6-1-2014

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

Xiuxiu Sun
Wayne State University

Zhe Wang
School of Biological and Agricultural Engineering, Jilin University, China

Hoda Kadouh
Wayne State University

Kequan Zhou
Wayne State University, kzhou@wayne.edu

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

Xiuxiu Sun [a], Zhe Wang [b], Hoda Kadouh [a], Kequan Zhou [a,*]

[a] Department of Nutrition and Food Science, Wayne State University, Detroit, MI 48202, United States, [b] School of Biological and Agricultural Engineering, Jilin University, No. 5988 Renmin Street, Changchun, Jilin 130025, China

[*]Corresponding author: Kequan Zhou, tel.: +1 313 577 3444; fax: +1 313 577 8616; email address: kzhou@wayne.edu.

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

(Pereda, Amica, & Marcovich, 2012). 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 (Chanwitheesuk, 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 (Chanwitheesuk, 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 =8.0×10^5 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 × 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 6×10^8 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 × 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 ± 2 °C) using a universal testing machine (PARAM XLW (B) Auto

155 Tensile Tester, Jinan, China) with a 200 N load cell according to the standard testing
156 method ASTM D882-01 (ASTM, 2001). Sample films, previously equilibrated at 23 ±
157 2 °C and 50 ± 2% RH, were cut into strips 15 mm wide and 130 mm long. Five
158 specimens from each film were tested. The initial grip separation and mechanical
159 crosshead speed were set at 80 mm and 50 mm/min, respectively.
160 TS (MPa) was calculated using the following equation:
161 $TS = F_{max}/A$; where F_{max} is the maximum load (N) needed to pull the sample apart; A
162 is cross-sectional area (m^2) of the samples.
163 EB (%) was calculated using the following equation:
164 $EB = (L/80)\times 100$; where L is the film elongation (mm) at the moment of rupture; 80 is
165 the initial grip length (mm) of samples.
166
167 **2.7 Physical properties**
168 **2.7.1 Water vapor permeability (WVP)**
169 The WVP of the films was determined by a Water Vapor Permeability Tester
170 (PERME TSY-TIL, Labthink Instruments Co., Ltd, Jinan, China) according to the
171 standard testing method ASTM E-96-95 (ASTM, 1995). Test cups were 2/3 filled with
172 distilled water. The test cups were tightly covered with circular film samples. Difference
173 in water vapor pressure between the inside and outside of the cup causes water vapor
174 diffusion through the sample. For each sample, five replicates were tested. The weight of

175 the cups was measured at 1 h intervals for 24 h. Simple linear regression was used to
176 estimate the slope of weight loss versus time plot.
177 WVP ($g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) was calculated using the following equation (Sztuka &
178 Kolodziejska, 2009): $WVP = (WVTR \times L)/\Delta p$; where *WVTR* (water vapor transmission
179 rate) is slope/film test area ($g/m^2 \cdot s$); *L* is film thickness (m); Δp is partial water vapor
180 pressure difference (Pa) between the two sides of the film.
181
182 **2.7.2 Oxygen permeability (OP)**
183 OP of the films was determined by a Gas Permeability Tester (GDP-C) (Brugger
184 Feinmechanik GmbH, Germany) according to the standard testing method ASTM
185 D3985-05 (ASTM, 2005). An edible film was mounted in a gas transmission cell to form
186 a sealed semi-barrier between chambers. Oxygen enters the cell on one side of the film
187 from a chamber which is at a specific high pressure and leaves from the other which is at
188 a specific lower pressure with a controlled flow rate (100 mL/min). The lower pressure
189 chamber was initially evacuated and the transmission of oxygen through the test
190 specimen was indicated by an increase of pressure. For each sample, at least five
191 replicates were tested. OP ($mol \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) was calculated using the following equation
192 (Ayranci & Tunc, 2003):

193 $OP = (M \times L)/(A \times T \times \Delta p)$; where M is the volume of gas permeated through the film
194 (mol); L is film thickness (m); A is the area of the exposed film surface (m^2); T is the
195 measured time interval (s); Δp is difference (Pa) between the two sides of the film.
196
197 **2.8 Microstructure properties**
198 **2.8.1 Fourier transform infrared spectroscopy (FT-IR)**
199 FT-IR was recorded on a Spectrum 400 FT-IR spectrometer (PerkinElmer Inc., USA).
200 Films were placed on the steel plate and measured directly in a spectral range of 650 to
201 4000 cm^{-1} at the resolution of 4 cm^{-1}, and the average of 128 scans was taken for each
202 sample.
203
204 **2.8.2 Scanning electron microscopy (SEM)**
205 The films were cut into small pieces (10 × 10 mm), dried and mounted on aluminum
206 stubs using a double-sided adhesive carbon tape and sputtered with a thin layer of gold.
207 Microstructures of the surface and cross-section of the dried films were observed by a
208 Scanning Electron Microscope (SEM, JSM-6510LV-LGS, JEOL Co., Ltd. USA) and
209 Field Emission Scanning Electron Microscope (FESEM, JSM-7600F, JEOL Co., Ltd.
210 USA), respectively. All samples were examined at an accelerating voltage of 15 KV and
211 magnified 10,000 X.
212

