

Article

Preparation and Characterization of Functional Films Based on Chitosan and Corn Starch Incorporated Tea Polyphenols

Lepeng Gao ¹ , Tao Zhu ¹, Fuyang He ¹, Zhiqiang Ou ¹, Jian Xu ^{1,2} and Lili Ren ^{1,2,*} 

¹ College of Biological and Agricultural Engineering, Jilin University, Changchun 130022, China; gaolp1818@mails.jlu.edu.cn (L.G.); zhutao1818@mails.jlu.edu.cn (T.Z.); hefy1818@mails.jlu.edu.cn (F.H.); ouzq1818@mails.jlu.edu.cn (Z.O.); xujian19@mails.jlu.edu.cn (J.X.)

² Key Laboratory of Bionic Engineering, Ministry of Education, Jilin University, Changchun 130022, China

* Correspondence: liliren@jlu.edu.cn; Tel.: +86-431-8509-5760 (ext. 613); Fax: +86-431-8509-5760 (ext. 888)

Abstract: The functional films based on chitosan and corn starch incorporated tea polyphenols were developed through mixing the chitosan and starch solution and the powder of tea polyphenols by the casting method. The objective of this research was to investigate the effect of different concentrations of tea polyphenols on the functional properties of the films. Attenuated total reflectance Fourier transform infrared spectrometry and X-ray diffraction were used to investigate the potential interactions among chitosan, corn starch and tea polyphenols in the blend films. Physical properties of the blend films, including density, moisture content, opacity, color, water solubility and water swelling, as well as morphological characteristics, were measured. The results demonstrated that the incorporation of tea polyphenols caused the blend films to lead to a darker appearance. The water solubility of the blend film increased with the increase of tea polyphenol concentrations, while moisture content and swelling degree decreased. The hydrogen bonding between chitosan, starch and tea polyphenols restricted the movement of molecular chains and was helpful to the stability of the blend films. The results suggested that these biodegradable blend films could potentially be used as packaging films for the food and drug industries to extend the shelf life to maintain their quality and safety.

Keywords: corn starch; chitosan; tea polyphenols; physical properties



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1. Introduction

In recent decades, with the rapid development of society, people have paid more and more attention to food quality and safety, and food packaging also ushered in great changes. The original food packaging materials are mainly petroleum-based plastics, and although widely used because of their good processing performance and excellent physical and chemical properties, their harm to the human body and environment can not be ignored, because they are not easy to degrade, and a large number of plastic particles will be produced in the process of manufacturing and circulation, which will cause destruction to the human body and environment. Therefore, the research and development of new, green, environmentally friendly, safe and degradable packaging materials have become a hot spot in the field of food materials. Due to the degradability and green safety of the natural biomass matrix, more directions are provided for the development and preparation of new active packaging materials, gradually attracting the attention of researchers [1].

Active packaging can extend the shelf life of food and improve the safety and sensory characteristics of food. The main active packaging systems can be divided into absorption and release systems, including water or liquid absorption, oxygen removal, carbon dioxide emission/absorption, ethylene removal/emission, odor and odor absorption or release, and antibacterial system. Antibacterial packaging is a very valuable research direction of active packaging. Adding antibacterial agents or using antibacterial polymers in the packaging system can affect the growth of microorganisms, which can kill or inhibit the

harmful microorganisms that pollute food, thus having a significant impact on the shelf life and quality of food [2].

Chitosan is one of the most abundant polysaccharides in nature next to cellulose. It is a derivative of chitin deacetylation and mainly comes from shellfish-processing waste. Chitosan has good physical and chemical properties, such as film-forming, moisture retention, moisture absorption and adsorption [3]. Different from other bio-based food packaging materials, chitosan has many physical and chemical properties that are harmless to the human body, such as non-toxic *in vivo* and *in vitro*, biodegradable, biocompatible, antibacterial activity and so on, which greatly promotes its application and development in the research field [4]. At the same time, it has the advantage of combining functional substances [5]. Functional substances can be added into chitosan-based films to expand the physical and chemical properties of chitosan. Many studies have proved that chitosan-based films and coatings are effective in food preservation [6]. However, chitosan has some limitations, such as low mechanical strength, thermal stability, water and gas barrier properties, limiting its use as degradable food packaging materials. Although chitosan is a promising active food packaging material, it has no significant antioxidant activity. Improving the antioxidant activity of chitosan can expand its application in active food packaging.

