CA₅MC</td>
<td>58.34 ± 0.16°</td>
<td>-1.41 ± 0.12°</td>
<td>12.6 ± 0.4°</td>
<td>12.6 ± 0.4°</td>
<td>96.4 ± 0.5°</td>
<td>37.37 ± 0.03°</td>
<td>56.47 ± 0.05°</td>
</tr>
<tr>
<td>MC</td>
<td>60.26 ± 0.03°</td>
<td>-2.20 ± 0.06°</td>
<td>0.10 ± 0.02°</td>
<td>2.21 ± 0.06°</td>
<td>177.5 ± 0.6°</td>
<td>33.29 ± 0.02°</td>
<td>60.20 ± 0.01°</td>
</tr>
</tbody>
</table>

Different letters within the same column indicate significant differences among films (p < 0.05). CA, chitosan ascorbate; MC, methylcellulose; CA₄MC₅ MC, chitosan ascorbate/methylcellulose composite films.

* Values are given as mean ± standard deviation (SD).
3.2.2. Thickness, density, and mechanical properties

Some physicochemical properties of films including thickness, density, and mechanical property are summarized in Table 2 and Fig. 3 respectively. The thickness of chitosan ascorbate film is measured as $54 \pm 7 \mu m$. Compared with pure chitosan ascorbate film, a decrease in thickness is observed for the composite films prepared from the mixing of chitosan ascorbate and methylcellulose, with values varied from $48 \pm 4$ to $41 \pm 4 \mu m$ as the methylcellulose increases in the mixture. This decrease in the thickness might be due to the strong inter-and intra-molecular hydrogen bonding interactions of methylcellulose and chitosan ascorbate, which led to a compact internal structure. On the basis of this, the effect of the methylcellulose ratio on the density of films marks a complete opposite trend to that on the thickness. According to the testing results, the density of films increases when the methylcellulose content increases.

Tensile strength (TS) and elongation at break (EB) are the major indicators to evaluate the mechanical strength and flexibility of food packaging films [31]. Average values of TS and EB of pure chitosan ascorbate and methylcellulose films and composite films are shown in Table 2 and the characteristic stress-strain curves of these films are illustrated in Fig. 3. The TS and EB values of chitosan ascorbate film are $17 \pm 2 \text{ MPa}$ and $8.0 \pm 1.8\%$, respectively. The breaking of the salt-forming reaction of chitosan with ascorbic acid to crystalline structure as well as hydrogen-bond interaction of chitosan matrix might give a stiff and rather brittle morphology of chitosan ascorbate film [14]. Inversely, methylcellulose film exhibits a strong mechanical property with average TS and EB values of $55 \pm 6 \text{ MPa}$ and $36 \pm 3\%$. However, addition of a little methylcellulose into the chitosan ascorbate matrix to prepare CAxMC film causes a decrease in the TS value, which might be ascribed to the stronger destructive force from methylcellulose for the internal structure of chitosan ascorbate film than the hydrogen-bond binding force of both. Gratifyingly, the values of TS of the composite films in this study all significantly increase as methylcellulose level further increases. The enhanced TS for the composite films might be on account of the entanglements and physical-crossing of methylcellulose and chitosan ascorbate by inter-and intra-molecular hydrogen bonding interactions, which increased the density of the structure (shown in Table 2) and formed a stronger and more compact internal network. Meanwhile, the increase in EB values is observed due to the increased amount of methylcellulose in the composite films. Similar tendency was observed by Arif Kilbar and Us [31], where the TS and EB values of methylcellulose–starch blend films simultaneously increased as the methylcellulose level increased. It was inferred that the increase of the moisture content in the polymeric matrix facilitated the mobility and the flexibility of polymeric chains for the films containing a greater proportion of methylcellulose (shown in Table 3), based on a plasticizing effect of water molecules similar to glycerol [29,32].