213 **2.9 Statistical analysis**

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

216

217 **3. Results and discussion**

218 **3.1 Antimicrobial properties**

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

226 The results show that the log reductions of *B.subtilis*, ranged from 1.24 to 5.75, are
227 demonstrated to be higher than other bacteria. The minimum inhibitory concentration
228 (MIC) of chitosan against *B. subtilis* is 0.10 g/L (Yadav & Bhise, 2004). The log
229 reductions of *E. coli* ranges from 0.57 to 2.31. The MIC of chitosan against *E. coli* is 0.75
230 mg/mL (Tao, Qian, & Xie, 2011) and gallic acid demonstrated significant antimicrobial
231 activity against *E. coli* (MIC=1 g/L) (Binutu & Cordell, 2000). Combining gallic acid
232 with chitosan shows a potent antimicrobial effect according to our results. The log

233 reductions of *S. typhimurium* ranged from 1.07 to 1.75. Furthermore, the combination of
234 gallic acid in chitosan films exhibited obvious reduction in the growth of *L. innocua*,
235 resulting in an approximate 2.5-log reduction. *Listeria* growth inhibition was recorded for
236 gallic acid at 0.45 g/L (Aissani, Coroneo, Fattouch, & Caboni, 2012). The diameters of
237 the zone of inhibition (mm) of chitosan against *E. coli* and *B. subtilis* were 18 mm and 40
238 mm respectively (Yadav & Bhise, 2004), which verified that *B. subtilis* is more sensitive
239 than *E. coli* to chitosan.

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

244

245 **3.2 Mechanical properties**

246 Mechanical properties are important to edible films, because adequate mechanical
247 strength ensures the integrity of the film and its freedom from minor defects
248 (Murillo-Martinez, Pedroza-Islas, Lobato-Calleros, Martinez-Ferez, & Vernon-Carter,
249 2011). Table 1 shows mechanical property values of four edible films after conditioning
250 at 23 ± 2 °C and 50 ± 2% RH. Differences in the TS and EB of F0, F1, F2 and F3 were
251 observed and could be attributed to the addition of gallic acid interacting with chitosan
252 and forming new linkages that affect film structure.

253 Our chitosan control film (F0) had TS and EB values of 13.876 MPa and 32.36%,
254 respectively (Table 1). These values are comparable to the previous reports with TS and
255 EB in the range of 12-20 MPa and 17-42%, respectively (Vargas, Albors, Chiralt, &
256 Gonzalez-Martinez, 2009). The TS and EB of chitosan films are affected by the type of
257 chitosan used, the presence of glycerol, and the temperature during film drying (Pereda,
258 Amica, & Marcovich, 2012). Interestingly, the incorporation of 0.5 g/100 g and 1.0 g/100
259 g gallic acid into chitosan films significantly increased its TS (P<0.05). The addition of a
260 relatively lower dose of gallic acid (F1) exhibited the highest TS among the films, which
261 could be attributed to the formation of intermolecular hydrogen bonding between the
262 NH_3^+ of the chitosan backbone and the OH^- of gallic acid (Sun, Liu, Li, Lv, Li, Xu, et al.,
263 2011). The intermolecular hydrogen bonding between chitosan and gallic acid could
264 enhance the cross-linkage, which decreases the molecular mobility and the free volume of
265 chitosan (Pasanphan & Chirachanchai, 2008). This phenomenon was reported by other
266 researchers in similar systems. For example, the cross-linking of chitosan-olive oil
267 emulsion as well as chitosan-oleic acid films resulted in an increased TS due to the
268 enhancement of the structural bonds in the polymer network (Pereda, Amica, &
269 Marcovich, 2012; Vargas, Albors, Chiralt, & Gonzalez-Martinez, 2009). However, when
270 the added concentration of gallic acid is higher than 0.5 g/100 g, the TS of the resulting
271 films decreased with increasing gallic acid concentration. As we can see, the TS of F3
272 (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,

293 2001). In addition, carboxyl groups and hydroxyl groups of gallic acid are hydrophilic
294 groups, which might promote water transfer in the matrix (Sanchez-Gonzalez, Chafer,
295 Chiralt, & Gonzalez-Martinez, 2010).