Starch is one of the most abundant natural polymers with wide sources and low prices. It has become a promising food packaging and preservation material because of its renewable, biodegradable, edible and low cost [7]. Starch can be processed by the existing plastic processing technology, the method is in the presence of water or other plasticizers, through mechanical energy and thermal energy to destroy the starch granule structure. Although starch-based materials are known to have excellent oxygen barrier properties [8], their applications are limited due to their poor moisture resistance, brittleness and low tensile strength. A common way to overcome these shortcomings and provide further functional properties is to mix starch with other natural biopolymers to form composites [9]. Chitosan has been previously added into starch matrices to prepare starch-based active packaging displaying antimicrobial properties. The effect of chitosan concentration on physicochemical, mechanical and water vapor barrier properties as well as morphological characteristics of the corn starch/chitosan films was comprehensively studied [2].

Tea polyphenols are extracted from tea, containing catechins, flavonoids, anthocyanins and phenolic acids, but mainly catechins, whose content is more than 80% [10]. The results showed that tea polyphenols had antibacterial and antioxidant activities, and had a good prospect of antiseptic and antioxidant applications [11]. It has been shown that tea polyphenols could prolong the shelf life of fresh mutton and fresh-cut lettuce [12]. The antioxidant mechanism of polyphenols is mainly attributed to its ability to capture reactive oxygen species and chelate metal ions, which generate free radicals through the Fenton reaction [13].

The research proves that chitosan is one of the main raw materials for the packaging of active food. By adding plant extracts [14], green tea extracts [15], rosemary essential oil [16], *Zataria multiflora* Boiss essential oil and grape seed extract [17], the antioxidant activity of chitosan can be improved. Tea polyphenols are good natural antioxidants, which can be used as additives in chitosan film or coating, so as to expand the antioxidant activity of chitosan film and its application in active packaging. Therefore, the purpose of this study is to prepare environmentally friendly functional chitosan and starch film with different tea polyphenol concentrations as raw materials. The physical properties, including attenuated total reflection Fourier transform infrared spectroscopy, density, water content, opacity, color, water solubility, crystalline structure and morphology of corn starch/chitosan/tea polyphenols (CS/CH/TP) films with different tea polyphenol concentrations were measured.

2. Materials and Methods

2.1. Materials

Chitosan with degree of deacetylation of 80–95%, molar mass of 161.16 kDa and viscosity of 51 mPa × s (The viscosity was measured by dissolving 10 g of chitosan in 1 g/mL glacial acetic acid solution at 20 °C according to GB 29941-2013) was supplied by Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Tea polyphenols were supplied by Anhui Red Star Pharmaceutical Co. Ltd. (Xuancheng, China). The tea polyphenols used in this study were yellow or brown powder, purity was ≥80%, caffeine was ≤5% and ash was ≤3%. Catechins in the tea polyphenols were ≥50% and Epigallocatechin gallate (EGCG) in the catechins was ≥30%. Corn starch was supplied by Changchun Jincheng Corn Development Co. Ltd., Da Cheng Group (Changchun, China). Its moisture content was 12.8% and the amylose/amylopectin ratio was 28/72. Glycerol and acetic acid (36%) were obtained from Beijing Beihua Fine Chemicals Co. Ltd. (Beijing, China). All these materials were used 106 as received without further purification.

2.2. Preparation of Films

Chitosan was dissolved in an aqueous solution of acetic acid at a concentration of 2% (*v/v*) to form a chitosan solution (2 g/100 mL) and stirred at 60 °C at 800 rpm. Starch solution (5 g/100 mL) was prepared by dispersing corn starch in deionized water at 95 °C and stirring at 600 rpm for 60 min to accomplish a complete starch gelatinization. Glycerol was added to the chitosan solution and starch solution as a plasticizer at a concentration of 20 wt% dry weight of chitosan and starch, respectively. Then tea polyphenols were added into chitosan solution at concentrations of 0.5%, 1%, 2%, 3% dry weight of chitosan and starch at 60 °C, respectively with stirring at 200 rpm for 10 min. The blend film solutions were prepared by mixing the chitosan/tea polyphenols solution and starch/tea polyphenols solution in 1:1 weight ratio. After stirring at 600 rpm for 60 min at 60 °C and degassed, 40 g of blend film solution was dispensed into glass Petri dishes with a diameter of 60 mm for casting and dried at 60 °C for 8 h. The stripped films were kept in a chamber at room temperature and 75% relative humidity for 48 h prior to experimental use.

2.3. Characterization

2.3.1. Attenuated Total Reflectance Fourier Transform Infrared

Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectra of the obtained blend films were measured using a Nexus 670 FTIR spectrometer with ATR accessories with Ge crystals perform (Nicolet Instrument Corporation, Madison, GA, USA) to study the interaction of chitosan, starch and tea polyphenols in the films. The measurement probe was directly in contact with the surface of the films, and a spectral resolution of 4 cm⁻¹ was used, and 64 scans were acquired for each spectrum in the range from 4000 to 500 cm⁻¹.