3.2.3. Thermal analysis

The thermal stability of chitosan ascorbate, methylcellulose, and the composite films was evaluated using thermogravimetric analysis, and the resulting TGA and DTG curves are given in Fig. 4. Three stages of thermal decomposition in heating temperatures ranging of 25–600 °C are recorded in these thermograms and similar behaviors are observed for all the films. Initial weight loss at around 30–100 °C with <5 wt% degradation corresponds to the evaporation of unbound water [33]. The subsequent thermal decomposition occurs at about 100–225 °C. It was related to the degradation of glycerol used as a plasticizer in the film matrix [34]. The major decomposition of all films is observed at

![Fig. 2. UV–vis spectra of chitosan ascorbate/methylcellulose composite films. CA, chitosan ascorbate; MC, methylcellulose; CAxMCy, chitosan ascorbate/methylcellulose composite films.](image1)

![Fig. 3. Stress-strain curves of chitosan ascorbate/methylcellulose composite films. CA, chitosan ascorbate; MC, methylcellulose; CAxMCy, chitosan ascorbate/methylcellulose composite films.](image2)

<table>
<thead>
<tr>
<th>Film</th>
<th>Thickness ($\mu m$)</th>
<th>Opacity ($A_{500/\mu m}$)</th>
<th>Density (g/cm$^3$)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>$54 \pm 7$</td>
<td>$1.38 \pm 0.03$</td>
<td>$0.91 \pm 0.02$</td>
<td>$17 \pm 2^b$</td>
<td>$8.0 \pm 1.8^b$</td>
</tr>
<tr>
<td>CAxMC</td>
<td>$48 \pm 6^b$</td>
<td>$5.5 \pm 0.7^*$</td>
<td>$0.96 \pm 0.02^*$</td>
<td>$14 \pm 3^e$</td>
<td>$10 \pm 3^e$</td>
</tr>
<tr>
<td>CAMC</td>
<td>$49 \pm 6^b$</td>
<td>$4.96 \pm 0.19^b$</td>
<td>$0.99 \pm 0.01^b$</td>
<td>$18 \pm 4^e$</td>
<td>$15.3 \pm 0.8^e$</td>
</tr>
<tr>
<td>CAMC2</td>
<td>$48 \pm 5^b$</td>
<td>$3.54 \pm 0.17^e$</td>
<td>$1.04 \pm 0.03^b$</td>
<td>$23 \pm 6^d$</td>
<td>$18.2 \pm 1.3^d$</td>
</tr>
<tr>
<td>CAMC4</td>
<td>$44 \pm 5^*$</td>
<td>$2.59 \pm 0.08^d$</td>
<td>$1.04 \pm 0.01^b$</td>
<td>$35 \pm 3^e$</td>
<td>$24.4 \pm 1.8^d$</td>
</tr>
<tr>
<td>CAMC4</td>
<td>$41 \pm 4^d$</td>
<td>$2.49 \pm 0.15^d$</td>
<td>$1.09 \pm 0.01^b$</td>
<td>$50 \pm 5^e$</td>
<td>$27 \pm 4^d$</td>
</tr>
<tr>
<td>MC</td>
<td>$41 \pm 4^d$</td>
<td>$1.50 \pm 0.02$</td>
<td>$1.12 \pm 0.01^b$</td>
<td>$55 \pm 6^e$</td>
<td>$36 \pm 3^e$</td>
</tr>
</tbody>
</table>

* Values are given as mean ± standard deviation (SD). Different letters in the same column indicate significantly different $(p < .05)$. CA, chitosan ascorbate; MC, methylcellulose; CAxMCy, chitosan ascorbate/methylcellulose composite films.
around 225–450 °C, which was associated with the thermal degradation of chitosan and methylcellulose backbones. The maximum decomposition temperatures (DTG_{max}) of the composite films observed at around 376–385 °C, similar to that of pure methylcellulose film at 376 °C, are evidently above that of chitosan ascorbate film at 287 °C, but no significant influence (p > .05) of the chitosan ascorbate/methylcellulose ratio on DTG_{max} of the composite films is found. A more compact structure obtained by the hydrogen-bond binding interaction between two polysaccharides might result in an increase resistance of the composite films to thermal decomposition compared with chitosan ascorbate film. Additionally, it is interesting to note that there seems to be a positive correlation between methylcellulose concentration and the weight losses of the composite films in the third stage from TGA and DSC curves. The relatively low amount of char content in the formulations with the higher methylcellulose content was possibly due to the low content of flame retardant minerals in methylcellulose component used for the preparation of composite films.