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

303

304 **3.3.2 Oxygen permeability (OP)**

305 Oxygen is an essential component of lipid oxidation, which decreases food quality
306 and shortens shelf life (Sothornvit & Krochta, 2000). The OP values of the chitosan
307 edible films are shown in Table 2. The incorporation of gallic acid into the films plays an
308 important role in the improvement of OP. From the results, the OP value of F1 is the
309 lowest, which is significantly different from other films (p<0.05). The OP value of F3 is
310 1.39 ×10^{-18} mol·m^{-1}·s^{-1}·Pa^{-1}, being the highest, indicates that F3 is not qualified for good
311 oxygen prevention properties compared with the other films. The high OP value of F3
312 might be due to the non-cross-linking gallic acid particles scattered in the film which may

313 have decreased the intermolecular forces between polymer chains, thus increasing the
314 free volume and segmental motions(Sothornvit & Krochta, 2001), and resulting in the
315 formation of pores. This result can also be verified by Fig. 3d and Fig. 4d, where obvious
316 pores are shown. The OP values of these films ranging from 0.50 to 1.46 $\times 10^{-18}$
317 $mol \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$ show a better oxygen prevention property compared to wheat gluten film
318 (34.6×10^{-18} $mol \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) and soy protein film ($31.5 \times 10^{-18}$ $mol \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}$) (Choi &
319 Han, 2002; Mehyar & Han, 2004).
320
321 **3.4 Microstructure properties**
322 **3.4.1 Fourier transform infrared spectroscopy (FT-IR)**
323 FT-IR spectroscopy was employed to analyze the hydrogen bonds in the films. The
324 FT-IR spectra of control films and films containing gallic acid were shown in Fig. 2.
325 Figure 2a shows the F0 film spectrum, which is similar to the chitosan films developed
326 by others (Q. Li, Zhou, & Zhang, 2009).
327 To facilitate the coupling reaction with primary amine groups in chitosan, the
328 carboxylic group of gallic acid is activated by converting the carboxylic acid group into
329 ester, as reported previously (Lee, Lee, Lee, Kim, Lee, & Byun, 2005). Gallic acid could
330 be conjugated at C-2 to obtain an amide linkage, or at C-3 and C-6 to obtain an ester
331 linkage (Pasanphan & Chirachanchai, 2008). The spectra of F1, F2 and F3 films showed
332 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.

Acknowledgements

Authors recognize and appreciate the financial support from Wayne State University Graduate Research Fellow (UGRF).

References

Aissani, N., Coroneo, V., Fattouch, S., & Caboni, P. (2012). Inhibitory Effect of Carob (Ceratonia siliqua) Leaves Methanolic Extract on Listeria monocytogenes. *Journal of Agricultural and Food Chemistry, 60*(40), 9954-9958.

Alkan, D., Aydemir, L. Y., Arcan, I., Yavuzdurmaz, H., Atabay, H. I., Ceylan, C., & Yemenicioglu, A. (2011). Development of Flexible Antimicrobial Packaging Materials against Campylobacter jejuni by Incorporation of Gallic Acid into Zein-Based Films. *Journal of Agricultural and Food Chemistry, 59*(20), 11003-11010.

Ayranci, E., & Tunc, S. (2003). A method for the measurement of the oxygen permeability and the development of edible films to reduce the rate of oxidative reactions in fresh foods. *Food Chemistry, 80*(3), 423-431.

Bajpai, S. K., Chand, N., & Chaurasia, V. (2010). Investigation of Water Vapor Permeability and Antimicrobial Property of Zinc Oxide Nanoparticles-Loaded Chitosan-Based Edible Film. *Journal of Applied Polymer Science, 115*(2), 674-683.

Binutu, O. A., & Cordell, G. A. (2000). Gallic Acid derivatives from mezoneuron benthamianum leaves. *Pharmaceutical Biology, 38*(4), 284-286.

Bravin, B., Peressini, D., & Sensidoni, A. (2006). Development and application of polysaccharide-lipid edible coating to extend shelf-life of dry bakery products. *Journal of Food Engineering, 76*(3), 280-290.

Brown, C. A., Wang, B. W., & Oh, J. H. (2008). Antimicrobial activity of lactoferrin against foodborne pathogenic bacteria incorporated into edible chitosan film. *Journal of Food Protection, 71*(2), 319-324.

Chanwitheesuk, A., Teerawutgulrag, A., Kilburn, J. D., & Rakariyatham, N. (2007). Antimicrobial gallic acid from Caesalpinia mimosoides Lamk. *Food Chemistry, 100*(3), 1044-1048.

Chien, P. J., Sheu, F., & Yang, F. H. (2007). Effects of edible chitosan coating on quality and shelf life of sliced mango fruit. *Journal of Food Engineering, 78*(1), 225-229.

Choi, W. S., & Han, J. H. (2002). Film-forming mechanism and heat denaturation effects on the physical and chemical properties of pea-protein-isolate edible films. *Journal of Food Science, 67*(4), 1399-1406.

Coma, V. (2008). Bioactive packaging technologies for extended shelf life of meat-based products. *Meat Science, 78*(1-2), 90-103.

Darmadji, P., & Izumimoto, M. (1994). Effect of Chitosan in Meat Preservation. *Meat Science, 38*(2), 243-254.

Durango, A. M., Soares, N. F. F., Benevides, S., Teixeira, J., Carvalho, M., Wobeto, C., & Andrade, N. J. (2006). Development and evaluation of an edible antimicrobial film based on yam starch and chitosan. *Packaging Technology and Science, 19*(1), 55-59.