2.3.2. Thickness

The thickness of the film is measured by a hand-held digital micrometer (Mitutoyo Absolute, Tester Sangyo Co. Ltd., Tokyo, Japan) with a resolution of 0.001 mm at five different positions of each sample, and whichever is selected average value. These values are used for density and opacity.

2.3.3. Density, Moisture Content and Opacity

Film density was determined from the specimen weight and volume. The specimen volume was calculated from specimen area and thickness. The specimen thickness was measured by using a hand-held digital micrometer (Mitutoyo Absolute, Tester Sangyo Co. Ltd., Tokyo, Japan) with a precision of 0.001 mm at five different positions in each specimen and the average values were taken.

Moisture content of the blend film was determined by measuring weight loss of films upon drying in an oven at 105 °C for 24 h. All the tests were conducted in triplicate

and the means were reported. The moisture content was calculated according to the following equation:

$$\text{Moisture content(\%)} = \frac{M_w - M_d}{M_w} \times 100\% \quad (1)$$

where M_w is the weight of the film adjusted to moisture equilibrium at 75% RH and M_d is the dry weight of the film.

Opacity was determined according to the method of Park, Je, and Kim (2004) [18], and the absorbance of the blend film at 600 nm was measured with a UV spectrophotometer (Ruili Analytical Instruments, Beijing, China). The films were cut into rectangular pieces and placed directly into the spectrophotometer test chamber. An empty test chamber was used as a reference. The opacity of the film was calculated by the following equation:

$$O = \frac{\text{Abs}_{600}}{d} \quad (2)$$

where O is the opacity, Abs_{600} is the absorbance value at 600 nm, and d is the film thickness (mm).

2.3.4. Color Properties

The color of the films was evaluated using HunterLab ColorFlex (New Union Electronics Co., Ltd., Shanghai, China). The color values of L^* (luminosity), a^* (negative green; positive yellow) and b^* (negative blue; positive yellow) were measured. The standard plate CX 2064 was used as the standard. The total color difference (ΔE^*) and chromaticity (C^*) were calculated as follows:

$$\Delta E = \sqrt{\Delta a^2 + \Delta b^2 + \Delta L^2} \quad (3)$$

$$C = \sqrt{a^2 + b^2} \quad (4)$$

where $\Delta L = L^*_{\text{standard}} - L^*_{\text{sample}}$, $\Delta a = a^*_{\text{standard}} - a^*_{\text{sample}}$, $\Delta b = b^*_{\text{standard}} - b^*_{\text{sample}}$. The values of the color parameters of the standard plate are $L^* = 94.52$, $a^* = -0.86$, and $b^* = 0.68$. Each film was measured five times, once at the center and four times at the periphery.

2.3.5. Water Solubility

The water solubility (WS) of the blend film was defined as the percentage of dry matter of the specimen dissolved after 24 h of immersion in distilled water according to the method used by Khoshgozaran-Abras, Azizi, Hamidy, and Bagheripour-Fallah (2012) [12]. The blend films previously equilibrated at 0% RH were cut into 1 cm × 4 cm strips and the initial dry weight (W_i) was determined. Then the strips were immersed in 50 mL of distilled water and stirred at 100 rpm. After 24 h, the strips were removed and dried at 105 °C for 24 h to determine the final dry weight (W_f). All tests were performed in triplicate and the mean values were reported. WS was calculated according to the following equation:

$$\text{WS(\%)} = \frac{W_i - W_f}{W_i} \times 100 \quad (5)$$

where W_i was the initial dry weight of sample, W_f was the final dry weight of sample.

2.3.6. Swelling Degree in Water

The swelling degree (SD) of the blend film in water was determined by the method described in the reference [19]. The thin film specimens (1 cm × 4 cm samples) were weighed (W_0) and placed in a Petri dish, and 30 mL of distilled water was added, then the specimens were removed after 24 h. The water was gently wiped from the surface of the specimens with filter paper and finally weighed (W_t). Three parallel samples were used for

the experiment, and the results were averaged. The formula for the degree of swelling (%) was calculated as follows:

$$SD(\%) = \frac{W_t - W_0}{W_0} \times 100\% \quad (6)$$

where W_t is the mass of the specimen after swelling, W_0 is the mass of the specimen before swelling.

2.3.7. X-ray Diffraction

The specimens were analyzed by X-ray diffraction (XRD) using a D/max-2500 X-ray diffractometer (Rigaku Corporation, Tokyo, Japan). Cu-K α rays ($\lambda = 1.542 \text{ \AA}$) were used at 40 kV and 250 mA current, with a 2θ scan range of $3\text{--}50^\circ$ and a scan rate of $2^\circ/\text{min}$.

