The antimicrobial, mechanical, physical and structural properties of chitosan-gallic acid films

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The antimicrobial, mechanical, physical and structural properties of chitosan-gallic acid films

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Abstract

Chitosan films incorporated with various concentrations of gallic acid were prepared and investigated for antimicrobial, mechanical, physical and structural properties. Four bacterial strains that commonly contaminate food products were chosen as target bacteria to evaluate the antimicrobial activity of the prepared gallic acid-chitosan films. The incorporation of gallic acid significantly increased the antimicrobial activities of the films against *Escherichia coli*, *Salmonella typhimurium*, *Listeria innocua* and *Bacillus subtilis*. Chitosan films incorporated with 1.5 g/100 g gallic acid showed the strongest antimicrobial activity. It was also found that tensile strength (TS) of chitosan film was significantly increased when incorporating 0.5 g/100 g gallic acid. Inclusion of 0.5 g/100 g gallic acid also significantly decreased water vapor permeability (WVP) and oxygen permeability (OP). Microstructure of the films was investigated by Fourier transform infrared spectroscopy (FT-IR) and scanning electron microscopy (SEM) and it was found that gallic acid was dispersed homogenously into the chitosan matrix.

Key words: Chitosan, gallic acid, antimicrobial activity, mechanical properties, edible film
1. Introduction

The interest in the development of edible and biodegradable films for food packaging has recently been steadily increasing due to significant concerns about environmental pollution caused by non-biodegradable packaging materials and consumer demand for high quality food products (Bravin, Peressini, & Sensidoni, 2006). Newly developed packaging materials often have additional functional properties, such as antioxidant and antimicrobial properties, beyond their essential mechanical properties (Bajpai, Chand, & Chaurasia, 2010; Suppakul, Miltz, Sonneveld, & Bigger, 2003).

Antimicrobial packaging is showing a great potential in the future of active packaging systems through its promising proposed impact on shelf-life extension and food safety, via controlling spoilage and the growth of pathogenic microorganisms (Moreira, Pereda, Marcovich, & Roura, 2011). Therefore, research on new functional edible and biodegradable packaging materials should yield numerous potential applications.

Chitosan is a natural polysaccharide produced by deacetylation of chitin, which is the structural element of the crustacean’s shell, insect’s cuticle and cell walls of fungi. Chitosan films have been successfully developed and used for packaging foods such as fruits, vegetables, and meats (Chien, Sheu, & Yang, 2007; Darmadji & Izumimoto, 1994; Moreira, Pereda, Marcovich, & Roura, 2011). The elastic and transparent chitosan films are known for their solid mechanical properties and selective permeability for gases.
Moreover, they are less sensitive to water in comparison with hydroxylpropyl methylcellulose films (Sebti, Chollet, Degraeve, Noel, & Peyrol, 2007). These non-toxic, biodegradable, and biocompatible films also have unique antimicrobial properties (Durango, Soares, Benevides, Teixeira, Carvalho, Wobeto, et al., 2006). However, for certain food products, the limited antimicrobial activity of pure chitosan films does not reach the antiseptic level desired by packers (Ye, Neetoo, & Chen, 2008). For example, to enhance the efficacy of chitosan film against foodborne pathogens, nisin, potassium sorbate, and sodium benzoate, have been incorporated into the chitosan coating to extend the shelf-life of frankfurters (Samelis, Bedie, Sofos, Belk, Scanga, & Smith, 2002). The incorporation of an additional antimicrobial agent could enhance its antimicrobial activity and expand the scope of its application.

