The modification of pomegranate polyphenol with ultrasound improves mechanical, antioxidant, and antibacterial properties of tuna skin collagen-chitosan film

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Abstract

To produce an edible film with high mechanical and physicochemical properties, Tuna skin collagen-chitosan (TSC-CTS) composite films were prepared by incorporating ultrasound (UT) and pomegranate polyphenols including gallic acid (GA), tannic acid (TA), and ellagic acid (EA), respectively. The tensile strength and the DPPH scavenging activity of the GA-UT-TSC-CTS film (ultrasound frequency of 28 ± 0.5 kHz, power of 100 W/L, sweep frequency cycle of 100 ms, duty ratio of 77% and time of 10 min; GA concentration of 1.0 g/L and reaction time of 10 min) were increased by 47.03% and 24.16 folds, respectively compared to the control (TSC-CTS film). Meanwhile, light transmittance and water vapor permeability of the GA-UT-TSC-CTS film were decreased by 29.26% and 15.70%, respectively. These positive modification results were attributed to the altered structure during the film formation process, which were verified by Fourier transform infrared spectroscopy (FTIR), circular dichroism (CD), X-ray diffraction (XRD), and thermogravimetry results. Moreover, the GA-UT-TSC-CTS film possessed moderate thermal stability and color indexes and improved antibacterial activity. The antibacterial effect of the film against Bacillus subtilis was the highest, followed by Escherichia coli, Listeria monocytogenes, and Staphylococcus aureus. Overall, the combination modification of gallic acid and ultrasound was an efficient modification method to improve the mechanical, antioxidant, and antibacterial properties of edible TSC-CTS films.

Introduction

Edible films prepared from food-derived substances (e.g., protein or polysaccharide) should have moderate mechanical properties, good biodegradability, low antigenicity, excellent biocompatibility, and affordable price before commercialization [1,2]. Collagen-based films possess good rigidity but poor hydrophobicity [3]. Chitosan-based films have moderate antibacterial activity but are brittle with low elongation at break value [4]. Either collagen or chitosan is insufficient to form an eligible edible film using the sole substance. Therefore, collagen and chitosan have been crosslinked to compensate for their disadvantages [5]. Nevertheless, the mechanical strength of the collagen-chitosan composite film was still poor and cannot support its packaging application [6,7]. Thus, modification of the composite film was necessary.

The plant polyphenol possesses antioxidant and antibacterial activity [8,9]. The hydroxyl groups in plant polyphenol molecules could cross-link with proteins, polysaccharides, and other macromolecules through hydrogen bonds. Therefore, polyphenols are a good chemical modification method for the film. Zhang et al. [10] found the mechanical properties (e.g., hardness and elongation at break) of carp myofibril-based film were improved through the modification of ferulic acid (FA), tannic acid (TA), rosmarinic acid (RosA), and syringic acid (SA). He et al. [3] added procyanidins into collagen solution. The addition of procyanidins retained the tertiary structure of collagen. The hydrophobicity, heat stability, and water resistance of the collagen film were improved after procyanidins modification. Wang et al. [11] reported a similar trend that the mechanical performance of gelatin film was improved after tea polyphenols-chitosan incorporation. Pomegranate pericarp is rich in gallic acid (GA), tannic acid (TA), ellagic acid (EA), etc. These pomegranate pericarp polyphenols have multifunctional properties such as antioxidant, antibacterial, antivirus, blood lipid
reduction, and prevention of cardiovascular diseases [12]. Nonetheless, most of the pomegranate pericarp is discarded during industrial production. In a similar case, tuna protein has good bioavailability [13]. However, tuna skin, a byproduct of tuna processing with high collagen content, is usually discarded as waste [14].

In addition, ultrasound treatment is a good physical modification method to promote the binding of protein and sugar, and the functional properties of conjugates can be improved after ultrasound treatment [15–19]. However, there is no report about the combined modification of pomegranate polyphenols with ultrasound in the preparation of composite films.

Therefore, in this study, ultrasound (UT) and pomegranate polyphenols (i.e., GA, TA, and EA) were incorporated into the tuna skin collagen-chitosan (TSC-CTS) composite film. This process was optimized to obtain the film with the best mechanical properties, water solubility, water vapor permeability, light transmittance, antioxidant capacity, and color profiles. The underpinning knowledge towards the structural changes, heat stability, and antibacterial activity was also studied to reveal the correlations between the modification of ultrasound and pomegranate polyphenols and the structural and functional properties of the film.