2.3.8. SEM

The surface morphology of the blend film was studied by scanning electron microscope (SEM) using a scanning electron microscope (Zeiss Evo 18 SEM, Jena, Germany). A thin layer of gold was plated on the surface of thin film by the gold sputtering method, and then was observed and photographed.

2.3.9. Statistical Analysis

The difference between factors and levels was evaluated by the analysis of variance (ANOVA). Duncan's multiple range tests were used to compare the means to identify which groups were significantly different from other groups ($p < 0.05$). All data are presented as mean \pm standard deviation.

3. Results and Discussion

3.1. ATR-FTIR Analysis

ATR-FTIR is a very powerful tool for detecting possible interactions between film components. When starch, chitosan and tea polyphenols were mixed together, the characteristic peak spectrum could reflect the effect of physical and chemical interaction. The ATR-FTIR spectra of the CS/CH/TP film are shown in Figure 1. In the spectrum of corn starch, the characteristic peak at 1630 cm^{-1} was due to the presence of bound water, the characteristic peak at 3420 cm^{-1} was due to the presence of hydroxyl groups (O-H), and the characteristic peak at 1540 cm^{-1} was due to the C-O in the C-O-H groups caused by stretching vibration. The peak at 1470 cm^{-1} was due to the stretching vibration of C-O in the group. In the chitosan spectroscopy experiment, due to the stretching vibration of the N-H and hydrogen-bonded carboxyl groups, there was a wide frequency band in the range $3800\text{--}3500 \text{ cm}^{-1}$. The peak at 1630 cm^{-1} in the spectrum of starch was related to the presence of bound water, while the peak located at 1650 cm^{-1} of chitosan was associated with amide-I stretch and the peak of N-H bending located at 1590 cm^{-1} [20,21]. Absorption in the range of $1680\text{--}1480 \text{ cm}^{-1}$ was related to the vibrations of carbonyl bonds (C=O) of the amide group CONHR and to the vibrations of protonated amine group [20]. Bending vibrations of methylene and methyl groups were also visible at the peaks of 1380 cm^{-1} and 1420 cm^{-1} , respectively [20,22].

When tea polyphenols, chitosan, starch and glycerol are blended, compared to the spectra of pure chitosan film and starch-only film, as shown in Figure 1, it can be seen that the amino peak of the chitosan at 1590 cm^{-1} shifts to 1550 cm^{-1} in the blend film. The N-H peak in chitosan at 1650 cm^{-1} and -OH in starch at 1630 cm^{-1} moved to 1640 cm^{-1} . The characteristic peak of chitosan at 3390 cm^{-1} and starch at 3420 cm^{-1} shifted to a lower frequency, and the peak of the hydrogen-bonded carboxyl group becomes a strong absorption peak at 3290 cm^{-1} . These shifts indicated that the formation of inter- and intramolecular hydrogen bonding had taken place between chitosan, starch, glycerol and tea polyphenols [23–25]. which were consistent with the results of Shao et al [26]. In this work, the strong absorption peak of CS/CH/TP film at 3290 cm^{-1} had a larger and wide wavenumber than that at 3270 cm^{-1} of CS/TP film, and the wavenumber of the N-H

bending peak at 1550 cm^{-1} was also greater than that of CS/TP film without chitosan [27]. These indicated that chitosan affected the structure of the composite membrane, causing these two characteristic peaks to shift to a higher wavenumber. Similar phenomena can also be observed in other experimental studies [2].

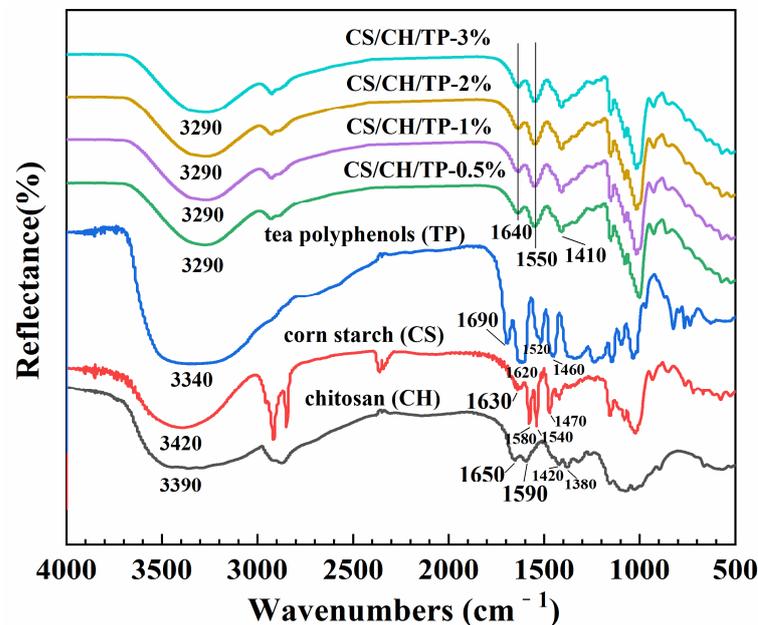


Figure 1. FT-IR spectra for chitosan film, starch film, tea polyphenols and CS/CH/TP films.

