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Characterization of Chitosan-Based Active Film Incorporating with Gallic Acid

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ABSTRACT: This study aims to investigate the effects of different gallic acid contents (0, 0.5, 1.0, and 1.5 mM) on the thickness (µm), moisture content (MC, %), water solubility (WS, %), water vapor permeability (WVP, mg mm/m^2 h kPa), and antioxidant activity (mg Trolox equivalent (TE)/g dry film) of chitosan films. The films were prepared from chitosan (1% w/v) combined with gallic acid to reach the concentrations of 0 (control), 0.5 (GA1), 1.0 (GA2), and 1.5 mM (GA3). The thickness of the gallic acid incorporated chitosan films was not significantly different. It was in the range of 33.267 ± 9.430 to 45.100 ± 10.484 µm, with the overall average of 38.225 µm. Chitosan films with various gallic acid contents expressed similar WS, at 3.699 % in terms of overall mean. Significant differences were observed in the MC, WVP, and antioxidant capacity expressed as DPPH radical scavenging activity of the gallic acid-chitosan films. The lowest MC was found in chitosan film with 1.0 mM gallic acid (GA2) (21.042 ± 1.979 %), whereas the highest was the sample with 0.5 mM gallic acid (GA1) (32.046 ± 0.854%). As the level of gallic acid elevated, WVP of the films declined but their DPPH radical scavenging activity enhanced significantly. GA3 was chosen as the best treatment since the chitosan films at 1.5 mM gallic acid demonstrated the lowest WVP and highest antioxidant activity, at 0.833 mg mm/m^2 h kPa and 13.548 mg TE/g dry film, respectively.

KEYWORDS: active film, chitosan, gallic acid, water vapor permeability, DPPH scavenging

I. INTRODUCTION

In recent years, traditional packaging is made from plastic materials that are widely used in food industry in order to preserve and protect food. Although plastics have been one of the most common packaging materials due to its convenience, availability, lightweight, low price and processing properties, the utilization of synthetic packaging films is witnessing a significant increase in the universal problems. From the manufacturing of plastic materials, there are a huge amount of fossil fuels consuming finite and non- renewable resources. The disposal of plastic is not biodegradable and takes thousands of years to decompose resulting in pollution and harm to animals as well as humans [1]. To solve the problems generated by plastic waste, environmentally friendly materials, known as innovative packaging, have been researched and developed as alternatives. The active biodegradable film is one kind of intelligent packaging with the addition of additives to improve protective properties of packaging as well as to extend the product's shelf life.

The biopolymers are commonly available for biodegradability for making edible film, biodegradable packaging. Chitosan is a kind of linear cationic polymer made from the deacetylation of chitin, a naturally occurring polysaccharide. The chemical structure of chitosan is a random linear polysaccharide, consisting of β-(1,4)-N-acetyl-D-glucosamine and β-(1,4)-D-glucosamine [2]. Chitosan is poorly soluble in water as its limitation although chitosan has a high biological activity with wide applications. Chitosan has been investigated a lot in the food packaging industry because of its film-forming properties and biological capabilities, such as biodegradability, biocompatibility, and nontoxicity [3, 4].

In order to enhance properties of the packaging, the additive compounds can be blended composition. The natural materials and agent compounds from animal and plant sources are significantly increased in science research. The chitosan-based coatings were investigated by adding polyphenols, are well-known as natural antioxidants. Gallic acid (3,4,5-trihydroxybenzoic acid, or GA), is a common polyphenolic molecule and an abundant phenolic acid [5]. It is commonly found in tea, nuts, wine, fruits, and vegetables, such as tea leaves, grapes, cherries, and longan seeds. Gallic acid is a non-toxic material and plays roles as a cross-linking agent and antioxidant. Gallic acid is a potential material in order to develop active biodegradable packaging. Gallic acid was observed to improve mechanical properties such as elongation at break, reduce UV absorption, and enhance antioxidant activities measured by radical scavenging assay and the ferric reducing ability on the research of gelatin-based films [6].

Therefore, the objectives of the research are to investigate the incorporation of various gallic acid contents (0, 0.5, 1.0, and 1.5 mM) into chitosan-based films. Furthermore, determination of the effect of gallic acid on physical properties of films such as moisture content, water solubility, water vapor permeability, as well as the antioxidant properties of films were tested.

