Journal of Food Measurement and Characterization (2022) 16:3926–3934
https://doi.org/10.1007/s11694-022-01495-y

ORIGINAL PAPER

The effects of tannic and caffeic acid as cross-linking agents on the physicochemical, barrier, and mechanical characteristics of cold-water fish gelatin films

Elnaz Parsaei[1] · Abdorreza Mohammadi Nafchi[1,2,3] · Leila Nouri[1] · A. A. Al-Hassan[4]

Received: 8 March 2022 / Accepted: 3 June 2022 / Published online: 28 June 2022

Abstract
This study aimed to evaluate the effects of tannic acid (TA) and caffeic acid (CA) as cross-linking agents on the physicochemical and mechanical properties of cold-water fish gelatin films. TA and CA (1%, 3%, and 5% of dried gelatin) were incorporated into the fish gelatin film solutions, and film samples were prepared using the solvent casting method. The gelatin film without phenolic acids was considered as the control sample. The results demonstrated that establishing cross-linking bonds between gelatin polymer and phenolic acids improved the film stability, and water solubility decreased from ~ 100 to ~ 40%. The lowest amount of oxygen permeability and the highest darkness were observed in the films containing the highest levels of phenolic acids. The incorporation of TA and CA into gelatin films also increased mechanical strength from ~ 28 to ~ 50 MPa. In conclusion, these results indicated that fish gelatin films cross-linked by two natural cross-linking agents (CA and TA) might be used as edible films and biomaterials, and TA is a more effective phenolic compound than CA.

Keywords Edible film · Fish gelatin · Cross-linking · Tannic acid · Caffeic acid

Introduction

Food packaging develops the shelf life of the packaged product and improves the quality and safety of foods by preventing the destruction of products and the growth of microorganisms [1, 2]. Due to the disadvantages of packaging films based on petroleum materials, biopolymers have attracted much attention because these natural biofilms, unlike synthetic films, are non-toxic, accessible, biodegradable, and eco-friendly. Biopolymer-based films can be made from various natural sources, including carbohydrates, proteins, lipids, or combinations [3, 4]. Among different biopolymers, gelatin is one of the most widely used protein-based biopolymers for producing biodegradable, edible films [5].

Gelatin is a biopolymer extracted from collagen, and its structure order is less than that of collagen. The properties of gelatin are highly dependent on its primary collage source and the methods of gelatin manufacture [6, 7]. This biopolymer has biocompatibility, biodegradability, low cost, non-toxic, strong hydrogen bonds, and good solubility in water. However, it has high degradability, water sensitivity, and low mechanical strength. Therefore, to prepare edible films based on gelatin, this biopolymer must be combined with other polymers, or suitable additives should be used in its formulation [8, 9]. The primary source of gelatin is extracted from the bones and skin of mammalian animals. However, due to the dangers of diseases related to pigs and cows and religious issues, attention has been focused on using gelatin-based marine sources such as fish gelatins [10, 11].

One of the effective methods to overcome the limitations of protein-based biopolymers is using cross-linking agents to formulate biopolymer-based edible films [12, 13]. The cross-linkers are compounds or agents that cause special chemical reactions in polymer solutions. Choosing a suitable cross-linker agent to improve the quality of biopolymers is very

✉ Abdorreza Mohammadi Nafchi
fstiau@gmail.com; amohammadi@usm.my

1 Department of Food Science and Technology, Damghan Branch, Islamic Azad University, Damghan, Iran

2 Food Technology Division, School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia

3 Cluster of Green Biopolymer, Coatings & Packaging, School of Industrial Technology, Universiti Sains Malaysia, 11800 Penang, Malaysia

4 Department of Food Science and Human Nutrition, College of Agriculture and Vit. Medicine, Qassim University, Burydah 51452, Saudi Arabia

