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Optimization and release evaluation for tea polyphenols and chitosan composite films with regulation of glycerol and Tween

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Abstract

In order to optimize the performance of chitosan-tea polyphenols composite films, effects of glycerol and Tween on the antioxidative activities and physical performance of chitosan composite films were evaluated. The results indicated that both flexibility and water vapour permeability of chitosan/tea polyphenols films were improved with the addition of glycerol, meanwhile the solubility and swelling degree decreased. Moreover, the release time of antioxidants from films was delayed with the addition of glycerol. The presence of Tween 20 had no significant effect on the tensile strength of films, but changed the DPPH radical scavenging activity depending on the blend ratio of glycerol and Tween. DSC, FTIR and X-ray analysis showed strong interaction among chitosan, tea polyphenols, glycerol and Tween. The optimum ratio was 0.5% tea polyphenols, 1.2% glycerol and 0.1% Tween. This study provided a reference that an optimized chitosan/tea polyphenols composite film could be obtained by the regulation of glycerol and Tween.

Keywords: chitosan film; green tea polyphenols; optimization; antioxidant ability; X-ray diffraction.

Practical Application: Provide a better understanding in the application of antioxidant active chitosan-tea polyphenols composite films.

1 Introduction

In recent years, great challenges in food packaging have been the establishing of active biodegradable films. Active film is a new-type packaging material that possesses antimicrobial or antioxidative activities to control the microbial growth and food oxidation, thus extending the shelf life of the food. While antioxidant packaging film is the hot topic of research in food packaging at present (Buonocore et al., 2003; Rojas-Graü et al., 2009). In addition, with the improving of people's safety awareness, active packaging tends to choose natural materials comparing with the chemical synthetic agents (Blanco-Fernandez et al., 2013; Tovar et al., 2005).

Chitosan is a natural cationic polysaccharide obtained by deacetylation of chitin which extracted from arthropods, such as shrimp and crab shells (Dutta et al., 2009; Ying et al., 2011). As an environment-friendly material, chitosan has good biodegradablility, antioxidant activity and film-forming properties (Martins et al., 2012; Park & Zhao, 2004). Moreover, chitosan has good biocompatible characteristics and is capable of combining functional ingredients such as vitamin E, plant essential oil and tea extracts (Blanco-Fernandez et al., 2013; Ojagh et al., 2010; Park & Zhao, 2004). As a very popular beverage all over the world, tea has strong antioxidative activities due to its polyphenol ingredients (Ashrafi et al., 2017; Khan & Mukhtar, 2007). According to previous reports, the antioxidative activities of bioactive ingredients in tea polyphenols are twenty and thirty times higher than vitamin C and vitamin E, repectively, concerning the DPPH free radical scavenging activity (Bancirova, 2010; Prior et al., 2005; Vinson et al., 1995). Additionally, tea polyphenols could delay the oxidation of vegetable oil, soybean oil and dry-fermented sausage (Bozkurt, 2006; Yanishlieva & Marinova, 2001; Yin et al., 2012). When tea polyphenols were added into chitosan films, the antioxidant capacity of films was increased significantly. However, tea polyphenols tend to cause poor flexibility and destroy the chitosan composite films, which reduced the application of tea polyphenol films in food packaging (Peng et al., 2013). Moreover, little information focusing on the controlled release kinetics of antioxidative polyphenols from chitosan based membranes was reported.

Plasticizing agents such as glycerol, sorbitol and polyethylene glycol are usually used to improve the film flexibility and weaken the interaction among biopolymeric matrix (Park & Zhao, 2004; Srinivasa et al., 2007; Ziani et al., 2008). A concentration of 30% glycerol (w/w chitosan) was usually considered to gain good mechanical properties in chitosan based films according to the chitosan deacetylated degree. However, glycerol concentration remains uncertain with the application of exogenous additives in chitosan films. Surfactant is a kind of amphiphilic substance that is hydrophilic as well as hydrophobe (Chen e al., 2009). Tween, as a nonionic surfactant, can increase the stability of film solution and enhance the physical properties of chitosan-based film (Casariego et al., 2008). Furthermore, with the addition of surfactant, it is expected that water vapor permeability values

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can be decreased due to the hydrophobic characteristic of surfactant (Ziani et al., 2008). However, there is little work on the synergistic influences of glycerol and surfactant on properties of chitosan/tea polyphenols films, especially the release of active constituents from composite films.

Therefore, the main aim of this work was to: 1) assess the combined influences of glycerol and Tween on antioxidative capacity of chitosan/green tea polyphenols films; 2) analyze the crystallinity and infrared spectra of the films, as well as thermal properties, to better understand the relationship between the release degree and structure of the films.