II. MATERIALS AND METHODS

A. Materials

Chitosan was purchased from local company in Ninh Thuan province had a medium molecular weight with degree of deacetylation of 75-85%, viscosity 200- 800 cP, ash content ≤ 1.0%, moisture content ≤ 10%, and solubility in acidic solution. Gallic acid 2,2-diphenyl-1-picrylhydrazyl (DPPH), Trolox were purchased from Sigma (USA). Magnesium nitrate, sodium chloride, calcium chloride, and glycerol, acetic acid, methanol were purchased from Xylong, China. All other reagents used were of analytical grade.

B. Film formation

Chitosan solution (1%, w/v) was prepared following modified method of Ge at al. [7], by dissolving chitosan in an aqueous solution of acetic acid (2%, w/w) at 25 °C and stirring with a magnetic stirrer (MSH-20A, Daihan, Korea) for 4 h. After that, 30% glycerol (w/w chitosan) was added as the plasticizer and the solution was stirred for 1 h. Then gallic acid was added into solutions to reach a final concentration of 0.5, 1.0, and 1.5 mM, labelled as GA1, GA2, and GA3, respectively. The film without gallic acid was a control sample. All the solutions were homogenized by using a centrifuge (Universal 320R, Hettich, Germany) at 3,500 rpm for 10 min, in order to remove undissolved particles. Finally, these film-forming solutions were casted over the petri dishes and dried at 25 °C for 48 h. Then, the dried films were carefully peeled from the plates and stored with Mg $(NO_3)_2$ saturated solution (50 % relative humidity) for 24 h at 25 °C before further tests.

C. Characterization of the films

1) The thickness of film:

The film thickness was measured by using a digital micrometer at ten random positions along the samples. The mean values were used to calculate.

2) Moisture content and water solubility:

Moisture content (MC) and water solubility (WS) of the films were evaluated according to previously reported method of Ge et al. [7]. The film strips (2 cm × 2 cm) were accurately weighed (W_0) and dried in a vacuum drying oven at 105 ℃ for 24 h after which they were reweighed (W_1). The dried film was then placed in a tube which was filled with 30 mL distilled water and kept for 24 h at room temperature. Finally, the undissolved film was recovered and dried at 50 ℃ for 24 h and weighed as W_2. The film MC and WS were calculated as follows:

Moisture content (%) = $\frac{(W_0 - W_1)}{W_2} \times 100$

Water solubility (%) = $\frac{(W_1 - W_2)}{W_1} \times 100$

where W_0 (g) was the initial mass of the film sample, W_1 (g) was the weight of the dry initial film sample and the W_2 (g) was the final dry mass of the film sample.

3) Water vapor permeability:

The water vapor permeability (WVP) of the films was determined gravimetrically according to the method of Siripatrawan and Harte [8]. Film samples was sealed to special cups containing silica gel in order to provide 0% RH, then placed into a desiccator at room temperature and 95% RH maintained by saturated NaCl solution. The cup was weighed periodically (everyday) using a 4-digital analytical balance with a precision of 0.1 mg until steady state was reached. The water vapor transmission rate (WVTR) of the films was determined from the plot of weight gained versus time. The slope of the linear portion of this plot was represented the steady state amount of water vapor diffusing through the film per unit time. The slope was a regression coefficient of 0.99 or greater. The WVP of the films was calculated by multiplying the WVTR with the film thickness and dividing that by the water vapor pressure difference across the films. This test was conducted triplicate. The WVTR and WVP was determined by using the following equations:

$$WVTR = \frac{\Delta w}{A \Delta t}$$

$$WVP = WVTR \frac{x}{\Delta p}$$

where WVP is the water vapor permeability coefficient (g mm m^{-2} h^{-1} kPa^{-1}), x is the film thickness (mm), Δp is the partial water vapor pressure gradient between the inner (p1) and outer (p2) surface of the film in the chamber (kPa).

4) DPPH radical scavenging activity:

The samples for measurement of antioxidant properties were prepared by following the method of Ballester-Costa with modification [9]. Each film sample (0.01 g) was extracted in 15 mL of methanol absolute. The mixture was shaken for 1 min and then left for 30 min. Then, the mixture was used to determine the antioxidant properties by applied DPPH assay.