3.2. Physicochemical Properties

Compared with CS/CH film, CS/CH/TP film had high oxidation resistance and could play a good role in some applications. Table 1 lists the effects of tea polyphenol concentrations on the physicochemical properties of the blend film. The concentrations of tea polyphenols were chosen according to the literature, where the composite film with 1 wt% TP exhibited the best antibacterial activities and [26]. The thickness of the film can play a good role in protecting food, and it will also affect the opacity of the film and the sensory effects of customers, as well as the barrier properties [28]. Because the addition of tea polyphenol was very low, the influence of tea polyphenol concentrations on the thickness of the blend film was relatively small [29]. It can be seen from Table 1 that the density of the blend film increased with the increase of the content of tea polyphenols.

Table 1. Density, Moisture Content and Opacity of the Chitosan/Corn Starch/Tea Polyphenol Films with Different TP Concentrations.

Film Formulation	Thickness(mm)	Density (g/cm^{-3})	Moisture Content (%)	Opacity ($\text{Abs}_{600}\text{ mm}^{-1}$)
CS/CH/TP-0.5%	0.22 ± 0.02 a	1.42 ± 0.04 a	19.16 ± 0.03 d	1.79 ± 0.05 b
CS/CH/TP-1%	0.23 ± 0.01 b	1.48 ± 0.30 b	18.78 ± 0.01 c	2.06 ± 0.11 c
CS/CH/TP-2%	0.22 ± 0.01 a	1.51 ± 0.07 c	18.61 ± 0.01 b	2.78 ± 0.07 d
CS/CH/TP-3%	0.24 ± 0.02 c	1.53 ± 0.13 d	18.40 ± 0.04 a	3.29 ± 0.03 a

Values are given as mean \pm standard deviation. Different letters in the same column indicate significantly different ($p < 0.05$) when analyzed by Duncan's New Multiple Range Test.

Tea polyphenol concentration also affected the moisture content and opacity of the blend film. The moisture content of the blend films decreased with the increase of tea polyphenol concentration, which was attributable to the reduction of hydrophilicity in the film [30]. This was because the interaction between chitosan and tea polyphenols would reduce the utilization of hydroxyl groups and amino groups, and the effect of hydrogen

bonds would reduce the hydrophilicity of the blend film. The reduced availability of hydroxyl groups might result in a decrease in moisture content [30,31].

Opacity is related to the orderly regions formed by the film [32]. The presence of tea polyphenols in the blend film might prevent or reduce the intensity of scattered light passing through the film, resulting in a higher opacity value. It must be pointed out that this might benefit the performance of food packaging, especially for the packaging of light-sensitive products, because it could block ultraviolet rays, which will help prevent lipid oxidation induced by light [33]. This is because as the opacity increases, the light transmittance of the composite film decreases, which can reduce the influence of ultraviolet radiation. Compared with the thymol composite film, the opacity of the tea polyphenol composite film was less than that of the thymol composite film, which could not only reduce light radiation, but also ensure the visibility of the packaged product.

3.3. Color Properties

The optical properties of the polymer film depend on the nature of the additives used in their formulation [34]. A color experiment of the blend film was carried out and the color properties of the film are shown in Table 2. With the increase of chitosan concentration, the *L* value of the CS/CH/TP film decreased. The smaller the *L* value was, the darker the color of the film was. In addition, the values of *a** and *b** increased with the increase of chitosan concentration, and the color gradually changed to red and yellow. With the increase of tea polyphenols, the value of *b** increases, showing a tendency to turn yellow, which was consistent with Shao's experiment results, where the film was added with corn starch phosphate and carboxymethyl cellulose [26]. With the increase of chitosan concentration, the total color difference ΔE value increased, and the color of the blend film became more abundant. The color of the chitosan film after adding tea polyphenols was richer than that of the composite film with lemon essential oil [35]. The value of *C** also increased with the increase of chitosan concentration, which is similar to the experimental results in Wang's report [27].

Table 2. Color Values of the Chitosan/Corn Starch/Tea Polyphenol Films with Different TP Concentrations.