Hager, A. S., Vallons, K. J. R., & Arendt, E. K. (2012). Influence of Gallic Acid and Tannic Acid on the Mechanical and Barrier Properties of Wheat Gluten Films. *Journal of Agricultural and Food Chemistry, 60*(24), 6157-6163.

Khan, A., Khan, R. A., Salmieri, S., Le Tien, C., Riedl, B., Bouchard, J., Chauve, G., Tan, V., Kamal, M. R., & Lacroix, M. (2012). Mechanical and barrier properties of nanocrystalline cellulose reinforced chitosan based nanocomposite films. *Carbohydrate Polymers, 90*(4), 1601-1608.

Komes, D., Horzic, D., Belscak, A., Ganic, K. K., & Vulic, I. (2010). Green tea preparation and its influence on the content of bioactive compounds. *Food Research International, 43*(1), 167-176.

Lee, S., Lee, J., Lee, D. Y., Kim, S. K., Lee, Y., & Byun, Y. (2005). A new drug carrier, Nalpha-deoxycholyl-L: -lysyl-methylester, for enhancing insulin absorption in the intestine. *Diabetologia, 48*(3), 405-411.

Li, A. J., Chen, J. X., Zhu, W. M., Jiang, T., Zhang, X. H., & Gu, Q. Q. (2007). Antibacterial activity of gallic acid from the flowers of Rosa chinensis Jacq. against fish pathogens. *Aquaculture Research, 38*(10), 1110-1112.

Li, Q., Zhou, J. P., & Zhang, L. N. (2009). Structure and Properties of the Nanocomposite Films of Chitosan Reinforced with Cellulose Whiskers. *Journal of Polymer Science Part B-Polymer Physics, 47*(11), 1069-1077.

Mehyar, G. R., & Han, J. H. (2004). Physical and mechanical properties of highamylose rice and pea starch films as affected by relative humidity and plasticizer. *Journal of Food Science, 69*(9), E449-E454.

Moreira, M. D., Pereda, M., Marcovich, N. E., & Roura, S. I. (2011). Antimicrobial Effectiveness of Bioactive Packaging Materials from Edible Chitosan and Casein Polymers: Assessment on Carrot, Cheese, and Salami. *Journal of Food Science, 76*(1), M54-M63.

Murillo-Martinez, M. M., Pedroza-Islas, R., Lobato-Calleros, C., Martinez-Ferez, A., & Vernon-Carter, E. J. (2011). Designing W-1/O/W-2 double emulsions stabilized by protein-polysaccharide complexes for producing edible films: Rheological, mechanical and water vapour properties. *Food Hydrocolloids, 25*(4), 577-585.

Park, H. J., & Chinnan, M. S. (1995). Gas and Water-Vapor Barrier Properties of Edible Films from Protein and Cellulosic Materials. *Journal of Food Engineering, 25*(4), 497-507.

Pasanphan, W., Buettner, G. R., & Chirachanchai, S. (2010). Chitosan gallate as a novel potential polysaccharide antioxidant: an EPR study. *Carbohydrate Research, 345*(1), 132-140.

Pasanphan, W., & Chirachanchai, S. (2008). Conjugation of gallic acid onto chitosan: An approach for green and water-based antioxidant. *Carbohydrate Polymers, 72*(1), 169-177.

Pereda, M., Amica, G., & Marcovich, N. E. (2012). Development and characterization of edible chitosan/olive oil emulsion films. *Carbohydrate Polymers, 87*(2), 1318-1325.

Ramos-Garcia, M., Bosquez-Molina, E., Hernandez-Romano, J., Zavala-Padilla, G., Terres-Rojas, E., Alia-Tejacal, I., Barrera-Necha, L., Hernandez-Lopez, M., & Bautista-Banos, S. (2012). Use of chitosan-based edible coatings in combination with other natural compounds, to control Rhizopus stolonifer and Escherichia coli DH5 alpha in fresh tomatoes. *Crop Protection, 38*, 1-6.

Ramos, O. L., Santos, A. C., Leao, M. V., Pereira, J. O., Silva, S. I., Fernandes, J. C., Franco, M. I., Pintado, M. E., & Malcata, F. X. (2012). Antimicrobial activity of edible coatings prepared from whey protein isolate and formulated with various antimicrobial agents. *International Dairy Journal, 25*(2), 132-141.

Ravishankar, S., Zhu, L. B., Olsen, C. W., McHugh, T. H., & Friedman, A. (2009). Edible Apple Film

Wraps Containing Plant Antimicrobials Inactivate Foodborne Pathogens on Meat and Poultry Products. *Journal of Food Science, 74*(8), M440-M445.

Samelis, J., Bedie, G. K., Sofos, J. N., Belk, K. E., Scanga, J. A., & Smith, G. C. (2002). Control of Listeria monocytogenes with combined antimicrobials after postprocess contamination and extended storage of frankfurters at 4 degrees C in vacuum packages. *Journal of Food Protection, 65*(2), 299-307.

Sanchez-Gonzalez, L., Chafer, M., Chiralt, A., & Gonzalez-Martinez, C. (2010). Physical properties of edible chitosan films containing bergamot essential oil and their inhibitory action on Penicillium italicum. *Carbohydrate Polymers, 82*(2), 277-283.