important because the agent used must not have health problems [14]. Various chemicals such as sulfuric acid, hydrochloric acid, and sodium hydroxide are commonly used to enhance the physical and mechanical properties and reduce the sensitivity to the moisture of biopolymer-based films. However, the use of these compounds has environmental issues and can be toxic to the consumer [15]. Nowadays, cross-linked compounds obtained from natural sources have attracted much attention because these compounds, especially phenolic acids, indicate remarkable antioxidant and antimicrobial activity and improve the functional properties of the biocomposite films [14]. Scientific research has shown that tannic acid and caffeic acid are two effective crosslinking agents for the gelatins. In addition to improving the mechanical strength of gelatin-based edible films, it causes antioxidant and antimicrobial activities in the biopolymer films [16, 17]. Tannic acid (TA) is a natural amphiphilic polymer that belongs to the group of phenolic acids. In its structure, hydrophilic hydroxyl groups are located in a hydrophobic macromolecular backbone [18]. TA can improve the physical properties and the strength of the biopolymer-based films by binding with the gelatin [19, 20]. This natural additive can modify the gelatin polypeptide network and increase the cross-linkages between the chains [21]. Caffeic acid (CA) is one of the secondary metabolites produced by plants, which is in the category of the phenolic compounds and has antioxidant and antimicrobial activities [22]. Various studies have been performed on the cross-linking of proteins using CA. The results showed that cross-linking between proteins and CA leads to the formation of covalent bonds and thus improves the mechanical properties of the protein-CA complex [23]. So far, various studies have been performed on the effect of natural cross-linking agents on the characteristicts of gelatins obtained from different sources, however, few studies have been conducted on the characterization of biocomposite films prepared with cross-linked gelatin with natural cross-linking agents and information in this area is limited. Therefore, this study aimed to investigate the effect of TA and CA at different levels of 1%, 3%, and 5% on improving the moisture resistance and mechanical strength of cold-water fish gelatin-based biocomposite films.

Materials and methods

Materials

Cold-water fish gelatin (CAS Number: 9000-70-8) was obtained from Sigma-Aldrich (St Louis, MO) without further treatment or purification. Tannic acid (TA), caffeic acid (CA), glycerol, sorbitol, and other chemicals were obtained from Merck Co. (Darmstadt, Germany).

Preparation of gelatin-based edible films with phenolic acids

To prepare the gelatin films, 5 g of cold-water fish gelatin was initially dissolved in 50 mL of deionized water at 60 °C. Tannic acid and caffeic acid were dissolved in a concentration of 1%, 3%, and 5% w/v in 50 mL of deionized water separately. The pH of solutions was adjusted above the 9 with NaOH 1.0 N. The solutions were then stirred continuously at 60 °C for 1 h. Oxygen was bubbled into the systems throughout the reaction, and a homogeneous solution was obtained [20]. The fish gelatin solution and the solutions of TA and CA were then mixed separately and placed on a magnetic stirrer for one hour in the same conditions as before. A mixture of sorbitol and glycerol (40% g/g dried gelatin) was added to the films as a plasticizer [24]. After cooling the film solutions at room temperature, a suitable volume of the solutions was cast onto Plexiglas plates with dimensions of 16×16 cm^2 and a thickness of 2 mm. The plates containing film solutions were placed in an oven at 40 °C for 20 h to dry the films. The film samples were kept in a desiccator (at 20 ± 5 °C temperature and 50 ± 5% RH) before characterization.

Measurement of the film water solubility

To determine the solubility of gelatin films in water, the samples (2.5×2.5 cm) were first dried in an oven at 105 ± 2 °C for 24 h and were weighed (W1). The dried films were then placed in a container containing 50 mL of distilled water for 24 h. After this time, the samples were separated from the water by filter paper, and after drying in an oven at 105 ± 2 °C for 24 h, their weight was recorded (W2). The water solubility of film samples was calculated using the following equation [25]:

$$\text{Solubility } (\%) = \frac{\text{W1} - \text{W2}}{\text{W1}} \times 100$$

Measurement of the film water absorption capacity (WAC)

The film samples (20×20 mm) were kept in calcium sulfate (at 0% RH) for 24 h. Then, the films were conditioned in a desiccator containing saturated calcium nitrite solution at 22 ± 2 °C. After that, the weight of the samples was measured at certain intervals of periods until reaching an equilibrium state. Finally, the water absorption capacity of the films was obtained using the following equation [26]:

$$\text{WAC}\left(\frac{\text{g water}}{\text{g dried film}}\right) = \frac{\text{absorbed water weight}}{\text{dried film weight}}$$

Measurement of the film water vapor permeability (WVP)

The water vapor permeability of gelatin film samples was determined according to the ASTM E96-16 standard method [27]. The WVP test was done at room temperature (25 ± 3 °C). The test cup was filled with water, and the sample was cut to the cup size and placed on the cup using a dough. The cup was weighted and placed inside a desiccator (0% RH). Then, the cup was weighed every 2 h and continued until seven points. Then, the weight versus time graph was drawn, and it was used to determine the water vapour transmission rate (WVTR). The WVP of gelatin films was obtained using the following equation:

$$WVP = \frac{\text{WVTR} \times \text{average thickness}}{\text{water vapor pressure differences on both sides of the film surface}}$$

Measurement of oxygen permeability

The oxygen permeability of the gelatin films was determined using a Mocon Oxtran 2/21 system (Minneapolis, USA) according to the ASTM D3985-17 standard method [28]. Briefly, samples were conditioned at 25 °C and 55% RH for 48 h. Then the thickness of the film was measured and mounted into the equipment cell. Finally, the oxygen permeability of the gelatin film samples was determined using WinPermTM permeability software according to the convergent method.