3.2.4. Water content, water solubility, swelling degree, and water vapor permeability

Water content, water solubility, and swelling degree of the neat chitosan ascorbate, methylcellulose, and the composite films are shown in Table 3. The water content of chitosan ascorbate film is 13.6 ± 0.8%, but it increases gradually until 27.3 ± 2.6% with a higher methylcellulose concentration. This increase in water content could be attributed to the hygroscopic character of methylcellulose [19]. Films were subject to the swelling and matrix dissolution when immersed in water at room temperature. Methylcellulose film could be in whole to dissolve in water with 100% water solubility, which led to fact that it was hard to evaluate the swelling degrees of the composite films with high methylcellulose ratios. But the addition of methylcellulose into the polymeric matrix promotes an increase in swelling degree values at the ratio of methylcellulose/chitosan ascorbate from 1:4 to 1:1. Besides, a trend toward an increase on water solubility with the increasing on the methylcellulose concentration is also detected. The results might be explained by the higher hydrophilic character of methylcellulose, which could cause the higher water binding capacity, and the disruption of the intra- or inter-molecular hydrogen bond networks. The results suggested that these composite films with a high rate of water solubility and swelling degree can be removed from food easily after washing with water, which makes it promising applicable packaging biomaterial for the water-fast food.

Water vapor permeability of the prepared films was affected by the ratio of chitosan ascorbate to methylcellulose in the matrix. The addition of methylcellulose enhances the water vapor barrier properties of the composite films with the lowered WVP values. This might be because the great moisture retention of methylcellulose in the polymeric matrix led to greater resistance to the diffusion of the water molecules. With the increase of methylcellulose, the WVP values of the composite films gradually increase until the maximum WVP value (3.11 ± 0.14 × 10^{-19} g·m/m²·Pa·s) is obtained at the ratio of methylcellulose/chitosan ascorbate of 1:1, which was probably due to the increase of the polymer chains mobility and the void spaces between polymer chains. But when the concentration of methylcellulose is increased further, the decrease in the WVP values is then detected. This decrease could be explained by the formation of a more compact network structure, and there was little room for water molecules to pass through.

3.3. Antioxidant activity

The DPPH radical scavenging assay and the reducing power assay were performed to evaluate the antioxidant effects of the films, and the results are presented in Fig. 5. And the EC_{50} value (shown in Table 3), usually defined as the concentration of antioxidant with 50% scavenging rate against free radicals, was also used as a scavenging performance indicator against DPPH radical.

No significant antioxidant property is observed in the neat methylcellulose film with the DPPH scavenging ratio of close to 0% and the

![Fig. 4. TGA and DTG curves of chitosan ascorbate/methylcellulose composite films. CA, chitosan ascorbate; MC, methylcellulose; CAxMC, chitosan ascorbate/methylcellulose composite films.](image)

Table 3

<table>
<thead>
<tr>
<th>Films</th>
<th>Water content (%)</th>
<th>Water solubility (%)</th>
<th>Swelling degree (%)</th>
<th>Water vapor permeability (×10^{-19} g·m/m²·Pa·s)</th>
<th>EC_{50} (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>13.6 ± 0.8</td>
<td>35.3 ± 1.5</td>
<td>15 ± 4</td>
<td>3.34 ± 0.09</td>
<td>-0.100</td>
</tr>
<tr>
<td>CAxMC</td>
<td>18.4 ± 1.2</td>
<td>31.1 ± 1.2</td>
<td>35.3 ± 1.4</td>
<td>2.89 ± 0.12</td>
<td>0.215 ± 0.005</td>
</tr>
<tr>
<td>CAxMC</td>
<td>19.2 ± 2.1</td>
<td>45 ± 3</td>
<td>43 ± 5</td>
<td>3.02 ± 0.10</td>
<td>0.299 ± 0.014</td>
</tr>
<tr>
<td>CAMC</td>
<td>21.5 ± 0.8</td>
<td>53 ± 3</td>
<td>58 ± 7</td>
<td>3.11 ± 0.14</td>
<td>0.617 ± 0.023</td>
</tr>
<tr>
<td>CAMC2</td>
<td>21.9 ± 1.7</td>
<td>61 ± 5</td>
<td>/</td>
<td>2.93 ± 0.12</td>
<td>1.30 ± 0.04</td>
</tr>
<tr>
<td>CAMC4</td>
<td>24.1 ± 1.5</td>
<td>73 ± 8</td>
<td>/</td>
<td>2.85 ± 0.21</td>
<td>1.443 ± 0.026</td>
</tr>
<tr>
<td>C</td>
<td>27.3 ± 2.6</td>
<td>100</td>
<td>/</td>
<td>2.78 ± 0.10</td>
<td>1.60a</td>
</tr>
<tr>
<td>MC</td>
<td>27.3 ± 2.6</td>
<td>100</td>
<td>/</td>
<td>2.78 ± 0.10</td>
<td>1.60a</td>
</tr>
</tbody>
</table>