Film Formulation	<i>L</i> *	<i>a</i> *	<i>b</i> *	ΔE *	<i>C</i> *
CS/CH/TP-0.5%	59.55 ± 0.73 b	11.36 ± 0.30 a	32.02 ± 0.69 a	48.51 ± 0.85 b	33.97 ± 0.65 a
CS/CH/TP-1%	64.62 ± 2.37 c	10.84 ± 0.72 a	32.04 ± 1.76 a	44.87 ± 2.38 a	33.82 ± 1.88 a
CS/CH/TP-2%	60.60 ± 0.74 b	14.28 ± 0.40 b	40.70 ± 0.54 b	54.59 ± 0.96 c	43.14 ± 0.64 b
CS/CH/TP-3%	49.13 ± 1.54 a	11.37 ± 0.82 c	52.69 ± 0.71 c	72.82 ± 1.71 d	57.25 ± 0.96 c

The color values of *L**: luminosity, *a**: negative green and positive yellow, *b**: negative blue and positive yellow. Values are given as mean ± standard deviation. Different letters in the same column indicate significantly different ($p < 0.05$) when analyzed by Duncan's New Multiple Range Test.

3.4. Water Solubility and Swelling Analysis

The solubility of film is an important index in food preservation film. For the cling film covering fruits and vegetables, a low-solubility film is required to ensure the integrity of the structure. For some edible films for packaging candies and cakes, high-solubility films should be selected [36]. The water solubility is that water molecules can form hydrogen bonds with groups such as $-NH_2$ or $-OH$ on the surface of the composite membrane during the initial stage of blend hydration [37]. Starch is a polysaccharide, which is hydrophilic in nature and has poor barrier properties. Chitosan has high hydrophobicity, and its presence could reduce the water solubility of starch-based films [38,39]. The presence of tea polyphenols could increase the water solubility of starch-based films. As mentioned earlier, the higher hydrophilicity of tea polyphenols may be responsible for the greater interaction between the film matrix and water. As shown in Figure 2a, with the gradual increase of the concentration of tea polyphenols, the water solubility of the blend film increased.

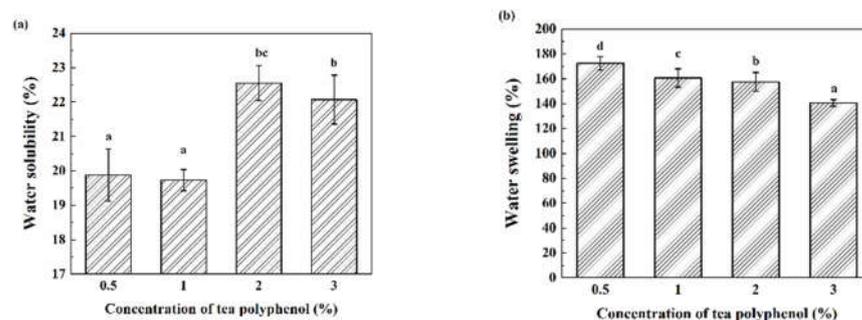


Figure 2. (a) Water solubility of CS/CH/TP films and (b) water swelling of CS/CH/TP films. Values are given as mean \pm standard deviation. Different letters indicate significantly different ($p < 0.05$) when analyzed by Duncan's New Multiple Range Test.

The swelling degree of the membrane is a commonly used physical quantity to characterize the swelling degree of polymer. The swelling is due to the microstructures of the composite films that can absorb and retain the solvent for a long time [40]. The swelling degree is also related to the water temperature, the data in this work were obtained at room temperature [41]. According to Figure 2b, we can see the swelling degree of CS/CH/TP film decreased with the increase of tea polyphenol concentration. Compared with chitosan and starch blend film [42], the swelling degree of CS/CH/TP film was larger. This is due to the hydrophilicity of tea polyphenols with a large number of hydroxyl groups and the hydrogen bond between the hydroxyl group of tea polyphenols and the amino group of chitosan, which leads to the increase of swelling degree.

Swelling capacity refers to the water retention capacity of the film. The blend film is dominated by free hydrophilic groups and free volume. Therefore, the swelling ability of the blend film was a function of its hydrogen bond and crystallinity [43]. The amorphous region of the blend film could hold water, and some inter-molecular hydrogen bonds might form a framework for the film expansion. However, as the content of tea polyphenols increased, the swelling degree of the blend film tended to decrease. The reason could be explained that the addition of tea polyphenols caused the structure of the membrane to change from an amorphous region to an ordered region, which weakened the swelling ability.