Film color indexes measurement

To determine the color parameters of the gelatin film samples, including L* (lightness), a* (redness/greenness), and b* (yellowness/blueness), a colorimeter (Minolta CM-3500D; Minolta Co. Ltd., Osaka, Japan) was used [29]. Before determining the film samples' color, the device was calibrated using the standard white screen. Total color changes (ΔE) of samples were calculated using the following equation:

$$\Delta E = \sqrt{(L_0 - L_t)^2 + (a_0 - a_t)^2 + (b_0 - b_t)^2}$$

Mechanical properties

Mechanical properties of gelatin films, including tensile strength (TS), Young's modulus (YM), and elongation at the breakpoint (EB), were measured using the ASTM D 882–18 [30] method using a texture analyzer (Stable Micro Systems, Surrey, England). For each sample, three 50 mm × 150 mm strips were tested. Before the textural test, the film samples were conditioned at 25 °C at 55% relative humidity. The length of initial grip separation and the cross-head speed was 30 mm and 50 mm/min, respectively [30]. The textural test was performed in at least three replications.

Statistical analysis

The data obtained from the experiments were analyzed statistically using IBM SPSS software version 22.0 (IBM Corp., New York). One-way analysis of variance (ANOVA) and Duncan multi-range post hoc test were used to identify the significant difference between film samples at $p < 0.05$.

Results and discussion

Water solubility of the gelatin films

The water solubility amounts of cold-water fish gelatin films containing different levels of TA and CA are shown in Fig. 1. The incorporation of TA and CA into the formulation of gelatin-based film samples had a significant effect on the solubility of the produced films ($p < 0.05$). The control film had the highest percentage of water solubility (96.33%), similar to the amounts reported in research conducted by Araghi et al. [22]. Other researchers have also reported the high water solubility of fish gelatin [31]. Incorporation of TA and CA significantly reduced the water solubility of gelatin films which is related to the reaction of polymers with hydroxyl and carbonyl groups that causes the formation of hydrogen bonds or covalent bonds and the formation of cross-links and therefore reduces the water solubility of the polymers. Cross-linkers can reduce the number of

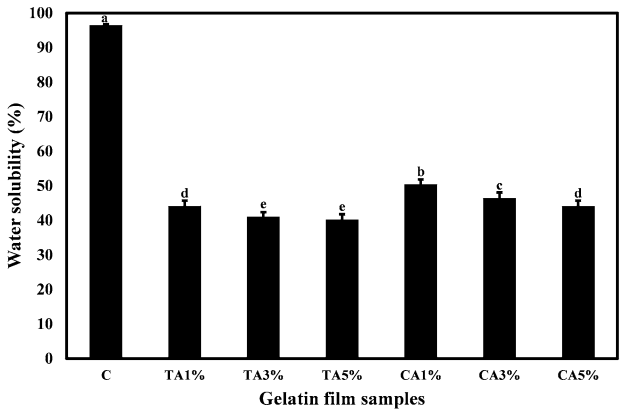

Fig. 1 The water solubility (%) of the cold-water fish gelatin-based films contain different TA and CA levels. Bars represent mean (n = 3) ± SD. Different letters on the bars indicate significant differences at 5% level of probability among films. TA and CA represent tannic acid and caffeic acid, respectively

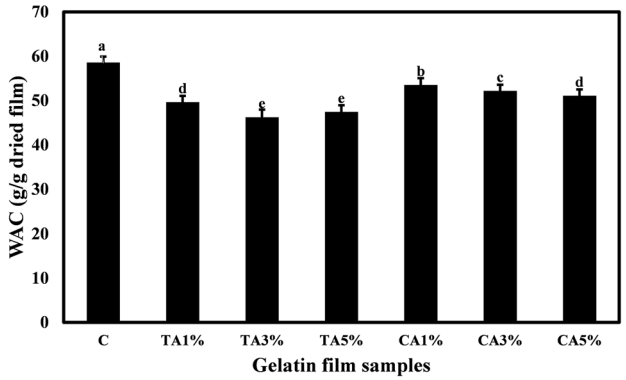

Fig. 2 The water absorption capacity (g/g dried film) of the cold-water fish gelatin-based films containing different levels of TA and CA. Bars represent mean (n = 3) ± SD. Different letters on the bars indicate significant differences at 5% level of probability among films. TA and CA represent tannic acid and caffeic acid, respectively