2. Materials and methods

2.1. Materials and reagents

Tuna skin was obtained from Ningbo Today Food Co. Ltd. (Zhejiang, China). Acetic acid, pepsin (1800 U/g), sodium chloride, chitosan, 10201, and CICC 20658, Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Acetic acid, pepsin (1800 U/g), sodium chloride, chitosan, 10201, and CICC 20658, Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Escherichia coli CICC 20658, Bacillus subtilis CICC 10732, Staphylococcus aureus CICC 10201, and Listeria monocytogenes ATCC 19111 were ordered from China Center of Industrial Culture Collection (Beijing, China). 1,1-Diphenyl-2-picryl-hydrazyl (DPPH), GA, TA, and EA were purchased from Sigma Company (St. Louis, MO, USA). All reagents used in the experiments were of analytical grade.

2.2. Methods

2.2.1. Extraction of tuna skin collagen

Tuna skin collagen (TSC) was extracted according to a previously described method [20] with some modifications. Briefly, tuna skin was pretreated and soaked in 0.5 mol/L acetic acid solution at the skin-to-solution ratio of 1:70 (w/v). Then, pepsin (0.6 mg/mL) was added to the above solution at pH 2.0 for the enzyme extraction. Other operations were the same as the literature reported method. The extracted TSC (the content of 90%) was stored at a 4°C refrigerator.

2.2.2. Ultrasound and polyphenol modified TSC-CTS film

Firstly, the TSC-CTS film was prepared according to a reported method [6] with some modifications. After adding glycerol (25 g), 2 g of TSC and 8 g of CTS in 100 mL distilled water were cross-linked at 55°C for 10 min under ultrasound treatment (sweep frequency of 28 ± 0.5 kHz, power density of 100 W/L, sweep frequency cycle of 100 ms, and pulse duty ratio of 77%), followed by a 50 min pomegranate polyphenol modification at the 55°C water bath. The total cross-linking time, including the ultrasonic time and polyphenol reaction time, was 1 h. After that, the cross-linking reaction was terminated by the fast cooling in an ice water bath. The ultrasound and polyphenol treated TSC-CTS solution was transferred to a disposable dish (90 mm in diameter) and dried at 40°C for 24 h. The dried film was stored in a desiccator at 25°C and relative humidity of about 58%.

To determine the best pomegranate polyphenol for film modification, GA, TA, and EA were added to the UT-TSC-CTS solution at the same concentration (0.5 g/L), respectively. Based on our preliminary results (shown in section 3.1), GA was selected for further optimization. GA was incorporated into the UT-TSC-CTS solution at different concentrations (0.1, 0.5, 1.0, 1.5, and 2.0 g/L) and a variety of reaction times (5, 10, 15, 20, and 25 min) to optimize the reaction conditions. The prepared GA-UT-TSC-CTS films were used for further tests. The TSC-CTS film without any ultrasound and polyphenol modification was prepared under the same conditions and selected as the control.

2.2.3. Determination of mechanical properties

The mechanical properties including tensile strength (TS) and elongation at break (EAB) were measured according to a previously reported method [21]. For this purpose, the film was cut into strips (20 × 60 mm). These strips were tested in a texture analyzer (TA-XT2i, Stable Micro Systems Company, England). The stretching rate and gauge length were set to 60 mm/min and 40 mm, respectively. All determinations were carried out in octuplicate and were averaged. The TS and EAB were calculated by using Eq. (1) and (2), respectively:

\[ TS = F/(d \times W) \]  

(1)

where, TS is the tensile strength (MPa), F is the maximum tension when the film breaks (N), d is the thickness of the film (mm), and W is the width of the film (mm).

\[ EAB = (L' - L_0') \times 100/L_0' \]  

(2)

where, EAB represents elongation at break (%), L’ represents the line distance when the film breaks (mm), and L₀’ indicates the line distance of the original film (mm).

2.2.4. Measurement of water solubility

The film was oven-dried to the equilibrium weight. The weight of this dried film was recorded as W_i (g). Then, this film was soaked in 50 mL distilled water at room temperature (25°C) for 24 h. After thoroughly drying in a 105°C oven, the weight of the film was obtained and recorded as W_f (g). All the measurements were carried out in triplicates. The water solubility (WS) was calculated by using Eq. (3) [22].

\[ WS(\%) = (W_f - W_i) \times 100/W_i \]  

(3)

2.2.5. Measurement of water vapor permeability

About 20 mL distilled water was transferred to a batch of 50 mL beakers. The beakers were closely covered by the film and put inside a desiccator containing silicon. The desiccator was stored in a 22°C oven. The weight of the film was recorded every 2 h with at least 6 collected data points. The water vapor permeability (WVP, g/m.s.Pa) was calculated using Eq. (4) [22]. Tests and measurements were carried out in triplicates.

\[ WVP = (\Delta m \times x)/(A \times \Delta P \times t) \]  

(4)

where, x is the thickness of the film (m), A is the contact area of the film (18.08 \times 10^{-4} m^2), \Delta m is the weight of the water passing through the film (g), t represents the test time (s), and \Delta P is the pressure differences between the two sides of the film (3179 Pa).