3.5. XRD Analysis

Figure 3 shows the XRD patterns of starch, chitosan, tea polyphenols and CS/CH/TP films. The diffraction peaks of corn starch are 14.9° , 17.0° , 18.1° , 22.8° , consistent with previous publications [44]. The first two peaks indicated that the integration of water molecules in the crystal lattice forms a hydrated microcrystalline structure. The characteristic peak of chitosan at 19.7° indicates the amorphous structure of chitosan. After adding chitosan and tea polyphenols to corn starch, the above diffraction peaks disappeared. It was known from the literature that when tea polyphenols were mixed with starch, different concentrations of tea polyphenols will not produce obvious diffraction peaks [45]. At 19.5° , the blend film showed a broad peak. This shows that the hydrogen bonding between chitosan and starch and tea polyphenols restricts the movement of molecular chains and inhibits the crystallization process. The crystalline structures of the blend film with different tea polyphenol concentrations were almost the same. The characteristic peak of starch and tea polyphenols disappeared for the blend film, and the peak at 19.7° of chitosan moved to a lower degree (19.5°). These observations suggested that the interaction between chitosan, starch and tea polyphenols formed. Liu et al. [46] reported the effect of glycerol concentration on the crystalline characteristics of starch–chitosan film, where the extent of crystallinity of the starch–chitosan films decreased with the increase of the glycerol concentration. The CS/CH/TP films had only one diffuse peak located at 19.5° , and compared to chitosan film and starch film, the degree of crystallinity decreased and several peaks disappeared in

the CS/CH/TP films containing high glycerol (20%, *w/w*) concentration, which were in agreement with the results of Liu et al. [46].

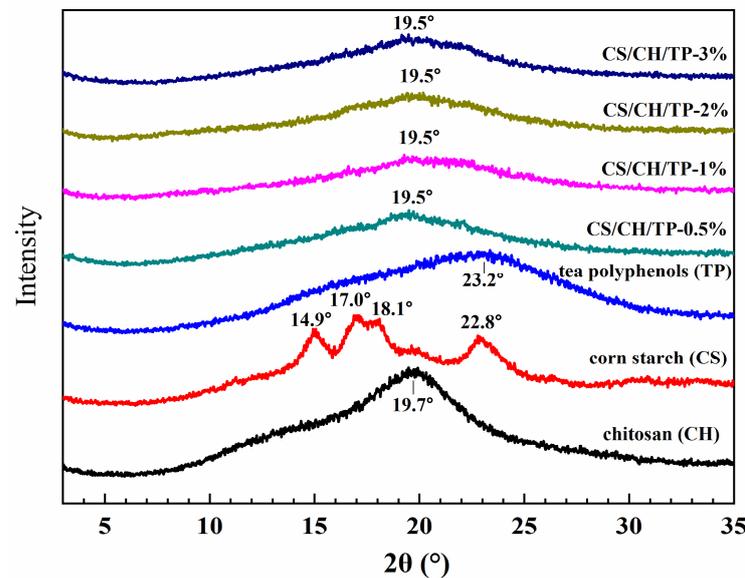


Figure 3. X-ray diffraction patterns of CS film, CH film, tea polyphenols and CS/CH/TP films.

3.6. Film Morphology

The microstructure and polymer compatibility of the CS/CH/TP films were studied by scanning electron microscope. This is an indicator to observe the structural integrity of the film, and it can also affect the barrier properties of the film [47]. Figure 4 shows the morphological characteristics at the surfaces of CS/CH/TP films. It can be seen that the CS/CH/TP films showed smooth surfaces and compact structures without particle and porous structures, without separation of phases between chitosan, starch and tea polyphenols, which indicated that chitosan, starch and tea polyphenols formed hydrogen bonds. Through hydrogen bond and hydrophobic interaction, the cross-linking between the molecules became closer, which was helpful to the stability of the CS/CH/TP films [48]. In addition, chitosan, starch and plasticizer were highly compatible, so the surface structure of the blend film was continuous and was no separation between the polymers. While the small debris in the material may be due to the frost cracking of the film before SEM analysis.