Different antimicrobial chemicals such as organic acids, inorganic gases, metals or ammonium compounds have been incorporated into plastic packaging materials (Suppakul, Miltz, Sonneveld, & Bigger, 2003). However, because of environmental problems associated with chemicals and plastics and the health concerns of the consumers, extensive studies have been conducted to use natural bioactive agents including antimicrobial enzymes, essential oils, bacteriocins, and phenolic compounds in biodegradable or edible packaging materials (Coma, 2008; Ramos-Garcia, Bosquez-Molina, Hernandez-Romano, Zavala-Padilla, Terres-Rojas, Alia-Tejacal, et al.,
2012; Vodnar, 2012). For instance, edible chitosan films containing lactoferrin as a natural antimicrobial agent were developed and shown to exhibit significant antimicrobial activity against both *Listeria monocytogenes* and *Escherichia coli* O157:H7 (Brown, Wang, & Oh, 2008). Chitosan-based formulations with lime or thyme essential oil, beeswax, and oleic acid were found effective in inhibiting *Escherichia coli* DH5a (Ramos-Garcia, et al., 2012). Others have incorporated oleoresins and tea extracts into chitosan films to improve their antimicrobial activity against *Listeria monocytogenes* (Vodnar, 2012).

The use of phenolic compounds and extracts in active packaging attracts a particular interest since these compounds show potent antimicrobial activity in food systems and their intake can make a contribution to human health (Komes, Horzic, Belscak, Ganic, & Vulic, 2010). Gallic acid is a widely available phenolic acid that has been shown to possess strong antimicrobial activity (Chanwitheeuk, Teerawutgulrag, Kilburn, & Rakariyatham, 2007). Gallic acid extracted from *Caesalpinia mimosoides* Lamk (Leguminosae) exhibited the activity against the bacteria *Salmonella typhi* and *Staphylococcus aureus* with MIC values of 2.50 and 1.250 g/L, respectively (Chanwitheeuk, Teerawutgulrag, Kilburn, & Rakariyatham, 2007). Gallic acid purified from the flowers of *Rosa chinensis* Jacq. has also been shown to posses significant antibacterial activity against pathogenic *Vibrios* species (A. J. Li, Chen, Zhu, Jiang, Zhang, & Gu, 2007). All of these reports in the literature have indicated promising
potential in using gallic acid to develop antimicrobial packaging materials against pathogens and spoilage bacteria.

In addition, gallic acid appears to enhance elasticity, thus acting as a plasticizer and eliminates classical brittleness and flexibility problems (Alkan, Aydemir, Arcan, Yavuzdurmaz, Atabay, Ceylan, et al., 2011; Hager, Vallons, & Arendt, 2012). Gallic acid incorporation during the formation of chitosan-gallic acid polymers yielded a conjugate with a superior hydroxyl radical scavenging capacity (Pasanphan, Buettner, & Chirachanchai, 2010). This is an encouraging aspect of gallic acid used in manufacturing food packaging chitosan films. Thus, our purpose is to evaluate the potential to develop a new cost-effective edible chitosan film with improved antimicrobial and mechanical properties by incorporating a widely accessible natural antimicrobial compound.

2. Materials and methods

2.1 Film-making materials

Chitosan (95-98% deacetylated, $M_V = 8.0 \times 10^5$ Da) (Moreira, Pereda, Marcovich, & Roura, 2011) and glacial acetic acid (99%, analytical reagent grade) were obtained from Sigma-Aldrich Co. (St. Louis, MO, USA); Glycerol, as a plasticizing agent, and gallic acid, as an antimicrobial agent, were purchased from Fisher Scientific Inc. (Pittsburgh, PA, USA).
2.2 Film preparation

The edible films were prepared by dissolving 1 g of chitosan in 100 g of 1% acetic acid solution and stirred, at room temperature, until chitosan was completely dissolved. Glycerol at 0.3 g/100 g was added as a plasticizer. Film without gallic acid was designated as film 0 (F0) which was used as a control. Gallic acid was added at varying concentrations: 0.5 g/100 g in film 1 (F1), 1.0 g/100 g in film 2 (F2) and 1.5 g/100 g in film 3 (F3), respectively. Equal volumes (150 mL) of the film solutions were spread on glass plates (200 x 200 mm) and dried for 12 h at 35 ± 2 °C in an incubator (New Brunswick Scientific Excella* E24, Fisher Scientific Inc. PA, USA). The films were removed from the glass plate with a thin spatula and conditioned at 23 ± 2 °C and 50 ± 2% relative humidity (RH) before running further tests.