2 Materials and methods

2.1 Materials and reagents

Food grade chitosan (CH) in this study was obtained from Shandong Aokang Biotechnology Company (Jinan, China) with a deacetyl degree of 90.2% and a viscosity of 62cps at room temperature. Tea polyphenols obtained from Fuzhou Corona Company (Fuzhou, China) with 99.9% of tea polyphenols, 81.2% of total catechins and 51.7% of epigallocatechin gallate were applied to prepare the composite films. Acetic acid, anhydrous calcium chloride, Tween 20, Glycerol, 1,1-diphenyl-2-picrylhydrazyl (DPPH) and other chemical reagents were of analytical grade.

2.2 Preparation of films

The chitosan films were obtained according to our previous method with some modifications (Peng et al., 2013). A concentration of 1% acetic acid solution was used to dissolve the chitosan (2%, w/v) with a 90-1 model magnetic stirring apparatus (Shanghai Huxi Device Company, China) at 25 °C for 4 h. Then glycerol (Gly) was added at different concentrations of 30%, 45% and 60% (w/w chitosan). Tween 20 (T) (5%, mass ratio to chitosan weight) was mixed into these solutions when needed and stirred for 1 h. Then, 0.5% (g/100mL film solutions) green tea extracts (GTE) were added into polymer solutions and dissolved completely. The film-forming solutions were homogenised with a T25 model dispersion homogenizer (IKA Instrumental Equipment Company, Germany) for 2 min at 13,500 rpm. The solutions were then degasified with a vacuum pump for 1 h at room temperature. Final samples were named CH-GTE-nGly-T, and n value is the added content of glycerol relative to initial chitosan weight.

Films were obtained by casting 200g film solutions on the horizontal glass tray (25 cm×25 cm) and allowed to dry for 48 h (25±1 °C, 60±2% RH). Finally, the films were peeled off and placed in desiccators with a relative humidity of 53% at room temperature for 48 h.

2.3 Rheological behaviour

On the basis of the reports of Sánchez-González et al. (2010), rheological behaviour of film solutions was determined by a RST plus cylinder rheometer (AMETEK Brookfield Commercial Enterprise Co. Ltd., USA) equipped with rotating circular column (12.50 mm radius). The shear stress (σ) was obtained at 25 °C with shear rate (γ) ranged from 0 to 512 s^{-1}. The rheological curves were mathematically fitted the Ostwald de Waale model with the following Equation 1. Apparent viscosity was determined at 100 s^{-1}. The measurements were repeated three times.

$$\sigma = k\gamma^{n} \tag{1}$$

where k is consistency index (k) and n is the flow behaviour index.

2.4 Film thickness, Water Vapour Permeability (WVP), water content

Film thickness was determined by a digital spiral micrometer with a precision of 0.01mm. Six random points at each sample were taken for obtaining the average value. Water content was carried out with an oven (Shanghai Yiheng Scientific Instruments Company, China) at 105 °C to get rid of water for 24 h.

WVP was measured using a modification method proposed by Talja et al. (2008). Films pieces (60 mm×60 mm) were placed onto the test cups (inradium 42mm, height 25mm) with full of anhydrous $CaCl_2$ (diameter < 2 mm, 20.5±0.2 g for each cup). The gap between film and $CaCl_2$ was less than 6 mm to ensure optimal moisture absorption. Then these cups were stored in desiccators which contained NaCl saturated solution to ensure the 75% RH at 25 °C. The cups were weighed after 7 d. The measurements were repeated five times and WVP was determined on basis of listed below formula (Equation 2):

$$WVP = \frac{mL}{At\Delta p} \tag{2}$$

Where m was moisture weight through the film (g), L was film thickness (m), A was the effective area (m^2), t was the permeation time (s), and Δp was steam pressure difference which the value was 2376.3 Pa at 75% RH and 25 °C.

2.5 The dissolution and swelling of films

Based on previous methods of Silva et al. (2009), the dry weight (M_1) of initial film (20 ×20 mm) was obtained in a vacuum oven at 70 °C for 24 h. Films were then immersed in 30 mL of deionized water at room air conditions for 24 h. After that these films pieces (M_2) were taken out and dried to constant weight (M_3) at 70 °C for 24 h. All the measurements were carried out in three replicates. Swelling behaviour and solubility were determined by Equation 3 and Equation 4, respectively:

$$\text{Film solubility} = \frac{(M_1 - M_3) \times 100}{M_1} \tag{3}$$

$$\text{Swelling degree} = \frac{(M_2 - M_1)}{M_1} \tag{4}$$

2.6 Mechanical properties

Based on the method of Park & Zhao (2004), mechanical properties were determined by a TA-XT 2i texture analyser (Stable Micro Systems Ltd., UK) with a 50 kg load cell. The films were equilibrated in cabinet at 53% RH and 25 °C for 2 days before measurement. The film samples (20mm width and 80mm length) were fixed on the pulling clamp (TA 96) for the measurement of tensile strength (TS) and elongation at break (EB). The initial clamp separations and tensile speed were carried out at 50 mm and 0.8 mm/s, respectively. The measurements were quickly carried out to minimize the water variances. The measurements were repeated eight times for each treatment.