The measurement of the DPPH radical scavenging activity was modified from the methodology described by Siripatrawan and Harte [8]. Briefly, 4 mg of DPPH was dissolved in 100 ml of methanol to obtain a concentration of 0.1 mM/ml. Subsequently, 0.1 ml of film extracted solution was mixed with 4.9 mL of 0.1 mM methanolic solution of DPPH. The mixture was vortexed using a vortex for 1 min and incubated in the dark at ambient temperature for exactly 30 min. The absorbance of the mixture was then measured at 517 nm using a spectrophotometer. Trolox solutions in the specific concentration range (0, 0.2, 0.4, 0.6, 0.8, and 1.0 mM) were used to construct a calibration curve. The concentration of free radical scavenging compounds in the samples was expressed in mg of Trolox equivalents (TE) per gram dry weight of the film sample, calculated by using an equation that was obtained from the standard graph. This estimation of free scavenging compounds in the samples was analyzed in three replications, and the results were averaged. The percentage of DPPH free radical quenching activity was determined using the following equation:

$$\text{DPPH scavenging effect}(\%) = \frac{A_{DPPH} - A_S}{A_{DPPH}} \times 100$$

where A_{DPPH} is the absorbance value of the methanolic solution of DPPH and A_S is the absorbance value of the sample extracts.

D. Data analysis

Statistical analysis was conducted by analysis of variance (ANOVA), and the significance of each mean value was evaluated with Fisher's multiple range tests using the Minitab software at a level of confidence of 95%. All experimental data was expressed as mean ± standard deviation (SD).

III. RESULTS AND DISCUSSION

A. Photographs and the thickness of films

The figure 1 shows all the types of film samples. The control chitosan films were the most transparent among all samples.

The average thickness values of chitosan-based films with various concentrations of gallic acid were in the range from 33.267 ± 9.430 to 45.100 ± 10.484 µm as can be seen in the table 1. The films thickness in all samples was not significantly different ($P \geq 0.05$). This indicates that the incorporation of gallic acid into chitosan-based film did not affect the change of films thickness. Liu et al. and Wu et al. also showed that the thickness of film incorporated with different phenolic acids were similar to the samples [10,11].

Figure 1. Photographs of films, control, GA1, GA2, and GA3, repestively.

B. Moisture content and water solubility

The moisture content and water solubility of the films may be used to evaluate their water resistance, which is one of the most important factors affecting chitosan films in the food industry.

The moisture contents of chitosan film and chitosan films incorporating with gallic acid were shown in Table 1. The increase of incorporating conccentration of gallic acid led to a significant decrease ($p < 0.05$) in the moisture content of films from 32.046 ± 0.854 % to 21.042 ± 1.979 %. However, there were no significant differences between the control and the GA1 film, the GA2 film and GA 3 film. According to Aljawish at al., the strong hydrogen bond interactions between water molecules and hydroxyl group -OH and amine group $-NH_2$ of chitosan led to the high moisture content of chitosan film [12]. Moreover, higher concentration of

gallic acid added could cause less hydrogen bond interactions. Therefore, chitosan-gallic acid films showed relatively lower moisture contents than the control chitosan film.

The table 1 demonstrates that the water solubility of films incorporated with gallic acid were not significantly different ($p > 0.05$) with the increase of gallic acid content. The water vapor values of all kinds of film samples were fluctuated from 3.336 ± 0.150 to 4.045 ± 0.580 %. The higher water solubility of the gallic acid-embedded films might be due to the presence of hydrophilic group in chitosan. The lower solubility would indicate higher water resistance; thus, the chitosan films combined with gallic acid might improve the limitation of pure chitosan films in liquid food applications. According to the research of Wang et al., phenolic compounds could lower the availability of hydroxyl and amino groups in chitosan, and thus limit the chitosan and water interactions [13].

Table 1. Thickness, moisture content and water solubility of chitosan film incorporating with various concentrations of gallic acid

Gallic Acid Content (mM)	*Thickness (µm)*	*Moisture Content (%)*	*Water Solubility (%)*
0	33.733 ± 5.845 [a]	31.821 ± 0.786 [a]	4.045 ± 0.580 [a]
0.5	33.267 ± 9.430 [a]	32.046 ± 0.854 [a]	3.555 ± 0.222 [a]
1.0	40.800 ± 6.798 [a]	21.042 ± 1.979 [b]	3.740 ± 0.125 [a]
1.5	45.100 ± 10.484 [a]	22.171 ± 0.837 [b]	3.336 ± 0.150 [a]

Values are given as mean ± standard deviation (SD). Different letters in the same column indicate significantly different ($p < 0.05$).