2.3 Bacterial strains and cultures

Two gram-negative bacteria: Escherichia coli 0157:H7 (ATCC 43895) and Salmonella typhimurium (ATCC 19585) and two gram-positive bacteria: Bacillus subtilis (ATCC 1254) and Listeria innocua (F4078) were used. E. coli was incubated in Luria-Bertani (LB) broth media, B. subtilis and L. innocua were incubated in Nutrient broth media, and S. typhimurium was incubated in Brain-heart infusion (BHI) broth media at 37 °C for 24 h.
2.4 Antimicrobial activity

Antimicrobial properties of the crafted films were determined by the log reduction method with a slight modification (Ravishankar, Zhu, Olsen, McHugh, & Friedman, 2009). Briefly, culture medium broth was inoculated with certain amount of suspension of bacteria. The bacterial concentration in the seeding culture was approximately $6 \times 10^8$ CFU/mL. Serial dilutions of the suspension were performed and the optical density values were tested to achieve a standard curve. Square film pieces ($20 \times 20$ mm) were sterilized and introduced into a test tube containing 5 mL fresh suspension of bacteria and incubated at 37 °C for 24 h. Optical density of culture media was measured at 620 nm using a Perkin-Elmer HTS 7000 Bio Assay reader, and cell concentrations were determined. All samples/standards were run in triplicates.

2.5 Film thickness (FT)

FT was measured with a 0-25 mm dial thickness gauge with an accuracy of ±0.01 mm in five random locations for each film. Averages were calculated for mechanical properties, water vapor permeability and oxygen permeability.

2.6 Mechanical properties

Tensile strength (TS) and elongation at break (EB) tests were performed at room temperature ($23 \pm 2 ^\circ C$) using a universal testing machine (PARAM XLW (B) Auto
Tensile Tester, Jinan, China) with a 200 N load cell according to the standard testing method ASTM D882-01 (ASTM, 2001). Sample films, previously equilibrated at 23 ± 2 °C and 50 ± 2% RH, were cut into strips 15 mm wide and 130 mm long. Five specimens from each film were tested. The initial grip separation and mechanical crosshead speed were set at 80 mm and 50 mm/min, respectively.

TS (MPa) was calculated using the following equation:

\[ TS = \frac{F_{\text{max}}}{A} \]

where \( F_{\text{max}} \) is the maximum load (N) needed to pull the sample apart; \( A \) is cross-sectional area (m²) of the samples.

EB (%) was calculated using the following equation:

\[ EB = \left( \frac{L}{80} \right) \times 100 \]

where \( L \) is the film elongation (mm) at the moment of rupture; 80 is the initial grip length (mm) of samples.

### 2.7 Physical properties

#### 2.7.1 Water vapor permeability (WVP)

The WVP of the films was determined by a Water Vapor Permeability Tester (PERME TSY-TIL, Labthink Instruments Co., Ltd, Jinan, China) according to the standard testing method ASTM E-96-95 (ASTM, 1995). Test cups were 2/3 filled with distilled water. The test cups were tightly covered with circular film samples. Difference in water vapor pressure between the inside and outside of the cup causes water vapor diffusion through the sample. For each sample, five replicates were tested. The weight of
the cups was measured at 1 h intervals for 24 h. Simple linear regression was used to estimate the slope of weight loss versus time plot.

\[ WVP (g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}) \] was calculated using the following equation (Sztuka & Kolodziejska, 2009):

\[ WVP = \frac{WVTR \times L}{\Delta p} \]

where \( WVTR \) (water vapor transmission rate) is slope/film test area (g/m\(^2\)·s); \( L \) is film thickness (m); \( \Delta p \) is partial water vapor pressure difference (Pa) between the two sides of the film.