2.7 Film colour

The film colour was detected by WSC-S colorimeter (Shanghai Jingke Industrial Company, China). The CIE Lab was carried out to display the difference in colour, where L* means from black to white (0-100), a* and b* represent red/green and yellow/blue, respectively. Total colour difference (ΔE) was obtained according to the following formula. The measurements were repeated four times for each sample (Equation 5).

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{0.5} \tag{5}$$

2.8 Release of antioxidative agents from films

According to the methods of Mayachiew & Devahastin (2010), film pieces (20 mm square) were immersed into 100 mL of deionized water and stirred magnetically at 200 rpm. Afterwards the samples (1 mL) were extracted and blended with 4 mL DPPH-methanol solution (150 μmol/L). Meanwhile, 1 ml of distilled water was added into container to cover the loss. The mixture was kept in the dark for 30 min and measured at 516 nm with a UV 2100 spectrophotometer (Unico Instruments Co., Ltd., Shanghai, China). Three repetitions were carried out for each treatment. Results were expressed by DPPH radical scavenging activity according to the following formula (Equation 6):

$$\text{DPPH radical scavenging activity (\%)} = \frac{(1 - A_{sample}) \times 100}{A_{control}} \tag{6}$$

A_{sample} and $A_{control}$ stand for the absorbance of the sample and control solution, respectively.

2.9 Thermal analysis

Thermal properties were measured using a differential scanning calorimetry (DSC) and was carried out on the Q2000 DSC equipment (TA Instruments Company, USA). After stored at 53% RH and 25 °C for 2 days, film samples (5 mg) was hermetically sealed in a criterion aluminium tray, then chilling down to -10 °C based on the usual procedure. The thermograms were received with the temperature elevated from -10 °C to 360 °C at the heating speed of 20 deg/min.

2.10 X-ray diffraction analysis

X-ray diffraction (XRD) was determined with a D/max-2200 X-ray diffractometer (Japan Rigaku Company, Japan) at the temprature of 25 °C. The measurement was determined at 40 kV and 30 mA with a copper tube. Film sample (diameter 1.8 cm) was placed on a glass plate. XRD was carried out with the scattering range of 5-35° (2θ) at a scan rate of 4° min^{-1}.

2.11 FTIR analysis

After dried in a container including silica-gel desiccant for 14 d, attenuated total reflectance -fourier transform infrared spectrometry (ATR-FTIR) of the films was processed with a Spectrum 100 spectrophotometer (Perkin Elmer, Inc., Waltham, USA). The spectra curves were obtained by 32 scans ranged from 4000 to 650 cm-1 with a resolution of 4 cm-1.

2.12 Statistical analysis

Statistical analyses were carried out by a SPSS software (version 17.0). The variable data analysis was carried out by ANOVA and differences ($p < 0.05$) between average value was evaluated by Duncan's multiple range test.

3 Results and discussion

3.1 Rheological behaviour

The addition of tea polyphenols significantly decreased the consistency index (k) and apparent viscosity of film solutions (Table 1). The decrease of apparent viscosity reflected the weakened intermolecular force in chitosan film solution. Dutta et al. (2009) reported that the intermolecular force was related to the amount of hydrogen bonding in film solution. The addition of tea polyphenols could weaken the hydrogen bonding force due to the interaction between benzene ring of tea polyphenols and chitosan.

The apparent viscosity of chitosan/green tea polyphenols films decreased with the increase of glycerol concentration. Likewise, with the addition of Tween 20, the composite films showed a lower apparent viscosity. Casariego et al. (2008) reported the values of surface tension and adhesion coefficients decreased with the increasing glycerol concentration. Ziani et al. (2008) also reported that glycerol could interact with chitosan chains and decrease intermolecular attraction. Also the introduction of Tween into starch film caused a decrease of surface tension

Table 1. The rheological behaviour of chitosan/glycerol/green tea polyphenols composite films.

Films[1]	n	k (Pa·s)^n	Viscosity (Pa·s)(100s^{-1})
CH-30%Gly	0.791±0.004a	0.741±0.003a	0.321±0.003a
CH-GTE-30%Gly	0.796±0.003b	0.721±0.003b	0.306±0.006b
CH-GTE-45%Gly	0.798±0.004b	0.718±0.001bc	0.299±0.002c
CH-GTE-60%Gly	0.807±0.002cd	0.716±0.002cd	0.297±0.003c
CH-GTE-30%Gly-T	0.798±0.005b	0.717±0.003cd	0.298±0.003c
CH- GTE-45%Gly-T	0.805±0.003c	0.715±0.002cd	0.294±0.002c
CH- GTE-60%Gly-T	0.809±0.002d	0.713±0.002d	0.289±0.004d

Variable data are represented as average value and standard deviation. Significant differences ($p < 0.05$) are shown in different little letters. [1]CH: chitosan; GTE: green tea extracts; Gly: glycerol; T: tween.

and could lead to the decline of binding force in hydroxyl groups in film solutions (Zhong & Li, 2011). Therefore, the decrease in apparent viscosity could be due to the decrease of intermolecular force in chitosan/tea polyphenols film solutions by the incorporation of glycerol and Tween 20.