Different letters in the same column indicate significantly different (p < .05). CA, chitosan ascorbate; MC, methylcellulose; CAxMC, chitosan ascorbate/methylcellulose composite films. * Values are given as mean ± standard deviation (SD).
negligible reducing power. But the chitosan ascorbate film exhibits the remarkable DPPH radical scavenging activity with scavenging values of 83.56 ± 2.17% at the minimum testing concentration of 0.10 mg/mL and EC50 value of below 0.10 mg/mL. Meanwhile, methylcellulose as film is also observed to possess the strong reducing power with the absorbance value at 700 nm of 4.23 ± 0.20 at the maximum testing concentration of 1.60 mg/mL. It has been reported that ascorbate could be allowed to act as a one-electron donor to damage active oxidizing radicals by a reversible and thermodynamically favorable oxidation, and thus empowered chitosan ascorbate film with the strong antioxidant ability [35]. After the mixing of methylcellulose and chitosan ascorbate, the DPPH radical scavenging effect and reducing power of the composite films show a noticeable concentration-dependent manner within the tested dosage and the highest scavenging rate and absorbance value at 700 nm are reached at 1.60 mg/mL. A continuously enhanced tendency in the DPPH radical scavenging ability is found for CAMC4, CAMC, CAMC2, CAMC, CA2MC, and CA4MC with the decreased EC50 values of 1.443 ± 0.026, 1.30 ± 0.04, 0.617 ± 0.023, 0.299 ± 0.014, and 0.215 ± 0.005 mg/mL, respectively. It can be seen that the DPPH radical scavenging ability increases significantly (p < .05) with increasing ratio of chitosan ascorbate in the matrix. Meanwhile, a same trend is observed in the reducing power assay. It can be seen from Fig. 5 (b) that the reducing power of five composite films increases as follows: CAMC4 < CAMC2 < CAMC < CA2MC < CA4MC, which suggested that a more remarkable reducing power could be obtained with increasing amount of chitosan ascorbate and further proved the main function of ascorbate in the antioxidant power of the composite films.

4. Conclusions

In the present study, antioxidant composite films were fabricated based on the blending of chitosan ascorbate and methylcellulose at different ratios. The chitosan ascorbate/methylcellulose ratios in the matrix showed great influence on the physicochemical properties and antioxidant activities of the composite films. A great proportion of methylcellulose in the polymer formulations increased the mechanical property, thermal stability, whistish index, water solubility, and moisture barrier properties of the composite films. Meanwhile, the increase in chitosan ascorbate concentration contributed to a more valuable film with more powerful UV–vis light absorption capacity and the stronger antioxidant activity due to the strong electron donating capability of ascorbate moiety with a conjugated PI electron system. Taken together, it could be seen that the blending of chitosan ascorbate and methylcellulose might achieve more integration and advantage complementation of the structural compactness of methylcellulose and the superior oxidation resistance property of chitosan ascorbate. This study provides an important insight for the combining of chitosan ascorbate and methylcellulose as blends to create new promising biomaterials used in the field of food packaging.

CRediT authorship contribution statement

Wenqiang Tan: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft Jingjing Zhang: Investigation, Validation. Xiang Zhao: Investigation, Validation. Qing Li: Data curation, Formal analysis, Writing - review & editing. Fang Dong: Conceptualization, Writing - review & editing. Zhaoyang Guo: Supervision, Writing - review & editing, Project administration.

Acknowledgments

The authors thank the financial support from the Natural Science Foundation of Shandong Province of China (ZR2019BD064 and ZR2017BD015), the National Key R&D Program of China (2019YFD0900705), the National Natural Science Foundation of China (41576156), and the Natural Science Foundation of Shandong Province Science and Technology Development Plan (2019GHY112010).

References