2.2.6. Measurement of light transmittance

The light transmittance of the film was determined according to a reported method [23] with some modifications. Briefly, the film was cut into 40 × 10 mm slices and placed on one side of a 1 cm cuvette. The absorbance (Abs) value of this cuvette was measured using a UV–Vis spectrophotometer (T6 New Century, General Analysis Beijing General Instrument Co., Ltd. Beijing, China) at 500 nm. The transmittance (T) was defined as follows:

\[ T(\%) = 10^{-2\times\text{Abs}} \]  

(5)
2.2.7. Measurement of antioxidant capacity

The antioxidant activity of the film was determined according to a previously reported method [24] with some modifications. For this purpose, the film (50 mg) was cut into fragments and dissolved in 2 mL methanol solution, which was soaked in a shaken water bath for 3 h. Then, approximately 0.5 mL of the above solution was mixed with 2 mL DPPH (0.2 mmol/L dissolved in ethanol) solution. The mixture was incubated in dark for 30 min. The absorbance values at 517 nm were measured using a UV spectrometer. The DPPH scavenging activity (%) was calculated using Eq. (6):

$$\text{DPPH scavenging activity} = \left(1 - \frac{\text{ABS}_1 - \text{ABS}_0}{\text{ABS}_1}\right) \times 100$$  \hspace{1cm} (6)

where, $$\text{ABS}_1$$ is the absorbance value of samples, $$\text{ABS}_0$$ is the absorbance value of control (0.5 mL sample mixed with 2 mL ethanol), and $$\text{ABS}_b$$ is the absorbance value of blank (0.5 mL methanol mixed with 2 mL DPPH solution).

2.2.8. Measurement of color profiles

The color profiles of the film were measured using a Color Quest XE colorimeter. Each measurement was carried out with 6 replications. Light to dark ($$L$$), red to green (a), and yellow to blue (b) values were reported. A higher $$L$$ value represents a lighter appearance, a higher a value represents a redder appearance and a higher b value means a yellower appearance. Color aberration was calculated using the following Eq. (7):

$$\Delta E = \sqrt{\Delta L^2 + \Delta a^2 + \Delta b^2}$$  \hspace{1cm} (7)

where, $$\Delta E$$ = color aberration, $$\Delta L = L - L_0$$, $$\Delta a = a - a_0$$, $$\Delta b = b - b_0$$, $$L$$, a, and b are the corresponding values of test samples. $$L_0$$, $$a_0$$, and $$b_0$$ are the corresponding values of control samples (the film without any ultrasound and polyphenol modification).

2.2.9. Determination of Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of the film were measured using a Fourier Transform Infrared Spectrometer (Nicolet is50, Thermo Electron Corporation, America) with a KRS-5 ATR probe. All spectra were obtained between 4000 and 500 cm\(^{-1}\) with 36 consecutive scans and a 4 cm\(^{-1}\) resolution.

2.2.10. Determination of circular dichroism (CD)

Secondary structural changes of the film were quantified using a CD instrument (J-815, JASCO Corporation, Japan). The molar ellipticity was recorded at 190–250 nm wavelength. The secondary structural features were calculated using CDpro software (IBM Corporation, NY, USA).

2.2.11. Determination of X-ray diffraction (XRD)

The crystallinity of the film was measured using an XRD instrument (D8 ADVANCE, Bruker AXS Inc., Madison, WI, USA).

2.2.12. Determination of thermogravimetry analysis (TGA)

The thermogravimetry analysis (TGA) of the film was measured using a synchronous thermal analyzer (STA449F3, NETZSCH Corporation, Germany). Briefly, five milligrams samples were accurately weighed and placed in a hermetically sealed stainless steel container. The container was heated in a range of 25–800 °C at a 10 °C/min heating rate. The nitrogen flow rate of the sample chamber was 70 mL/min.