3.2 Water properties of chitosan composite films

The changes of water content, water vapour permeability (WVP), thickness, swelling degree and film solubility were shown in Table 2. Results showed that the incorporation of glycerol and Tween 20 increased the thickness of composite films.

The incorporation of green tea polyphenols into chitosan film decreased water content and WVP (Table 2). The interaction between molecular bonding in cihtosan and tea polyphenols could be the main reason, which weakened the interaction between chitosan and water in the composite film (Gómez-Guillén et al., 2007). Similar results were also found in chitosan-based mineral and vitamin E composite films (Park & Zhao, 2004). However, the addition of glycerol increased the water content and WVP of composite film, respectively. As a small hydrophilic molecule, glycerol could be inserted into the polymeric chains and increase the water content as well as the migration of water vapour (Rodríguez et al., 2006; Srinivasa et al., 2007). The addition of surfactant in CH-GTE-Gly films had no significant effect on WVP except the film in 45% glycerol. This was not completely consistent with that reported by Rodríguez et al. (2006), which found a positive interaction between glycerol and Tween 20 in WVP. The reason could be attributed to the addition of green tea polyphenols that interrupted the intermolecular interactions in film matrix.

The low swelling degree and film solubility were found in the control film (Table 2) after dipping for 24h. The CH-GTE-Gly films had higher dissolution and swelling than the CH-Gly films. Ususlly, the dissolution and swelling in water of film involved the adsorption and diffusion of hydrone, ionization and dissociation of chemical bonds, and are also related to the degree of looseness of films (Mathew et al., 2006). In our study, the high swelling degree and solubility might be related to the destruction of crystal structure which resulted in the relaxation of chitosan film matrix, thus allowing more water into the matrix. However, the swelling and solubility of films significantly decreased with the increasing glycerol concentration. Silva et al. (2009) also found the similar results in pectin-glycerol films, where the increase in crosslinking degree among intermolecular chains caused an increased retractive force, and resulting in lower swelling degree. For this reason, the existence of glycerol could reconstruct the intermolecular cross-linking in the chitosan/green tea polyphenols films, thus resulting in less water through the matrix network.

The addition of Tween 20 in CH-GTE-Gly films had no significant effect on the film solubility. But Tween 20 increased the swelling degree of CH-GTE- 30%Gly films and CH-GTE-45%Gly films. Tween 20, with an HLB (Hydrophile-lipophile balance) of 16.7, was the most hydrophilic surfactant, which had a higher water permeability value. Both the types and the concentration of surfactants might have different effects on the hydrophilic performance of film (Rodríguez et al., 2006). The interaction among chitosan, green tea polyphenols, glycerol and Tween might be more complicated and needs further investigation.

3.3 Film colour

Table 3 showed the colour value L*, a*, b* and ΔE of chitosan-based films with different additives. The colour of chitosan control film was slightly yellow and the b* was 10.35. The addition of tea polyphenols significantly ($P < 0.05$) increased

Table 2. The water properties of chitosan/glycerol/green tea polyphenols composite films.

Films[1]	Thickness (μm)	Water content (%)	Swelling degree	Solubility in water (%)	WVP $(g.m^{-1}s^{-1}Pa^{-1}\times10^{-11})$
CH-30%Gly	71.6±3.1d	28.92±1.09bc	1.10±0.06a	15.21±1.34e	13.39±0.75b
CH-GTE-30%Gly	94.2±2.6c	22.99±2.42d	3.39±0.23d	34.17±0.25a	6.27±0.59e
CH-GTE-45%Gly	104.2±1.9b	29.99±0.27b	1.58±0.07b	28.42±0.17bc	8.84±1.16d
CH-GTE-60%Gly	111.0±3.5a	37.16±1.92a	0.97±0.11a	24.44±1.56d	15.74±0.49a
CH-GTE-30%Gly-T	109.2±3.6a	22.43±0.80d	4.39±0.52e	34.96±1.54a	7.08±0.99e
CH- GTE-45%Gly-T	112.6±4.7a	26.46±1.08c	2.14±0.10c	29.81±0.78b	10.72±1.07c
CH- GTE-60%Gly-T	114.8±4.4a	38.04±2.48a	0.89±0.06a	26.49±1.61cd	14.86±0.51a

Variable data are represented as average value and standard deviation. Significant differences ($p < 0.05$) are shown in different little letters. [1] CH: chitosan, GTE: green tea extracts, Gly: glycerol, T: tween.

Table 3. The colour of chitosan/glycerol/green tea polyphenols films.