### 2.7.2 Oxygen permeability (OP)

OP of the films was determined by a Gas Permeability Tester (GDP-C) (Brugger Feinmechanik GmbH, Germany) according to the standard testing method ASTM D3985-05 (ASTM, 2005). An edible film was mounted in a gas transmission cell to form a sealed semi-barrier between chambers. Oxygen enters the cell on one side of the film from a chamber which is at a specific high pressure and leaves from the other which is at a specific lower pressure with a controlled flow rate (100 mL/min). The lower pressure chamber was initially evacuated and the transmission of oxygen through the test specimen was indicated by an increase of pressure. For each sample, at least five replicates were tested. \( OP (mol \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}) \) was calculated using the following equation (Ayranci & Tunc, 2003):
\[ OP = \frac{(M \times L)}{(A \times T \times \Delta p)}; \] where \( M \) is the volume of gas permeated through the film (mol); \( L \) is film thickness (m); \( A \) is the area of the exposed film surface (m\(^2\)); \( T \) is the measured time interval (s); \( \Delta p \) is difference (Pa) between the two sides of the film.

2.8 Microstructure properties

2.8.1 Fourier transform infrared spectroscopy (FT-IR)

FT-IR was recorded on a Spectrum 400 FT-IR spectrometer (PerkinElmer Inc., USA). Films were placed on the steel plate and measured directly in a spectral range of 650 to 4000 cm\(^{-1}\) at the resolution of 4 cm\(^{-1}\), and the average of 128 scans was taken for each sample.

2.8.2 Scanning electron microscopy (SEM)

The films were cut into small pieces (10 × 10 mm), dried and mounted on aluminum stubs using a double-sided adhesive carbon tape and sputtered with a thin layer of gold. Microstructures of the surface and cross-section of the dried films were observed by a Scanning Electron Microscope (SEM, JSM-6510LV-LGS, JEOL Co., Ltd. USA) and Field Emission Scanning Electron Microscope (FESEM, JSM-7600F, JEOL Co., Ltd. USA), respectively. All samples were examined at an accelerating voltage of 15 KV and magnified 10,000 X.
2.9 Statistical analysis

Analysis of variance (ANOVA) was carried out using SPSS software (version 17). When the p-value was less than or equal to 0.05, the results were considered significant.

3. Results and discussion

3.1 Antimicrobial properties

To examine the antimicrobial properties of the studied edible films, *E. coli*, *S. typhimurium*, *B. subtilis*, and *L. innocua*, which are very significant pathogens in the food industry, were tested. The results are shown in Fig. 1. The edible films incorporated with different concentrations of gallic acid significantly improved the antimicrobial activities of the chitosan film against all the tested bacteria ($p<0.05$). The log reduction increases with the increase of gallic acid concentration, which illustrates the antimicrobial activity of gallic acid.

The results show that the log reductions of *B. subtilis*, ranged from 1.24 to 5.75, are demonstrated to be higher than other bacteria. The minimum inhibitory concentration (MIC) of chitosan against *B. subtilis* is 0.10 g/L (Yadav & Bhise, 2004). The log reductions of *E. coli* ranges from 0.57 to 2.31. The MIC of chitosan against *E. coli* is 0.75 mg/mL (Tao, Qian, & Xie, 2011) and gallic acid demonstrated significant antimicrobial activity against *E. coli* (MIC=1 g/L) (Binutu & Cordell, 2000). Combining gallic acid with chitosan shows a potent antimicrobial effect according to our results. The log
reductions of *S. typhimurium* ranged from 1.07 to 1.75. Furthermore, the combination of gallic acid in chitosan films exhibited obvious reduction in the growth of *L. innocua*, resulting in an approximate 2.5-log reduction. *Listeria* growth inhibition was recorded for gallic acid at 0.45 g/L (Aissani, Coroneo, Fattouch, & Caboni, 2012). The diameters of the zone of inhibition (mm) of chitosan against *E. coli* and *B. subtilis* were 18 mm and 40 mm respectively (Yadav & Bhise, 2004), which verified that *B. subtilis* is more sensitive than *E. coli* to chitosan.