2.2.13. Evaluation of antibacterial activity

The antibacterial activity of the film was quantified using the inhibition zone method according to the literature [24] with some modifications. For this purpose, the test bacteria were incubated in a sterile liquid medium and cultured in a constant temperature shaker (LRH-250, Shanghai Yiheng Scientific Instrument Co., Ltd., Shanghai, China) at 37 °C, 120 r/min for 24 h. The activated bacteria were then inoculated on sterile water. Such initial bacterial solution has a microbial content of $$10^{6-7}$$ CFU/mL. The initial bacterial solution (0.1 mL) was inoculated on LB solid medium. A sterile filter paper (6 mm diameter) with 10 μL film solution (dropped on the center of the filter paper) was placed on the LB solid medium. The filter paper dropped with sterile water was used as the blank. The LB solid medium was cultured in an incubator at 37 °C for 24 h. Finally, the diameter of the inhibition zone was measured. All measurements were repeated six times.

2.2.14. Statistical analysis

Each measurement was repeated at least three times. The data presented in this study are mean ± standard deviation. Statistical analysis was performed using SPSS (Version 19.0, IBM Corporation, NY, USA). Significance was calculated using Duncan’s test. The significance level was established at $$p < 0.05$$.

3. Results and discussion

3.1. Comparison of GA-UT, TA-UT, and EA-UT modification

The effects of GA-UT, TA-UT, and EA-UT modification on the mechanical properties, water solubility, water vapor permeability (WVP), transmittance, DPPH scavenging activity, and color profiles of the TSC-CTS film are presented in Fig. 1 and Table 1, respectively. Compared to the control without any ultrasound and polyphenol modifications, the GA-UT modified film possessed significantly the highest TS levels ($$p < 0.05$$) (Fig. 1A). This might be attributable to the high binding force (e.g., hydrogen bond) between the polyphenol hydroxyl groups and the collagen-chitosan molecules under the ultrasonic treatment (verified in section 3.3.1), which enhanced the endurance of psychological stress of the film. The GA-UT modified TSC-CTS film contained the highest TS (47.03% higher than that of the control), followed by the TA-UT modified film (35.80% higher than that of the control), and EA-UT modified film (24.07% higher than that of the control). Regarding the EAB value, it was significantly increased after an ultrasound and polyphenol modification ($$p < 0.05$$). This might be due to the altered structure of the composite film after an ultrasound and polyphenol modification (verified in section 3.3.1) [25–27]. The GA-UT modified TSC-CTS film contained the highest EAB (11.11% higher than that of the control), followed by the EA-UT modified film (6.73% higher than that of the control). The alterations of mechanical properties were similar to the literature. Wu et al. [28] incorporated tea polyphenols in pomelo peel film. The modification of tea polyphenols increased the TS value and the EAB value of the pomelo peel film. Ultrasound was also beneficial to the formation of the protein-chitosan polymer [15,19]. For the food packaging application, the improvements of TS and EAB are critical. Therefore, we recommended GA-UT modification for the improvement of mechanical properties.

Solubility of the TSC-CTS film was significantly increased ($$p < 0.05$$) after an ultrasound and polyphenol modification (Fig. 1B). The altered structure of the film after an ultrasound and polyphenol modification resulted in increased water solubility [29]. Compared to the control, the increase in water solubility of the EA-UT modified film was the highest (6.36%), followed by the GA-UT modified film (2.26%). However, the WVP of the film was significantly reduced ($$p < 0.05$$) after an ultrasound and polyphenol modification. This might be due to the hydrophobic groups being exposed on the surface of the complex structure after an ultrasound and polyphenol modification, which hindered water vapor penetration. Wu et al. [28] reported a similar result that the modification of tea polyphenols decreased WVP of pomelo peel film. The ultrasonic cavitation effect can also affect the structure of the complex [15–19], resulting in the decrease of WVP. The WVP of the food packaging film should be as low as possible to avoid the moisture exchange between the food and the external environment [30], which can improve food stability. Compared to the control, the decrease in WVP of the TA-UT modified film was the highest (20.74%), followed by the GA-UT modified film (15.70%). Therefore, GA-UT modification was
recommended, showing better performance in water solubility and good resistance to water vapor transmission.

The light transmittance of the film is presented in Fig. 1C. After an ultrasound and polyphenol modification, the light transmittance of the film was significantly reduced ($p < 0.05$). The EA-UT modified film had the lowest transmittance (75.89% lower than that of the control), followed by the TA-UT modified film (57.10% lower than that of the control), and the GA-UT modified film (29.26% lower than that of the control). TA and EA showed a dark-yellow appearance, which can reduce the light transmittance when combined with collagen-chitosan molecules. GA had a light-yellow surface color and hence exert less impact on light transmittance when combined with collagen-chitosan molecules. The light transmittance results were consistent with the color profiles (Table 1). Compared to the control, the $L$ value of the film was significantly reduced ($p < 0.05$) while the $a$ and $b$ values increased significantly ($p < 0.05$). EA-UT modified film possessed the highest $\Delta E^*$ value ($\Delta E^* = 32.35$) while the GA-UT modified film was most closely to the control in color ($\Delta E^* = 16.71$). Thus, GA-UT modification was able to obtain a composite film with the best appearance.