Films[1]	L*	a*	b*	ΔE
CH-30%Gly	89.67±0.32a	-2.99±0.37d	10.35±0.38d	16.76±0.36e
CH-GTE-30%Gly	62.84±0.73c	17.97±5.53a	27.81±3.90c	45.03±2.27c
CH-GTE-45%Gly	62.55±1.05c	16.36±1.17ab	28.09±0.84c	44.80±0.72c
CH-GTE-60%Gly	60.93±0.87d	16.39±1.27ab	32.65±1.20b	48.63±1.47b
CH-GTE-30%Gly-T	68.92±0.22b	9.78±0.37c	28.62±0.14c	39.22±0.21d
CH- GTE-45%Gly-T	62.91±0.35c	14.05±0.23b	34.17±0.25b	47.65±0.39b
CH- GTE-60%Gly-T	57.38±1.38e	17.31±0.88ab	39.99±3.44a	55.99±3.29a

Variable data are represented as average value and standard deviation. Significant differences ($p < 0.05$) are shown in different little letters. [1] CH: chitosan, GTE: green tea extracts, Gly: glycerol, T: tween.

the b* and ΔE of films and decreased L* value. The b* increased from 27.81 to 32.65 with the increasing glycerol concentration. In addition, the yellowness was increased even more with the presence of Tween 20 in composite films. The increase in film colour could be due to the natural colour of green tea polyphenol, glycerol and surfactant (Chillo et al., 2008).

3.4 Mechanical properties

As shown in Figure 1, the addition of tea polyphenols decreased the mechanical performance of chitosan composite films. Particularly, elongation at break significantly ($P < 0.05$) decreased from 32.02 to 7.72%. Similar results were also found by Sánchez-González et al. (2010) with the addition of bergamot essential oil into chitosan film. The decrease in elongation at break and tensile properties could be attributed to the reduction of the hydrogen bonding force and crystallinity in the chitosan film (Park & Zhao, 2004).

In order to improve the film flexibility, higher concentration of glycerol was added into the film matrix. As shown in Figure 1, the elongation at break significantly increased with the increasing glycerol concentration while the tensile strength decreased. The reason could be that glycerol decreased the hardness of the film matrix and increased the mobility of the molecular chains by reducing the intermolecular forces (Srinivasa et al., 2007). The presence of Tween 20 had no significant effect on the tensile intensity and flexibility of films except GTE-30%Gly-T film. Ziani et al. (2008) reported the addition of Tween into chitosan/glycerol films had no significant influence on the tensile performance of chitosan films (60.9% deacetylated degree), but caused a significant reduction of elongation at break in 96% deacetylated degree chitosan films. They speculated that the presence of Tween led to a disruption of the film structure which affected mechanical properties of film. However, compared with addition of glycerol, the mechanical properties were not affected so intensely as the addition of Tween. Therfore, in this work, the differences in blend ratio between glycerol and Tween could be the main reason which caused the differences in mechanical properties.

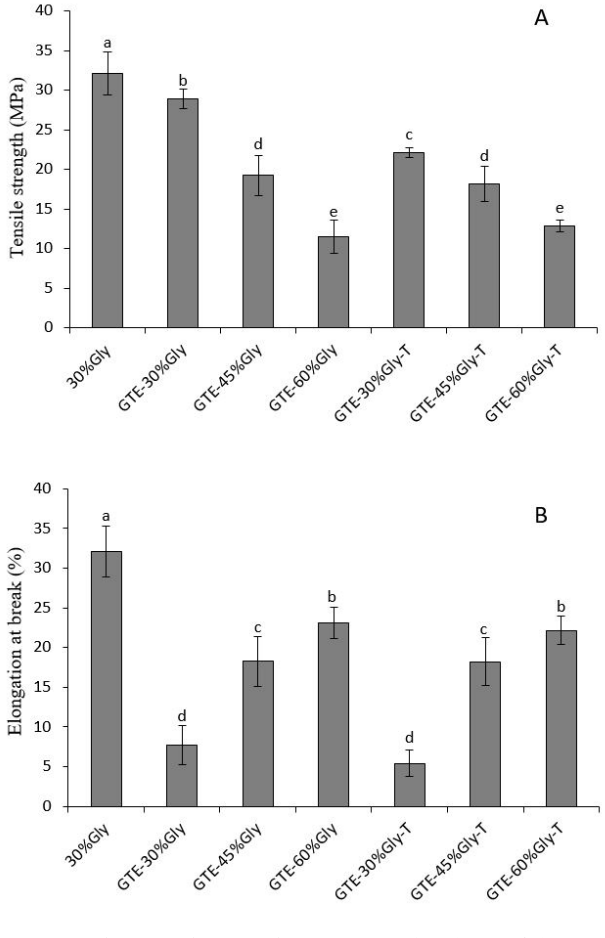

Figure 1. Tensile strength (A) and elongation at break (B) of chitosan/glycerol/green tea composite films. The error bars indicated the standard deviation.