Furthermore, the film showed a higher effectiveness against *B. subtilis* and *L. innocua* compared to *E. coli* and *S. typhimurium* which may be rationalized by the characteristic difference of the outer membrane between Gram-positive bacteria and Gram-negative bacteria (Ramos, Santos, Leao, Pereira, Silva, Fernandes, et al., 2012).

### 3.2 Mechanical properties

Mechanical properties are important to edible films, because adequate mechanical strength ensures the integrity of the film and its freedom from minor defects (Murillo-Martinez, Pedroza-Islas, Lobato-Calleros, Martinez-Ferez, & Vernon-Carter, 2011). Table 1 shows mechanical property values of four edible films after conditioning at 23 ± 2 °C and 50 ± 2% RH. Differences in the TS and EB of F0, F1, F2 and F3 were observed and could be attributed to the addition of gallic acid interacting with chitosan and forming new linkages that affect film structure.
Our chitosan control film (F0) had TS and EB values of 13.876 MPa and 32.36%, respectively (Table 1). These values are comparable to the previous reports with TS and EB in the range of 12-20 MPa and 17-42%, respectively (Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2009). The TS and EB of chitosan films are affected by the type of chitosan used, the presence of glycerol, and the temperature during film drying (Pereda, Amica, & Marcovich, 2012). Interestingly, the incorporation of 0.5 g/100 g and 1.0 g/100 g gallic acid into chitosan films significantly increased its TS ($P<0.05$). The addition of a relatively lower dose of gallic acid (F1) exhibited the highest TS among the films, which could be attributed to the formation of intermolecular hydrogen bonding between the $NH_3^+$ of the chitosan backbone and the $OH^-$ of gallic acid (Sun, Liu, Li, Lv, Li, Xu, et al., 2011). The intermolecular hydrogen bonding between chitosan and gallic acid could enhance the cross-linkage, which decreases the molecular mobility and the free volume of chitosan (Pasanphan & Chirachanchai, 2008). This phenomenon was reported by other researchers in similar systems. For example, the cross-linking of chitosan-olive oil emulsion as well as chitosan-oleic acid films resulted in an increased TS due to the enhancement of the structural bonds in the polymer network (Pereda, Amica, & Marcovich, 2012; Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2009). However, when the added concentration of gallic acid is higher than 0.5 g/100 g, the TS of the resulting films decreased with increasing gallic acid concentration. As we can see, the TS of F3 (9.207 MPa) was lower than that of F0 (13.876 MPa). It is possible that the excessive
gallic acid scattered in the film crack the inner structure of the film (Fig. 3d and Fig. 4d).

The decrease of EB values in F1-F3 films indicated that the incorporation of gallic acid into the chitosan film resulted in a strong reaction between filler and matrix, which decreased EB by the motion restriction of the matrix. The decreased EB values from 20% to 6% of chitosan films indicated that the incorporation of cellulose whiskers into the chitosan matrix resulted in strong interactions between matrix and filler, which restricted the motion of the matrix (Q. Li, Zhou, & Zhang, 2009).

3.3 Physical properties

3.3.1 Water vapor permeability (WVP)

Table 2 shows there was a significant difference between the WVP values of F0-F3 films incorporated with different gallic acid concentrations ($p<0.05$). When the added gallic acid was below 1.0 g/100 g, the WVP values of the films decreased significantly ($p<0.05$) with increasing gallic acid concentrations, which could be because the bulky benzene ring group of gallic acid obstructs the inter- and intra-molecular hydrogen bond network of chitosan (Pasanphan & Chirachanchai, 2008). However, when the concentration of gallic acid was higher than 1.0 g/100 g, the WVP of the film increased ($p<0.05$), which may be related to the excessive gallic acid scattered in the film (Fig. 3d and Fig. 4d) which subsequently decreased the intermolecular forces between polymer chains and increased the free volume and segmental motions (Sothornvit & Krochta, 2008).
In addition, carboxyl groups and hydroxyl groups of gallic acid are hydrophilic groups, which might promote water transfer in the matrix (Sanchez-Gonzalez, Chafer, Chiralt, & Gonzalez-Martinez, 2010).