Antioxidant activity of the film was significantly enhanced ($p < 0.05$) after an ultrasound and polyphenol modification. This might be due to the instinctive antioxidant property of polyphenols, which scavenges free oxygen radicals through phenolic hydroxyl groups. The antioxidant capacity of the GA-UT modified film was the highest (24.16 times higher than that of the control), followed by the TA-UT incorporated film (14.32 times higher than that of the control), and the EA-UT incorporated film (close to that of the control). This trend agreed with the free radical scavenging capacity of GA, TA, and EA [12].

Based on the mechanical properties, water solubility, WVP, transmittance, color profile, and antioxidant capacity, the GA-UT modification was most suitable for the TSC-CTS film modification. Therefore, GA-UT modified film (GA-UT-TSC-CTS) was used for further tests.

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**Table 1**
The effect of pomegranate polyphenol and ultrasound modification on the color profile of the film. GA-UT-TSC-CTS, TA-UT-TSC-CTS, and EA-UT-TSC-CTS represent the gallic acid, tannic acid, and ellagic acid with ultrasound modified TSC-CTS films. The different superscript letters of data under the same column indicate that they are significantly different ($p < 0.05$).

| Samples         | $L$   | $a$   | $B$   | $\Delta E^*$ |
|-----------------|-------|-------|-------|--------------|
| Control         | 94.91 | -1.32 | 11.60 | —            |
| Control         | 51.1  | 0.17  | 0.24  |              |
| GA-UT-TSC      | 80.25 | 6.11  | 14.55 | 16.71 ± 1.25 |
| CTS             | 1.03  | 0.54  | 0.92  |              |
| TA-UT-TSC      | 77.10 | 6.95  | 13.95 | 19.81 ± 0.87 |
| CTS             | 0.72  | 0.48  | 1.23  |              |
| EA-UT-TSC      | 63.67 | 5.90  | 15.76 | 32.35 ± 0.72 |
| CTS             | 0.65  | 0.38  | 1.27  |              |

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Fig. 1. (A) Mechanical properties, (B) Water solubility and Water vapor permeability (WVP), and (C) Transmittance and DPPH scavenging activity of the films. GA-UT, TA-UT, and EA-UT represent the gallic acid, tannic acid, and ellagic acid with ultrasound modified TSC-CTS films. Control is the unmodified TSC-CTS film. Different Arabic letters above each column indicate these samples had significant differences ($p < 0.05$).
3.2. Optimization of the GA-UT-TSC-CTS film

Fig. 2 and Table 2 show the effects of different GA concentrations on the mechanical properties, water solubility, water vapor permeability (WVP), transmittance, DPPH scavenging activity, and color profiles of the GA-UT-TSC-CTS film. TS value of the film reached the highest (25.60 MPa) at the GA concentration of 1.0 g/L (Fig. 2A). A lower GA concentration was not sufficient to strengthen the intermolecular force between GA and TSC-CTS; whereas a higher GA concentration can break down the structure of the TSC-CTS film and hence decreased the TS [31]. EAB of the film decreased significantly with the increase in GA concentration \( (p < 0.05) \), which was due to the increasingly loose film structure caused by increased GA concentration. At 0.1–1.0 g/L, the EAB of the film was moderate. These results agreed with the findings of Nie et al. [29]. Fig. 2B indicated the water solubility and WVP of the film. In the early stage, with the increasing hydrogen bonding between GA and TSC-CTS, the bonding force of the polymer became stronger, the film became insoluble and the water vapor could not pass through, so the water solubility and WVP decreased. At the later stage, with high GA concentration, the film structure became looser, which was conducive to water solubility and WVP increases. The lowest water solubility (27.07%) was observed at the GA concentration of 1.0 g/L. WVP followed a similar trend to water solubility, which reached the lowest value \( (5.25 \times 10^{-11} \text{ g/m·s·Pa}) \) at the GA concentration of 1.0 g/L. A low water vapor transmission coefficient is beneficial to improve the moisture resistance of the film, which can improve food stability. At 1.0 g/L, the WVP of the film was moderate. The light transmittance of the film at different GA concentrations significantly decreased \( (p < 0.05) \). This was because, with the increase of GA concentration, more GA combined with collagen and chitosan, and the gradual deepening of the color led to the decrease in film transmittance. This was consistent with the influence of GA concentration on film color (Table 2). With the increase of GA concentration, the \( L \) value of the film decreased significantly (from 85.15 to 70.49), while the \( a \) and \( b \) values increased significantly (from 3.85 to 9.97 and from 11.06 to 20.77). The color aberration (\( \Delta E \)) between GA-UT-TSC-CTS film and TSC-CTS film increased significantly. At 0.1–1.0 g/L, the light transmittance and color of the film were moderate. Moreover, Fig. 2C showed that the DPPH scavenging capacity of the film increased significantly with the increase of GA concentration \( (p < 0.05) \) until it reached the upper limit (92.11%) at 0.5–2.0 g/L. In the beginning, the hydroxyl groups on the film surface were correlated with the concentration of GA and hence increased the antioxidant activity. However, it reached equilibrium when the polymers were stabilized (i.e. the hydroxyl groups on the film surface were saturated). Considering these results altogether, the optimal GA concentration was recommended as 1.0 g/L and used in subsequent experiments.