3.5 Release of antioxidative agents from films

DPPH radical scavenging activity assay is a standard method to determine the antioxidative capacity of film in food industry (Prior et al., 2005; Xu et al., 2018). As shown in Figure 2, the control film had the lowest DPPH radical scavenging activity. With the addition of green tea polyphenols, the DPPH radical scavenging activity increased to 94.6% within 20 min.

The equilibration time, which was the time obtained the radical maximize scavenging activity, of GTE antioxidants was delayed with the addition of glycerol. As shown in Figure 2, the higher the glycerol concentration, the slower the release of antioxidative ingredients from films. The same trends were also observed in the presence of Tween 20. However, the release of antioxidative ingredients was slower in GTE-60%Gly-T film. The release of antioxidative ingredients from polysaccharide films included several steps. First of all, at the initial stage of release, water molecules gradually penetrated into the film inside, which resulted in the swelling of composite films. The swelling caused the diffusion of antioxidative ingredients into the outside solution until thermal balance was finished. Secondly, the film structure

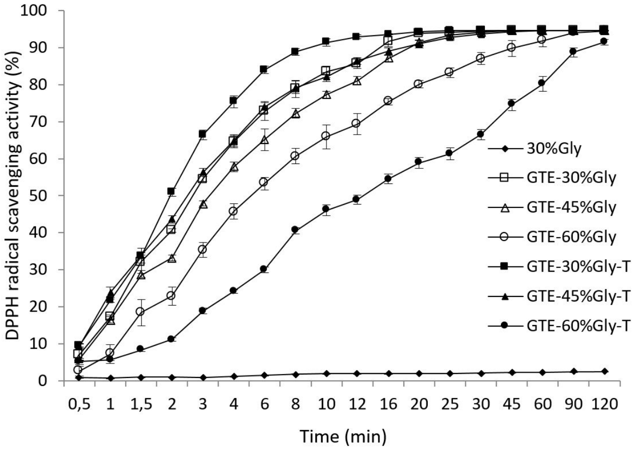

Figure 2. The DPPH radical scavenging activity of chitosan/glycerol/green tea composite films. The error bars showed the standard deviation.

became more loosely with the extension of time and led to a quick increase of release degree, as a result the DPPH radical scavenging activity increased significantly. Thirdly, when the film structure was completely destroyed, the release of antioxidative ingredients reached to the maximum (Buonocore et al., 2003; Mayachiew & Devahastin, 2010). Therefore, the differences in release kinetics might be caused by swelling, film solubility and the crytal structure of the different matrix components.

3.6 Thermal analysis

As shown in Figure 3, a thermodynamic peak at 162 °C was found in the DSC of the control film which was related to the α-relaxation of chitosan chain. Sakurai et al. (2000) reported that the endothermic peak of pure chitosan was at 205 °C. The lower endothermic peak in this work could be due to the differences of plasticizer, molecular weight and deacetylation degree of chitosan (Mathew et al., 2006). The phase transition temperature in the process of heat degradation was 271.2 ˚C for chitosan films in this work, and the same phenomenon was also observed by Park & Zhao (2004) in chitosan/vitamin E composite films and Suyatma et al. (2005) in chitosan/plasticizers films. The thermodynamic peaks around 61.9 °C, 159.6 °C and 202.3 °C were found in chitosan/tea polyphenol based films with the addition of green tea polyphenols (Figure 3b). Compared with the pure tea polyphenol powder (Figure 3h), the endothermic peak at 61.9 °C revealed the emission of unstable volatile components from cihtosan/tea polyphenol composite films. And the temperature at 159.6 °C and 202.3 °C was associated with the elimination reaction of NH_3, the depolymerization and decomposition of the polysaccharide backbone, repectively (Martins et al., 2012). With addition of tea polyphenols, the phase transition temperature of chitosan composite film was apt to transfer to lower temperatures (255.9 °C) and led to a decrease in thermal stability.