The WVP values of our crafted films were in the similar range of the previous reports (Pereda, Amica, & Marcovich, 2012; Sanchez-Gonzalez, Chafer, Chiralt, & Gonzalez-Martinez, 2010). In general, the WVP of chitosan films is lower than that of corn-zein film and wheat gluten film, but higher than that of hydroxypropylmethyl cellulose film (Park & Chinnan, 1995). Nonetheless, the WVP values of the films are all in the order of $10^{-10} \text{g} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{m}^{-2} \cdot \text{Pa}^{-1}$, which are qualified for preventing migration of moisture from fruits or vegetables.

### 3.3.2 Oxygen permeability (OP)

Oxygen is an essential component of lipid oxidation, which decreases food quality and shortens shelf life (Sothornvit & Krochta, 2000). The OP values of the chitosan edible films are shown in Table 2. The incorporation of gallic acid into the films plays an important role in the improvement of OP. From the results, the OP value of F1 is the lowest, which is significantly different from other films ($p<0.05$). The OP value of F3 is $1.39 \times 10^{-18} \text{mol} \cdot \text{m}^{-1} \cdot \text{s}^{-1} \cdot \text{Pa}^{-1}$, being the highest, indicates that F3 is not qualified for good oxygen prevention properties compared with the other films. The high OP value of F3 might be due to the non-cross-linking gallic acid particles scattered in the film which may
have decreased the intermolecular forces between polymer chains, thus increasing the free volume and segmental motions (Sothornvit & Krochta, 2001), and resulting in the formation of pores. This result can also be verified by Fig. 3d and Fig. 4d, where obvious pores are shown. The OP values of these films ranging from 0.50 to $1.46 \times 10^{-18}$ mol·m$^{-1}$·s$^{-1}$·Pa$^{-1}$ show a better oxygen prevention property compared to wheat gluten film ($34.6 \times 10^{-18}$ mol·m$^{-1}$·s$^{-1}$·Pa$^{-1}$) and soy protein film ($31.5 \times 10^{-18}$ mol·m$^{-1}$·s$^{-1}$·Pa$^{-1}$) (Choi & Han, 2002; Mehyar & Han, 2004).

3.4 Microstructure properties

3.4.1 Fourier transform infrared spectroscopy (FT-IR)

FT-IR spectroscopy was employed to analyze the hydrogen bonds in the films. The FT-IR spectra of control films and films containing gallic acid were shown in Fig. 2. Figure 2a shows the F0 film spectrum, which is similar to the chitosan films developed by others (Q. Li, Zhou, & Zhang, 2009). To facilitate the coupling reaction with primary amine groups in chitosan, the carboxylic group of gallic acid is activated by converting the carboxylic acid group into ester, as reported previously (Lee, Lee, Lee, Kim, Lee, & Byun, 2005). Gallic acid could be conjugated at C-2 to obtain an amide linkage, or at C-3 and C-6 to obtain an ester linkage (Pasanphan & Chirachanchai, 2008). The spectra of F1, F2 and F3 films showed significant peaks around 1700 cm$^{-1}$ and 1640 cm$^{-1}$, while F0 did not. These peaks
correspond to ester and amide linkages between chitosan and gallic acid, respectively (Pasanphan & Chirachanchai, 2008). Detected ester and amide linkages are unlikely due to either gallic acid or chitosan individually (Yu, Mi, Pang, Jiang, Kuo, Wu, et al., 2011). These results suggest the conjugation of the gallate group with chitosan in the films. A sharp peak at 3267 cm$^{-1}$, detected only in F3 but not in the other films, corresponds to -OH group. The peaks at 1610 cm$^{-1}$, 1201 cm$^{-1}$ and 1021 cm$^{-1}$ referred to the C=O, C-O, and O-H respectively. These peaks demonstrated the presence of -COOH in F3, which indicates the existence of excessive gallic acid in F3. From these results, it can be concluded that the gallate group of gallic acid was successfully cross-linked with chitosan via amide and ester linkages for F1 and F2, though there was more than enough unreacted gallic acid in F3 (Fig. 3d and Fig. 4d).