Effects of different reaction times are presented in Fig. 3 and Table 2, respectively. As illustrated in Fig. 3A, TS of the GA-UT-TSC-CTS film

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Fig. 2. (A) Mechanical properties, (B) Water solubility and Water vapor permeability (WVP), and (C) Transmittance and DPPH scavenging activity of the GA-UT-TSC-CTS film under different gallic acid (GA) concentration. GA-UT-TSC-CTS represents the gallic acid with ultrasound modified TSC-CTS film. Different Arabic letters above each column indicate these samples had significant differences \( (p < 0.05) \).
increased significantly with the increase of reaction time \( (p < 0.05) \) until it reached the upper limit (10 min, 25.02 MPa). In the beginning, the hydrogen bonding force of the film was correlated with the reaction time of GA and hence increased the TS. However, it reached equilibrium when the hydroxyl groups on the film surface were saturated. EAB showed a reversed trend, which decreased significantly with the increase of reaction time \( (p < 0.05) \) until it reached the equilibrium (10 min). Interestingly, at 10 min reaction time, the water solubility, WVP, and light transmittance also reached the lowest value (Fig. 3B and 3C). The DPPH scavenging activity of the GA-UT-TSC-CTS film was not significantly affected \( (p > 0.05) \) by the varied reaction time (Fig. 3C). As illustrated in Table 2, with the increase of reaction time, the L value of the film decreased significantly \( (p < 0.05) \), while the a and b values increased significantly \( (p < 0.05) \). At 5–25 min, the ΔE values between GA-UT-TSC-CTS film and TSC-CTS film were moderate. Considering these results altogether, a reaction time of 10 min was recommended to prepare the GA-UT-TSC-CTS film with the best quality. Overall, the optimized conditions for GA modification were GA concentration of 1.0 g/L and reaction time of 10 min.

### 3.3. Effect of GA-UT modification on the structural features of the film

#### 3.3.1. FTIR analysis

The FTIR results of the TSC-CTS film before and after GA-UT modification are presented in Fig. 4A. The GA-UT-TSC-CTS film and the TSC-CTS film showed broad peaks at 3272 cm\(^{-1}\) and 3289 cm\(^{-1}\), corresponding to the vibration of hydroxyl groups \( [32] \). After an gallic acid (GA) and ultrasound (UT) modification, the absorption peak was shifted to the lower wavenumber, indicating the increased hydrogen bonds were formed between GA and TSC-CTS and the formation of a new chemical compound GA-TSC-CTS. It was reported that sonication can change the force of the intermolecular/internal hydrogen bonding of the protein-polysaccharide polymer \( [19] \). Furthermore, the amide I, amide II, and amide III bands of the TSC-CTS film at 1648, 1558, 1411, and 1074 cm\(^{-1}\), relating to the stretching vibration of C=O, -COOH, C-N, and C-O \( [33] \), shifted to 1642, 1558, 1405, and 1069 cm\(^{-1}\) after a GA-UT modification, showing obvious shifts to the lower wavenumber. These shifts implied that the GA-UT modification weakened the covalent binding ability between collagen and chitosan. Overall, FTIR spectra confirmed that TSC-CTS bound with GA to form a new chemical compound GA-TSC-CTS, which was different from the original TSC-CTS. It was also speculated that the ultrasound and gallic acid modifications reduced the covalent bonds of the TSC-CTS film and loosened the film structure.