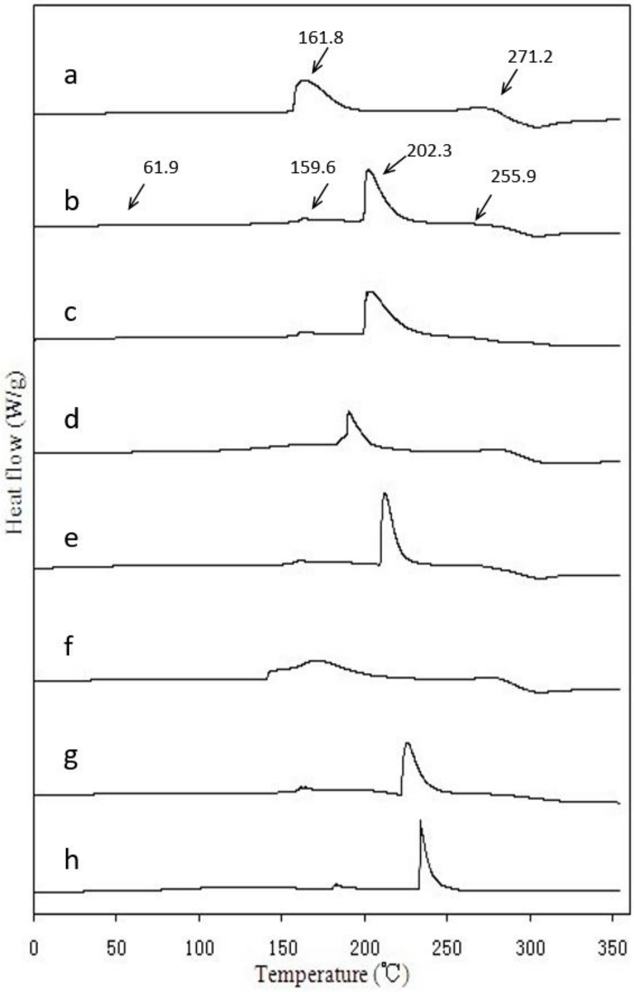

Figure 3. DSC analysis of chitosan/glycerol/green tea composite films. (a) CH-30%Gly control film, (b) CH-GTE-30%Gly film, (c) CH-GTE-45%Gly film, (d) CH-GTE- 60%Gly film, (e) CH-GTE-30%Gly-T film, (f) CH-GTE-45%Gly-T film, (g) CH-GTE-60%Gly-T film, (h) green tea extract powder.

The endothermic peak and phase transition temperature changed with the incorporation of glycerol and Tween 20. Glycerol in low concentration (<45%) decreased the phase transition temperature while high concentration of glycerol seemed to ameliorate the thermal properties. Suyatma et al. (2005) reported the glass transition temperature of glycerol films decreased with the increased contents of plasticizer from 5% to 40%. They found that plasticization decreased thermal stability by interrupting the interactions of polymer-polymer. Moreover, the crystallinity of chitosan composite films could have influence on thermal stability according to the reports of Martins et al. (2012). This could also be associated with the following X-ray diffraction analysis.

3.7 X-ray diffraction analysis

Figure 4 showed the X-ray diffraction patterns of all citosan composite films. The chitosan control film (Figure 4a) showed a semicrystallinity character. The diffraction peak was observed at

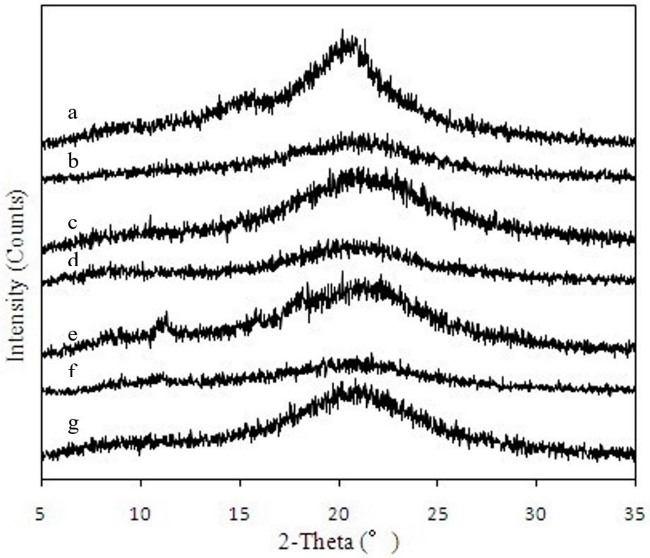

Figure 4. X-ray diffraction spectra of chitosan/glycerol/green tea composite films. (a) CH-30%Gly control film, (b) CH-GTE-30%Gly film, (c) CH-GTE-45%Gly film, (d) CH-GTE-60%Gly film, (e) CH-GTE-30%Gly-T film, (f) CH-GTE-45%Gly-T film, (g) CH-GTE-60%Gly-T film.

21.7° (2θ) in control film while the same results were also reported by Mathew et al. (2006) in chitosan films. The diffraction peak decreased significantly after green tea polyphenols were added into chitosan films. This could be due to the strong interactions between the functional group of chitosan and tea polyphenols which destroyed the internal crystal structure of chitosan matrix. The diffraction peak was reconstructed and reappeared with the increasing glycerol concentration. The addition of glycerol could result in redistribution of crystal type within the matrix which promoted the formation of the diffraction peak (Ziani et al., 2008). However, the peak value did not increase along with the increasing glycerol concentration, which implied that there was optimized glycerol content in chitosan/green tea polyphenols films. The presence of Tween 20 in the composite films caused complex changes in diffraction peak, which indicated the blend ratio of glycerol and Tween had remarkable influence on crystal properties of chitosan/tea polyphenols films.