C. Water vapor permeability

Water vapor permeability (WVP) represents the amount of water vapor passing through a material per unit area per unit time per unit barometric pressure. This is an important property in order to decide the appropriate food products for each packaging material. As shown in figure 2, the WVP decreased from 1.631 ± 0.130 to 0.833 ± 0.015 mg mm/ m^2 h kPa as the concentration of gallic acid increased into the chitosan matrix. The results showed that the WVP of the films significantly decreased ($p \geq 0.05$) with the effect of the incorporation of gallic acid. The gallic acid-chitosan films at the concentration of 1.0 and 1.5 mM (GA2 and GA3, respectively) expressed the lowest WVP values, at an average WVP value was 0.878 mg mm/ m^2 h kPa, which indicates the desirable property of food packaging. Similar results were also observed by many other researchers when incorporating other phenolic acid with chitosan films, such as gallic acid [6, 14], ferulic acid [1, 15, 16].

The increase in concentration of gallic acid into the chitosan film produced the films to become denser and less water vapor permeability. The decrease in WVP indicated the improved barrier property of gallic acid incorporating with chitosan-based film. The deterioration of barrier properties in watwer vapor was related to the hydrophilicity of chitosan [17]. The covalent bonding of gallic acid into the chitosan showed the limitation of the behavior of chitosan, decreased the affinity of gallic acid-chitosan films toward water [18]. Furthermore, the network between the benzene ring groups in gallic acid obstructed the inter- and intra-molecular hydrogen bond with chitosan, that caused the decrease in WVP [11].

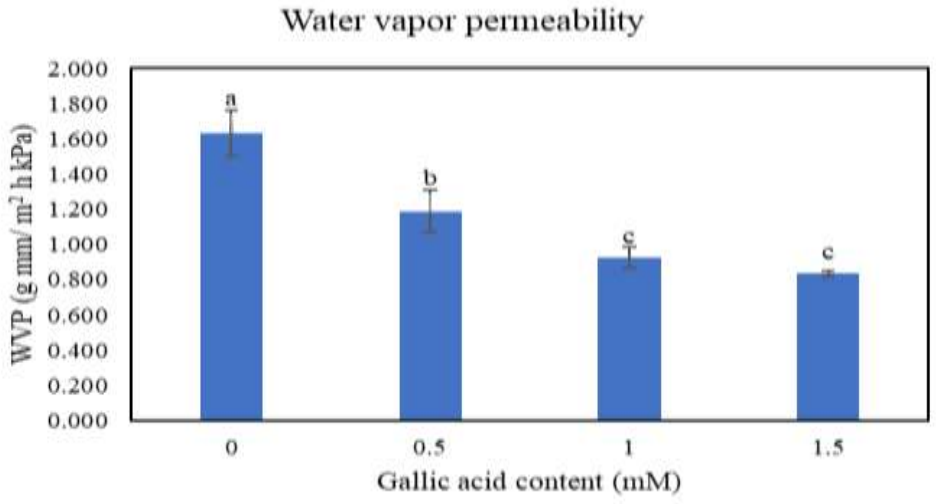

Figure 2. The water vapor permeability of chitosan film incorporating with various concentrations of gallic acid. ***Different letters indicate significantly different ($p < 0.05$).***

D. DPPH radical scavenging activity

In the DPPH test, the DPPH radical reacted with free radicals in the samples to change color from purple to a yellow-colored compound, diphenylpicrylhydrazine, and the extent of the reaction depends on the hydrogen donating ability of the antioxidants.