Sebti, I., Chollet, E., Degraeve, P., Noel, C., & Peyrol, E. (2007). Water sensitivity, antimicrobial, and physicochemical analyses of edible films based on HPMC and/or chitosan. *Journal of Agricultural and Food Chemistry, 55*(3), 693-699.

Shi, K., Huang, Y. P., Yu, H. L., Lee, T. C., & Huang, Q. R. (2011). Reducing the Brittleness of Zein Films through Chemical Modification. *Journal of Agricultural and Food Chemistry, 59*(1), 56-61.

Sothornvit, R., & Krochta, J. M. (2000). Oxygen permeability and mechanical properties of films from hydrolyzed whey protein. *Journal of Agricultural and Food Chemistry, 48*(9), 3913-3916.

Sothornvit, R., & Krochta, J. M. (2001). Plasticizer effect on mechanical properties of beta-lactoglobulin films. *Journal of Food Engineering, 50*(3), 149-155.

Sun, Y., Liu, Y., Li, Y. Z., Lv, M. Z., Li, P. W., Xu, H. L., & Wang, L. (2011). Preparation and characterization of novel curdlan/chitosan blending membranes for antibacterial applications. *Carbohydrate Polymers, 84*(3), 952-959.

Suppakul, P., Miltz, J., Sonneveld, K., & Bigger, S. W. (2003). Active packaging technologies with an emphasis on antimicrobial packaging and its applications. *Journal of Food Science, 68*(2), 408-420.

Sztuka, K., & Kolodziejska, I. (2009). The influence of hydrophobic substances on water vapor permeability of fish gelatin films modified with transglutaminase or 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC). *Food Hydrocolloids, 23*(3), 1062-1064.

Tao, Y., Qian, L. H., & Xie, J. (2011). Effect of chitosan on membrane permeability and cell morphology of Pseudomonas aeruginosa and Staphyloccocus aureus. *Carbohydrate Polymers, 86*(2), 969-974.

Vargas, M., Albors, A., Chiralt, A., & Gonzalez-Martinez, C. (2009). Characterization of chitosan-oleic acid composite films. *Food Hydrocolloids, 23*(2), 536-547.

Vodnar, D. C. (2012). Inhibition of Listeria monocytogenes ATCC 19115 on ham steak by tea bioactive compounds incorporated into chitosan-coated plastic films. *Chemistry Central Journal, 6*.

Wang, Z., Sun, X. X., Lian, Z. X., Wang, X. X., Zhou, J., & Ma, Z. S. (2013). The effects of ultrasonic/microwave assisted treatment on the properties of soy protein isolate/microcrystalline wheat-bran cellulose film. *Journal of Food Engineering, 114*(2), 183-191.

Wu, Q. X., Sakabe, H., & Isobe, S. (2003). Studies on the toughness and water resistance of zein-based polymers by modification. *Polymer, 44*(14), 3901-3908.

Yadav, A. V., & Bhise, S. B. (2004). Chitosan: A potential biomaterial effective against typhoid. *Current Science, 87*(9), 1176-1178.

509 Ye, M., Neetoo, H., & Chen, H. (2008). Control of Listeria monocytogenes on ham steaks by
510 antimicrobials incorporated into chitosan-coated plastic films. *Food Microbiology, 25*(2), 260-268.
511 Yu, S. H., Mi, F. L., Pang, J. C., Jiang, S. C., Kuo, T. H., Wu, S. J., & Shyu, S. S. (2011). Preparation and
512 characterization of radical and pH-responsive chitosan-gallic acid conjugate drug carriers.
513 *Carbohydrate Polymers, 84*(2), 794-802.
514

515

516 **Fig. 1. Antimicrobial properties of the edible gallic acid-chitosan versus**
517 **chitosan-only films** (The log reduction of cell number of *B. subtilis* (a)*, L. innocua* (b), *E.*
518 *coli* (c), and *S. typhimurium* (d)). F0 represents the edible film casted from chitosan
519 without gallic acid; F1 represents edible film casted from chitosan with 0.5 g/100 g gallic
520 acid (w/v); F2 represents edible film casted from chitosan with 1.0 g/100 g gallic acid
521 (w/v); F3 represents edible film casted from chitosan with 1.5 g/100 g gallic acid (w/v).
522 Bars with different letters indicate significant difference (p<0.05).
523
524 **Fig. 2. FT-IR spectra of the edible gallic acid-chitosan and chitosan-only films** (a.
525 represents the edible film casted from chitosan without gallic acid; b. represents edible
526 film casted from chitosan with 0.5 g/100 g gallic acid (w/v); c. represents edible film
527 casted from chitosan with 1.0 g/100 g gallic acid (w/v); d. represents edible film casted
528 from chitosan with 1.5 g/100 g gallic acid (w/v)).
529
530 **Fig. 3. SEM of surface of the edible gallic acid-chitosan and chitosan-only films** (a.
531 represents the edible film casted from chitosan without gallic acid; b. represents edible
532 film casted from chitosan with 0.5 g/100 g gallic acid (w/v); c. represents edible film
533 casted from chitosan with 1.0 g/100 g gallic acid (w/v); d. represents edible film casted
534 from chitosan with 1.5 g/100 g gallic acid (w/v)).
535