3.8 FTIR analysis

The intermolecular interaction in chitosan based composite film could be observed by the FTIP spectroscopy in Figure 5. The types of acid solution tended to change the spectra in chitosan based films (Zhong et al., 2011). Therefore, the peak at 3285 cm^{-1} reflected the overlapping of O-H bond and the N-H bond stretching. The peak around 2920 cm^{-1} and 2872 cm^{-1} reflected the hydrocarbon bond stretching (Mathew et al., 2006). The characteristic peak at 1642 cm^{-1} and1558 cm^{-1} was owing to the C=O stretching (amide I) and the N-H bending (amide II), respectively (Martins et al., 2012). The $-CH_2$ bending (1410 cm^{-1}), $-CH_3$ symmetrical deformation (1381 cm^{-1}) and C-O/C-C stretching (from 1152 cm^{-1} to 882 cm^{-1}) were also shown in Figure 5, which was consistent with reports of Lawrie et al. (2007).

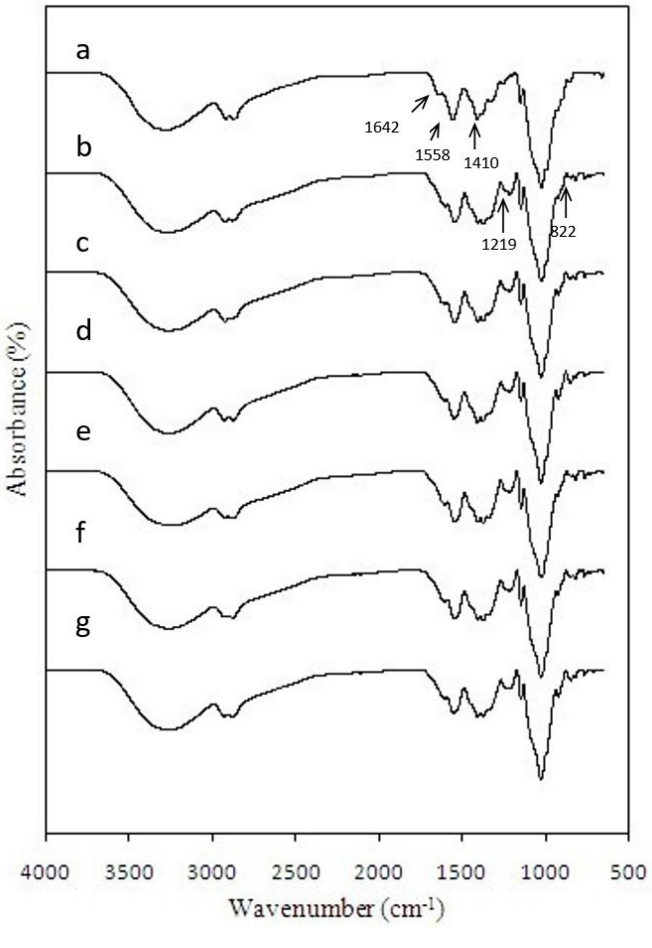

Figure 5. FTIR spectra of chitosan/glycerol/green tea composite films. (a) CH-30%Gly control film, (b) CH-GTE-30%Gly film, (c) CH-GTE-45%Gly film, (d) CH-GTE-60%Gly film, (e) CH-GTE-30%Gly-T film, (f) CH-GTE-45%Gly-T film, (g) CH-GTE-60%Gly-T film.

When green tea polyphenols were mixed into chitosan films (Figure 5b), the peaks around 1642 cm^{-1} and 1558 cm^{-1} transfered to lower wavenumber compared with the control film. Furthermore, some new peaks (from 1558 cm^{-1} to 882 cm^{-1}) were also observed in fingerprint region. The reason could be the effects of benzene ring (the main component in tea polyphenols) on film matrix.

Nevertheless, no remarkable difference in spectrum curves was surveyed with the addition of glycerol except the enhanced intensities in the region at 882 cm^{-1}. In addition, no new peak was observed with the addition of Tween. Ziani et al. (2008) also reported the similar phenomenon that surfactant had little influence on infrared spectra. However, according to the reports of Guerrero et al. (2010), the intensities might be affected because the peak of chitosan and glycerol overlapped from 800 cm^{-1} to 1150 cm^{-1}.

4 Conclusion

This work indicated that an optimized ratio of antioxidative film was 0.5% green tea polyphenols, 1.2% glycerol and 0.1% Tween in chitosan composite films. The presence of Tween 20 had no significant influence on the tensile strength and flexibility. However, water vapour permeability increased with the addition of glycerol and Tween, which was consistent with the intermolecular and crystal structure of composite films. The higher the glycerol concentration, the slower the release of antioxidative ingredients from films. The differences in release degree could be related to the different interactions between matrix components according to the results of FTIR and XRD. According to the demand of active packaging in food industry, future research trends should be focused on the controlled release of antioxidants from composite films.

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