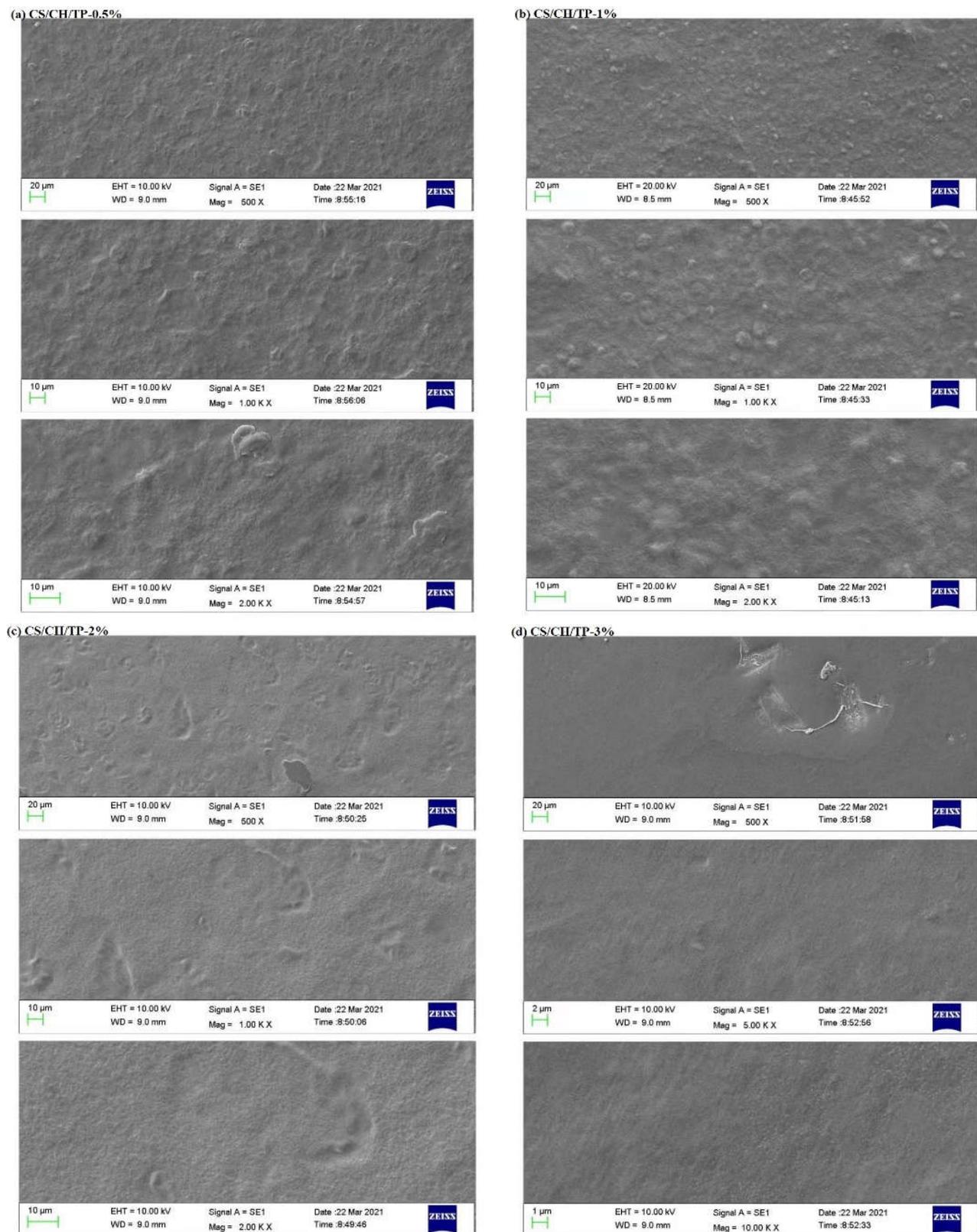


Figure 4. SEM micrographs of the surfaces of the blend films: (a) CS/CH/TP-0.5%, (b) CS/CH/TP-1%, (c) CS/CH/TP-2%, (d) CS/CH/TP-3%.

4. Conclusions

CS/CH/TP films with different tea polyphenol concentrations of 0.5, 1.0, 2.0 and 3.0%, were prepared successfully by a casting method. The incorporation of tea polyphenols into chitosan–starch film had an influence on the physicochemical properties of the obtained CS/CH/TP films. The shifts of the characteristic peaks in ATR-FTIR results confirmed that the formation of inter- and intra-molecular hydrogen bonding had taken place between chitosan, starch and tea polyphenols. As the content of tea polyphenols increased, the density of the CS/CH/TP film increased. The moisture content of the CS/CH/TP film decreased with the increase of tea polyphenol concentration. The incorporation of tea polyphenols gave rise to the CS/CH/TP film's darker and yellower appearance. With the increase of the tea polyphenol concentration, the water solubility of the CS/CH/TP film increased while the swelling degree decreased. The XRD patterns indicated that the integration of water molecules in the crystal lattice formed a hydrated microcrystalline structure and the amorphous structure of chitosan. The hydrogen bonding between chitosan, starch and tea polyphenols restricted the movement of molecular chains and inhibited the crystallization process. Chitosan, starch, tea polyphenols and plasticizer were highly compatible, so the surface of the CS/CH/TP film was continuous without any separation between the polymers. This study demonstrated an effective strategy to improve the performance of chitosan and corn starch film. Further, these promising results can improve the development of CS/CH/TP films as functional packaging films for the food and drug industries. The mechanical and barrier properties and the antimicrobial ability of the blend films against microorganisms and the shelf life of foods coated by the blend films will be carried out in the next work.

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