536 **Fig. 4. SEM of the cross-section of the edible gallic acid-chitosan and chitosan-only**
537 **films** (a. represents the edible film casted from chitosan without gallic acid; b. represents
538 edible film casted from chitosan with 0.5 g/100 g gallic acid (w/v); c. represents edible
539 film casted from chitosan with 1.0 g/100 g gallic acid (w/v); d. represents edible film
540 casted from chitosan with 1.5 g/100 g gallic acid (w/v)).
541
542

543 **Table 1. Mechanical properties of the edible gallic acid-chitosan and chitosan-only**

544 **films**

Film code	FT (mm)	TS (MPa)	EB (%)
F0	0.107 ± 0.006^{b}	13.876 ± 0.604^{c}	32.36 ± 1.18^{a}
F1	0.108 ± 0.009^{b}	23.773 ± 0.453^{a}	33.15 ± 2.53^{a}
F2	0.111 ± 0.001^{b}	18.394 ± 1.405^{b}	25.56 ± 0.58^{b}
F3	0.141 ± 0.001^{a}	9.207 ± 0.616^{d}	10.97 ± 0.95^{c}

545 F0 represents edible film casted from chitosan without gallic acid; F1 represents edible
546 film casted from chitosan with 0.5 g/100 g gallic acid (w/v); F2 represents edible film
547 casted from chitosan with 1.0 g/100 g gallic acid (w/v); F3 represents edible film casted
548 from chitosan with 1.5 g/100 g gallic acid (w/v). Superscripts in same column with
549 different letters indicate significant differences ($p<0.05$).

550

551

552 **Table 2. WVP and OP of the edible gallic acid-chitosan and chitosan-only films**

Film code	FT (mm)	WVP $(g \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}) \times 10^{-10}$	OP $(mol \cdot m^{-1} \cdot s^{-1} \cdot Pa^{-1}) \times 10^{-18}$
F0	0.107 ± 0.006^{b}	2.52 ± 0.03^{b}	1.35 ± 0.03^{a}
F1	0.108 ± 0.009^{b}	2.24 ± 0.05^{c}	0.56 ± 0.06^{c}
F2	0.111 ± 0.001^{b}	2.23 ± 0.04^{c}	0.90 ± 0.03^{b}
F3	0.141 ± 0.001^{a}	3.71 ± 0.07^{a}	1.39 ± 0.07^{a}

553 F0 represents edible film casted from chitosan without gallic acid; F1 represents edible
554 film casted from chitosan with 0.5 g/100 g gallic acid (w/v); F2 represents edible film
555 casted from chitosan with 1.0 g/100 g gallic acid (w/v); F3 represents edible film casted
556 from chitosan with 1.5 g/100 g gallic acid (w/v). Superscripts in same column with
557 different letters indicate significant differences ($p<0.05$)

558

559

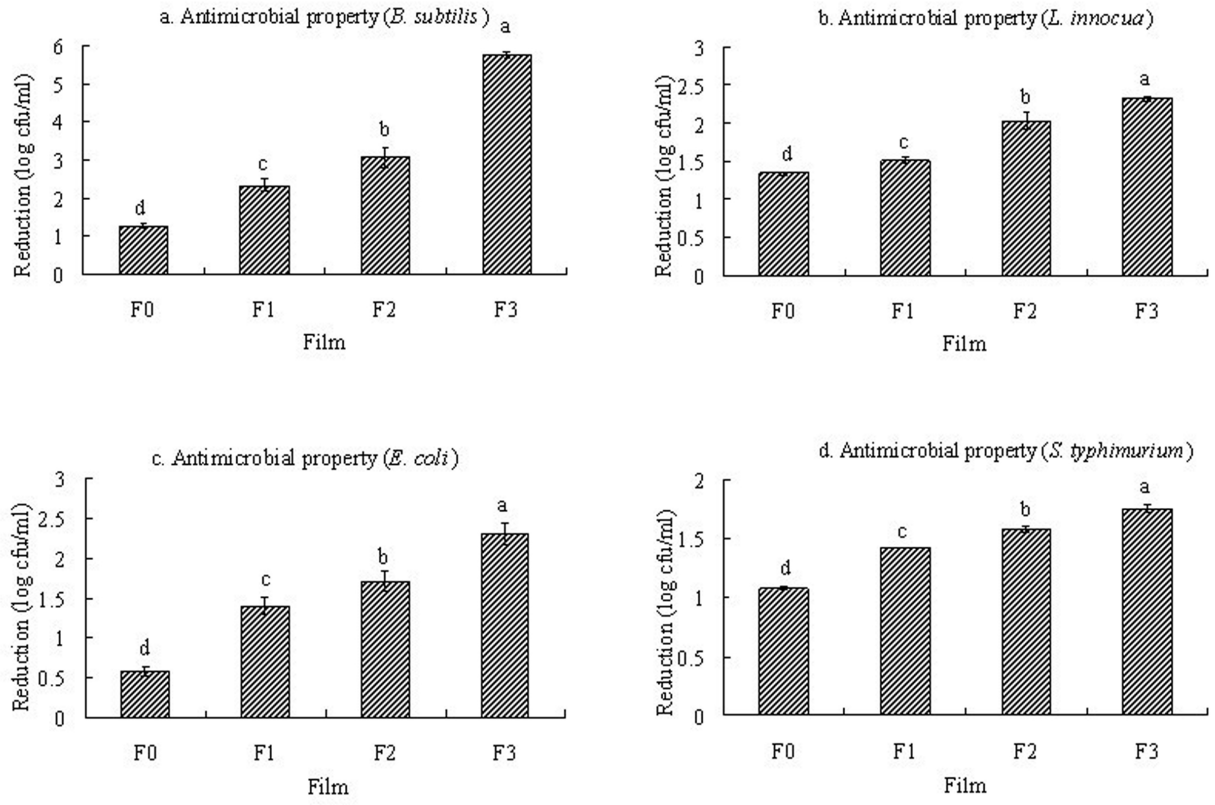
a. Antimicrobial property (B. subtilis)
Reduction (log cfu/ml)
Film
F0
F1
F2
F3
b. Antimicrobial property (L. innocua)
c. Antimicrobial property (E. coli)
d. Antimicrobial property (S. typhimurium)