#### 3.3.2. Secondary structure analysis

The secondary structural features of the TSC-CTS film before and after GA-UT modification are presented in Fig. 4B. The TSC-CTS film showed a negative peak at 198 nm. By contrast, the GA-UT-TSC-CTS film exhibited a negative peak at 196 nm. The shift of peak position implied the secondary structural features of the film were changed by the GA-UT modification. It was also found that ultrasonic treatment significantly changed the secondary structure of rapeseed protein isolate-dextran composite \( [19] \). The proportion of secondary structural features is also shown in Fig. 4B. GA-UT modification caused a reduced α-helix structure and β-sheet structure. This was compensated by an increase of β-turn structure and random coil. The reductions of α-helix and β-sheet and the increases in β-turn and random coil in GA-UT-TSC-CTS film indicated the polymer’s structure transferred from an ordered phase to an unordered phase. This conclusion of CD analysis was consistent with the FTIR results (shown in section 3.3.1), which confirmed that GA-UT modification resulted in a loose structure of the film.

#### 3.3.3. Crystallinity analysis

The crystallinity of the TSC-CTS film before and after GA-UT modification is presented in Fig. 4C. The TSC-CTS film showed a diffraction peak at 2θ = 19.85°, and the diffraction peak was slightly wider, indicating the amorphous structure of the film \( [34] \). After a GA-UT modification, the diffraction peak appeared at 22.14°, and the diffraction angle slightly shifted to the right. This indicated that GA-UT modification increased the crystallinity of the GA-UT-TSC-CTS film. This may be due to stronger molecular interactions between GA and TSC-CTS under the ultrasound, which altered composite structure. Qu et al. \( [19] \) found that ultrasonic treatment significantly changed the crystallinity of rapeseed protein isolate-dextran composite. It was also speculated that the formation of a novel crystalline structure for GA-TSC-CTS, which was different from the original TSC-CTS.

#### 3.3.4. Thermogravimetry analysis

TGA results of the TSC-CTS film before and after GA-UT modification are presented in Fig. 4D. TGA results represented the thermal degradation temperature of the film. The TGA curve included three thermal degradation stages. In the first stage (25–160 °C), the weight changes of the film reflected the loss of water \( [19] \). The weight loss rates of the TSC-CTS film and GA-UT-TSC-CTS film at this stage reached the maximum at 132 °C and 155 °C, respectively. It is known that H-bonds link these water molecules to polymer \( [24] \). FTIR results in section 3.3.1 confirmed that GA-UT-TSC-CTS film had a stronger hydrogen bond force than TSC-CTS film, so the evaporation of free water in the GA-UT-TSC-CTS film was slower and requires higher temperatures. In the second stage (160–270 °C), the weight loss of the film was attributed to the degradation of the complexes. In this stage, the thermal degradation temperature of the TSC-CTS film was changed from 209 °C to 213 °C after the GA-UT modification. The third stage of the TGA curve (270–800 °C) indicated the thermal degradation of collagen and chitosan residues. The degradation of collagen residue involves the breakdown of hydrogen bonds, electrostatic forces, covalent bonds, etc., whereas the degradation of chitosan correlated with the breakdown of glycosidic bonds \( [35] \). The thermal degradation temperature of the TSC-CTS was changed from 302 °C to 298 °C after the GA-UT modification. Comprehensively, these results indicated that the thermostatibility of the

### Table 2

The effect of gallic acid (GA) concentration and reaction time on the color profiles of the GA-UT-TSC-CTS film. GA-UT-TSC-CTS represents the gallic acid when the hydroxyl groups on the film surface were saturated. GA-UT-TSC-CTS represents the gallic acid when the hydroxyl groups on the film surface were saturated. EAB showed a reversed trend, which decreased significantly with the increase of reaction time \( (p < 0.05) \) until it reached the equilibrium (10 min).

| GA Concentration (g/L) | L | a | b | ΔE |
|------------------------|---|---|---|----|
| 0                      | 94.91 ± 0.132 ± 11.60 ± 0.24 | 0.51 | 0.17 | 0.24 |
| 0.1                    | 85.15 ± 3.85 ± 11.06 ± 0.24 | 1.22 | 0.50 | 0.24 |
| 0.2                    | 80.53 ± 5.43 ± 14.67 ± 1.68 | 0.87 | 0.66 | 0.82 |
| 1.0                    | 77.63 ± 7.38 ± 16.22 ± 1.90 | 0.87 | 0.66 | 0.82 |
| 1.5                    | 74.62 ± 8.02 ± 17.44 ± 23.10 | 0.61 | 0.72 | 0.61 |
| 2.0                    | 70.49 ± 9.97 ± 20.77 ± 26.28 | 0.65 | 0.60 | 1.22 |
| Reaction time (min)    | L | a | b | ΔE |
| 0                      | 94.91 ± 0.132 ± 11.60 ± 0.24 | 0.51 | 0.17 | 0.24 |
| 5                      | 81.06 ± 6.63 ± 15.81 ± 16.53 | 0.91 | 0.54 | 0.54 |
| 10                     | 77.78 ± 6.26 ± 15.47 ± 19.13 | 0.76 | 0.29 | 0.46 |
| 15                     | 77.70 ± 6.99 ± 14.61 ± 19.35 | 0.15 | 0.53 | 0.39 |
| 20                     | 77.22 ± 5.89 ± 14.92 ± 19.40 | 1.05 | 0.69 | 0.61 |
| 25                     | 75.63 ± 5.73 ± 15.69 ± 20.58 | 0.94 | 0.43 | 1.00 |
GA-UT-TSC-CTS film was better than that of the TSC-CTS film. The difference in heat resistance was due to the spatial conformation change of TSC-CTS film after a GA-UT modification (verified in section 3.3.1). It was reported that sonication can improve the thermal stability of the zein-chitosan complex [15].