The results showed that DPPH scavenging activity of the films were significant differences ($p < 0.05$). The control film containing only chitosan had the lowest DPPH radical scavenging capacity, at 1.743 ± 0.357 mM TE/g dry film. However, the chitosan films without gallic acid did not show the significant difference in DPPH scavenging activity as compared to the chitosan film containing 0.5 mM gallic acid, in the range of 1.743 to 2.218 mM TE/ g dry film. By contrast, the presence of phenol products onto chitosan improved the antioxidant properties [12], which means chitosan films incorporating with gallic acid exhibited much higher DPPH radical scavenging activity than the films without the addition of gallic acid. Moreover, the DPPH radical scavenging activity of gallic acid combined films increased with the increase of the gallic acid content. The DPPH scavenging activity of gallic acid films incorporating with chitosan increased from 2.218 ± 0.358 to 13.548 ± 1.878 mM TE/g dry film with increasing gallic acid content concentration as shown in Figure 3. The values of the gallic acid-chitosan films at the concentration of 1.0 and 1.5 mM were increased approximately 3.5 and 5 folds as compared to that of the lower concentration of gallic acid incorporating in chitosan film (0.5 mM), respectively. The research of Wu et al. also reported the similarity in antioxidant activity that gallic acid grafted to chitosan films were higher antioxidant activity with the increase in the grafting ratio of gallic acid [11]. There were some previous studies that observed the same increase trend in antioxidant activity [10, 12, 13].

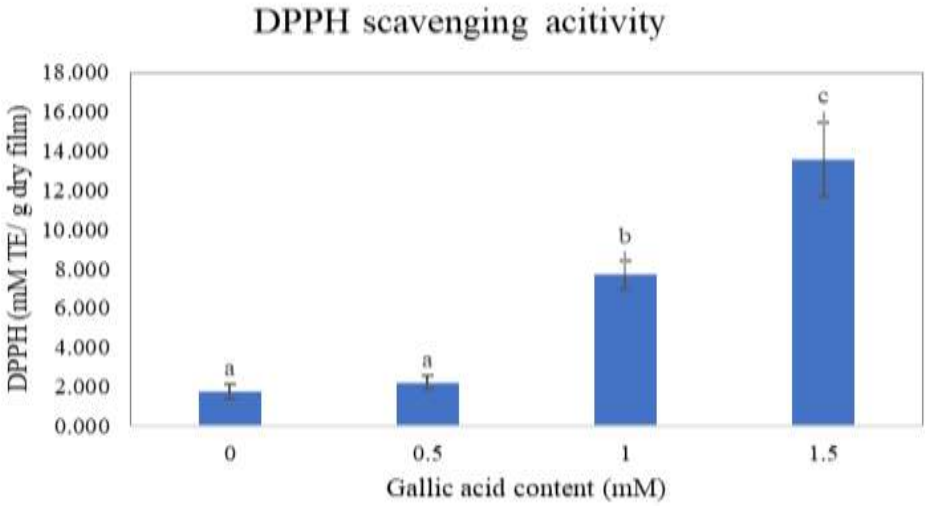

Figure 3. The DPPH scavenging activity of chitosan film incorporating with various concentrations of gallic acid.
Different letters indicate significantly different ($p < 0.05$).

The chitosan's scavenging process is based on the the reactions between free radicals with the remaining free amino groups in chitosan to produce stable macromolecule radicals, and these amino groups then could absorb a hydrogen ion from the solution to form ammonium groups [19]. Woranuch and Yoksan explained that the interaction between free radicals and the hydroxyl groups at the C3 or C6 positions, as well as the amino groups at the C2 position, should be linked to the antioxidant activity of chitosan film [17]. Therefore, the higher incorporating gallic acid contents contributes to the more effective antioxidant activity of gallic acid-chitosan films.

V. CONCLUSIONS

In this study, gallic acid was incorporated into chitosan films at four concentrations of 0, 0.5, 1.0, and 1.5 mM to investigate its effects on the thickness, moisture content, water solubility, water vapor permeability, and antioxidant activity of the films. The addition of gallic acid exerted no impacts on the thickness and WS of the chitosan films. The MCs of chitosan films at 1.0 and 1.5 mM gallic acid were significantly lower than those of 0.5 mM gallic acid samples and control. The WVP of the control was nearly double the values of 1.0 and 1.5 mM gallic acid samples. The chitosan films at 1.0 and 1.5 mM gallic acid exhibited 3.5- and 5-fold DPPH radical scavenging activity compared with the film containing 0.5 mM gallic acid. It is concluded that chitosan film containing 1.5 mM gallic acid was the best treatment with the lowest WVP and highest antioxidant capacity.

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