Fig. 1

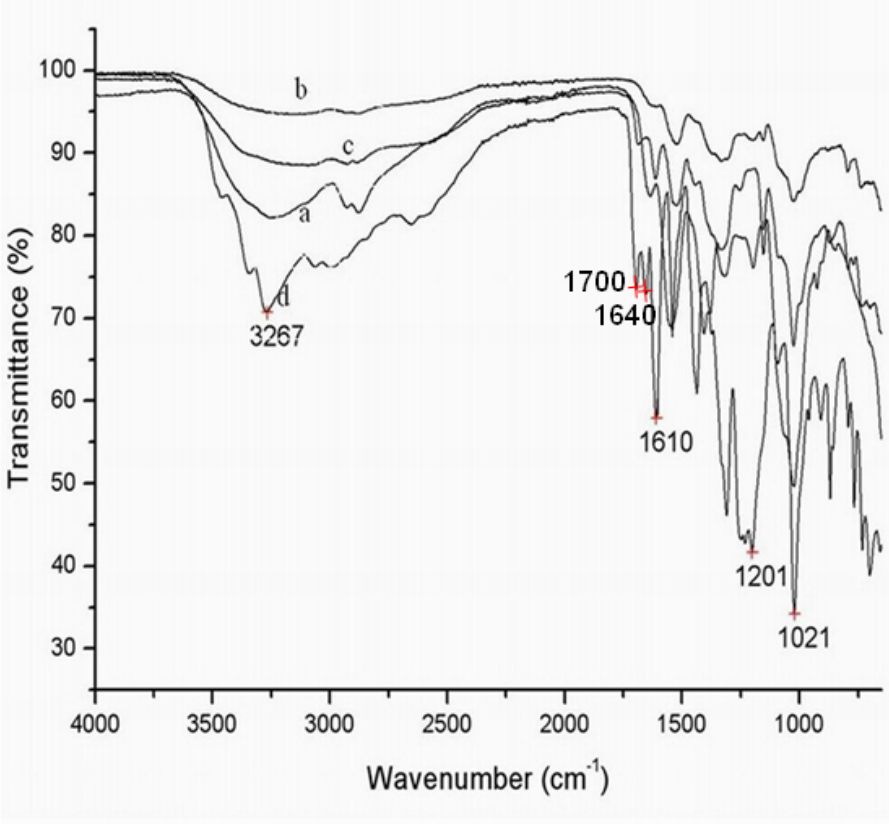
Transmittance (%)
Wavenumber (cm-1)
100
90
80
70
60
50
40
30
4000
3500
3000
2500
2000
1500
1000
a
b
c
d
3267
1700
1640
1610
1201
1021