3.4. Effect of GA-UT modification on the antibacterial activity of the film

The inhibitory zone diameters of the films against Escherichia coli, Bacillus subtilis, Staphylococcus aureus, and Listeria monocytogenes are presented in Table 3. After the GA-UT modification, the inhibitory zone diameters of the TSC-CTS film were significantly increased (p < 0.05). The chitosan in the composite film was responsible for microbial inhibition. Chitosans contain positively charged amino groups, which targeted the negatively charged cell membrane and destroyed the cell membrane structure [36,37]. The gallic acid showed a synergistic effect with the chitosan, further increasing the antibacterial capacity [6,8,9]. The GA-UT-TSC-CTS film showed the best inhibition effect against Staphylococcus aureus, followed by Escherichia coli, Listeria monocytogenes, and Bacillus subtilis. Similar results reported that the addition of tea polyphenol or gallic acid could improve the antibacterial activity of the composite film [6,9].

4. Conclusions

Combined modification of pomegranate polyphenol and ultrasound played a positive role in the TSC-CTS film modification. Compared to the TSC-CTS film, the GA-UT-TSC-CTS film showed the best modification effect, which increased the tensile strength by 47.03% and improved the antioxidant capacity by 24.16 folds. In addition, the water vapor permeability and the light transmittance were significantly decreased by the GA-UT modification. The increases in mechanical properties and antioxidant capacity, and the reductions of water vapor permeability and light transmittance in GA-UT-TSC-CTS film were conducive to the development of high-quality food packaging film, which can improve food stability. Such enhancements were due to the altered structure of the TSC-CTS film by the GA-UT modification. These findings were supported by the FTIR, CD, XRD, and TGA results. Furthermore, the GA-UT-TSC-CTS film had improved antibacterial activity. Overall, pomegranate polyphenol and ultrasound modification could be a practical approach for TSC-CTS modification. If the related more film function can be further studied, it will facilitate the application of polyphenol and ultrasound technology in an edible food packaging film modification.

Fig. 3. (A) Mechanical properties, (B) Water solubility and Water vapor permeability (WVP), and (C) Transmittance and DPPH scavenging activity of the GA-UT-TSC-CTS film under different reaction time of gallic acid (GA). GA-UT-TSC-CTS represents the gallic acid with ultrasound modified TSC-CTS film. Different Arabic letters above each column indicate these samples had significant differences (p < 0.05).
Fig. 4. (A) FTIR spectra, (B) CD spectra, (C) XRD diffraction, and (D) thermogravimetry curves of the tuna skin collagen-chitosan (TSC-CTS) film and the gallic acid with ultrasound modified tuna skin collagen-chitosan (GA-UT-TSC-CTS) film.
Table 3

Inhibitory zone diameters of the gallic acid with ultrasound modified tuna skin collagen-chitosan (GA-UT-TSC-CTS) film. The filter paper dropped with sterile water is used as the blank. Control is the unmodified TSC-CTS film. The different superscript letters of data under the same column indicate that they are significantly different (p < 0.05).

| Sample                  | E. coli (mm) | B. subtilis (mm) | S. aureus (mm) | L. monocytogenes (mm) |
|-------------------------|-------------|-----------------|---------------|----------------------|
| Blank                   | 6.02 ± 0.03² | 6.01 ± 0.02²     | 6.02 ± 0.03²  | 6.02 ± 0.02²         |
| Control                 | 7.70 ± 0.02² | 7.40 ± 0.02²     | 7.80 ± 0.03³  | 7.13 ± 0.02³         |
| GA-UT-TSC-CTS           | 8.43 ± 0.21¹ | 7.67 ± 0.11¹     | 8.61 ± 0.08⁰  | 7.78 ± 0.10⁰         |

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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