3.4.2 Scanning electron microscopy (SEM)

SEM was employed to observe the films’ surface morphology and cross-section as well as the homogeneity of the composite, the presence of voids, and the homogeneous structure of the films (Khan, Khan, Salmieri, Le Tien, Riedl, Bouchard, et al., 2012). The surface and cross-section morphologies of the films are shown in Fig. 3 and Fig. 4, respectively. Figure 3a and 3b shows a flat and smooth appearance and a good compact structure of the F0 and F1 films, respectively, which indicates that the mixtures of chitosan and glycerol, as well as chitosan, glycerol and gallic acid are homogenous in
these films. This is further supported by Fig. 4a and Fig. 4b, where the cross-section morphologies of both F0 and F1 films are also smooth. In Fig. 3c, the appearance of a white spot suggests some heterogeneity in the chitosan matrix when gallic acid was incorporated into chitosan. This phenomenon is further verified by Fig. 4c, where some bands are presented. Figure 3d and Fig. 4d show abundant plaques and obvious pores which interrupt the inner structure of the film (F3), therefore reducing the tensile strength and elongation at break by 33.6% and 66.1% compared to the pure chitosan film (F0), respectively. The interrupted inner structure also affects the permeability of the film (F3): the water vapor permeability and oxygen permeability were increased by 47.2% and 3.0%, respectively. Overall, these figures suggest that the films with lower concentrations of gallic acid (F1 and F2) have better mechanical and barrier properties compared to the film added with 1.5 g/100 g gallic acid (F3). Meanwhile, our results agree with the concept that surface properties are important to the barrier properties of films, where a homogeneous and smooth surface is usually preferred (Wang, Sun, Lian, Wang, Zhou, & Ma, 2013). Water permeability and moisture sensitivity of edible film were directly affected by its surface properties and hydrophobicity (Wu, Sakabe, & Isobe, 2003). For instance, films casted from unmodified zein showed higher water permeability and moisture sensitivity than modified zein films partially because the former films had larger water surface contact angles, while the modified zein films had stronger surface hydrophobicity through the acylation reaction (Shi, Huang, Yu, Lee, & Huang, 2011).
**4 Conclusions**

The results of this study suggest that chitosan films incorporated with gallic acid improved the antimicrobial properties of the film significantly, and the films reduced microbial growth by 2.5-log reduction. Furthermore, incorporation of lower concentrations of gallic acid (0.5 g/100 g) increased the TS of the chitosan film by 71.3%. It also improved the barrier properties of chitosan film by reducing WVP and OP by 11.1% and 58.5%, respectively. Surface morphology of the film with lower gallic acid concentration revealed a homogeneous structure. Overall, chitosan films with gallic acid could be used as novel food packaging material due to their excellent antimicrobial and mechanical properties.

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References


Fig. 1. Antimicrobial properties of the edible gallic acid-chitosan versus chitosan-only films (The log reduction of cell number of *B. subtilis* (a), *L. innocua* (b), *E. coli* (c), and *S. typhimurium* (d)). F0 represents the edible film casted from chitosan without gallic acid; F1 represents edible film casted from chitosan with 0.5 g/100 g gallic acid (w/v); F2 represents edible film casted from chitosan with 1.0 g/100 g gallic acid (w/v); F3 represents edible film casted from chitosan with 1.5 g/100 g gallic acid (w/v). Bars with different letters indicate significant difference (*p*<0.05).

Fig. 2. FT-IR spectra of the edible gallic acid-chitosan and chitosan-only films (a. represents the edible film casted from chitosan without gallic acid; b. represents edible film casted from chitosan with 0.5 g/100 g gallic acid (w/v); c. represents edible film casted from chitosan with 1.0 g/100 g gallic acid (w/v); d. represents edible film casted from chitosan with 1.5 g/100 g gallic acid (w/v)).