Fig. 2

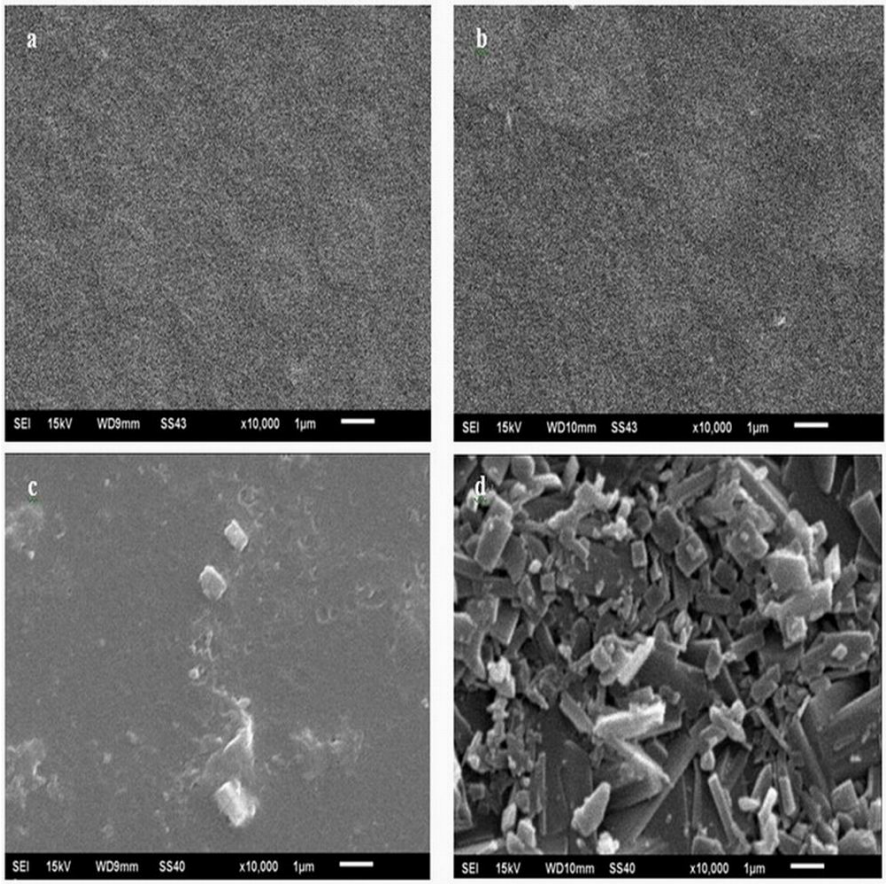
a
SEI 15kV WD9mm SS43 x10,000 1μm
b
SEI 15kV WD10mm SS43 x10,000 1μm
c
SEI 15kV WD9mm SS40 x10,000 1μm
d
SEI 15kV WD10mm SS40 x10,000 1μm

Fig. 3

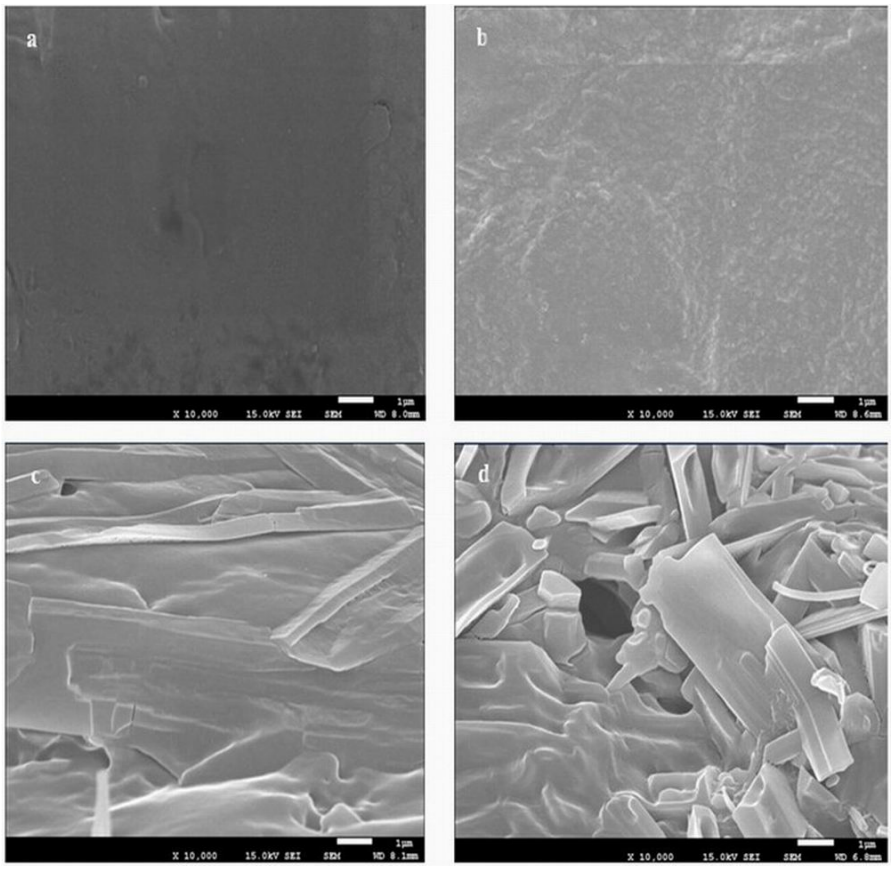
a
X 10,000 15.0kV SEI SEM WD 8.0mm 1μm
b
X 10,000 15.0kV SEI SEM WD 8.6mm 1μm
c
X 10,000 15.0kV SEI SEM WD 8.1mm 1μm
d
X 10,000 15.0kV SEI SEM WD 6.8mm 1μm

Fig. 4