Fig. 3. SEM of surface of the edible gallic acid-chitosan and chitosan-only films (a. represents the edible film casted from chitosan without gallic acid; b. represents edible film casted from chitosan with 0.5 g/100 g gallic acid (w/v); c. represents edible film casted from chitosan with 1.0 g/100 g gallic acid (w/v); d. represents edible film casted from chitosan with 1.5 g/100 g gallic acid (w/v)).
Fig. 4. SEM of the cross-section of the edible gallic acid-chitosan and chitosan-only films (a. represents the edible film casted from chitosan without gallic acid; b. represents edible film casted from chitosan with 0.5 g/100 g gallic acid (w/v); c. represents edible film casted from chitosan with 1.0 g/100 g gallic acid (w/v); d. represents edible film casted from chitosan with 1.5 g/100 g gallic acid (w/v)).
Table 1. Mechanical properties of the edible gallic acid-chitosan and chitosan-only films

<table>
<thead>
<tr>
<th>Film code</th>
<th>FT (mm)</th>
<th>TS (MPa)</th>
<th>EB (%)</th>
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<tr>
<td>F0</td>
<td>0.107 ± 0.006&lt;sup&gt;b&lt;/sup&gt;</td>
<td>13.876 ± 0.604&lt;sup&gt;c&lt;/sup&gt;</td>
<td>32.36 ± 1.18&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F1</td>
<td>0.108 ± 0.009&lt;sup&gt;b&lt;/sup&gt;</td>
<td>23.773 ± 0.453&lt;sup&gt;a&lt;/sup&gt;</td>
<td>33.15 ± 2.53&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>F2</td>
<td>0.111 ± 0.001&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.394 ± 1.405&lt;sup&gt;b&lt;/sup&gt;</td>
<td>25.56 ± 0.58&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>F3</td>
<td>0.141 ± 0.001&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.207 ± 0.616&lt;sup&gt;d&lt;/sup&gt;</td>
<td>10.97 ± 0.95&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

F0 represents edible film casted from chitosan without gallic acid; F1 represents edible film casted from chitosan with 0.5 g/100 g gallic acid (w/v); F2 represents edible film casted from chitosan with 1.0 g/100 g gallic acid (w/v); F3 represents edible film casted from chitosan with 1.5 g/100 g gallic acid (w/v). Superscripts in same column with different letters indicate significant differences (p<0.05).
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<thead>
<tr>
<th>Film code</th>
<th>FT (mm)</th>
<th>WVP ((g\cdot m^{-1}\cdot s^{-1}\cdot Pa^{-1})\times10^{-10})</th>
<th>OP ((mol\cdot m^{-1}\cdot s^{-1}\cdot Pa^{-1})\times10^{-18})</th>
</tr>
</thead>
<tbody>
<tr>
<td>F0</td>
<td>0.107 ± 0.006(^b)</td>
<td>2.52 ± 0.03(^b)</td>
<td>1.35 ± 0.03(^a)</td>
</tr>
<tr>
<td>F1</td>
<td>0.108 ± 0.009(^b)</td>
<td>2.24 ± 0.05(^c)</td>
<td>0.56 ± 0.06(^c)</td>
</tr>
<tr>
<td>F2</td>
<td>0.111 ± 0.001(^b)</td>
<td>2.23 ± 0.04(^c)</td>
<td>0.90 ± 0.03(^b)</td>
</tr>
<tr>
<td>F3</td>
<td>0.141 ± 0.001(^a)</td>
<td>3.71 ± 0.07(^a)</td>
<td>1.39 ± 0.07(^a)</td>
</tr>
</tbody>
</table>

F0 represents edible film casted from chitosan without gallic acid; F1 represents edible film casted from chitosan with 0.5 g/100 g gallic acid (w/v); F2 represents edible film casted from chitosan with 1.0 g/100 g gallic acid (w/v); F3 represents edible film casted from chitosan with 1.5 g/100 g gallic acid (w/v). Superscripts in same column with different letters indicate significant differences \((p<0.05)\).
Fig. 1
Fig. 3