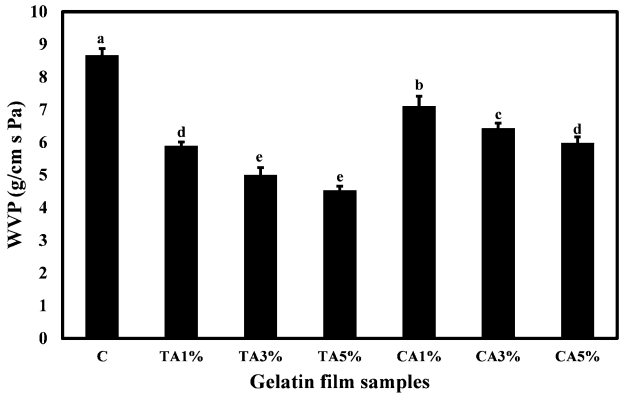

Fig. 3 The water vapor permeability (g/cm.s.Pa) of the cold-water fish gelatin-based films containing different levels of TA and CA. Bars represent mean (n = 3) ± SD. Different letters on the bars indicate significant differences at 5% level of probability among films. TA and CA represent tannic acid and caffeic acid, respectively

polar groups of the films and thus reduce their hydrophilicity, thereby reducing the water solubility of the film [32]. With increasing the levels of these two phenolic acids in the film formulation, a decrease in the water solubility of the samples was also observed ($p < 0.05$). The effect of TA in reducing the solubility of films was more significant than CA, so the solubility of the films containing TA was in the range of 40.10–43.97%, and the films containing CA were in the range of 43.98–50.22%. Cao et al. [33] attributed the decrease in the combination of gelatin with water due to increased cross-linking due to incorporating phenolic compounds. Line with the results of the present study, Choi et al. [16] also reported a significant decrease in the solubility of turmeric-gelatin-based films due to the incorporation of tannic acid, caffeic acid, and green tea extract. Menezes et al. [19] achieved similar results in studying the effect of tannic acid on the solubility of fish skin gelatin-based films. In the study of Tammineni et al. [34], the effect of different levels of tannic acid on the characteristics of gelatin-based films was investigated, and the results indicated a significant impact of this phenolic acid in reducing the solubility percentage of gelatin films. In general, the solubility of the films depends on the amount and type of cross-linker and the method used to evaluate the water solubility of the films.

Water absorption capacity (WAC) of the gelatin films

Edible films used for food packaging must have good moisture resistance and indicate less water absorption capacity. The WAC amounts of the films depend on their polarity, porosity, molecular structure, degree of cross-linking, and molecular interactions [35]. Figure 2 shows the WAC amounts of the fish gelatin-based films containing different levels of TA and CA. The highest WAC was observed in the control film (58.45 g/g dry film), and cross-linking by incorporating different levels of TA and CA in the fish gelatin reduced the WAC of the produced films significantly ($p < 0.05$). The film samples containing TA had water absorption capacity amounts in the range of 46.09–49.49 g/g dried film, and the films containing CA ranged from 50.95 to 53.37 g/g dried film. As can be seen, TA had a more significant effect on reducing the WAC of gelatin films than CA. Polyphenolic acids have several hydroxyl groups in their structure. They cause molecular interactions with the hydroxyl and amine groups in the gelatin structure, thus, reducing the WAC amounts of the films [8]. In agreement with the present study results, previous studies indicated that using cross-linking compounds in gelatin-based films increases the resistance of films to water [36, 37]. Taheri et al. [8] and Ge et al. [38] also achieved similar results. They demonstrated that adding tannic acid and rosmarinic acid to the gelatin-based films reduced the WAC amounts of the films, respectively, and by increasing their levels in the films, a decrease in the WAC amounts was observed.

Water vapour permeability (WVP) of the gelatin-based films

Food packaging materials with desirable barrier properties can improve packaging conditions by reducing moisture transfer between the food products and the surrounding environment [39]. In general, poor water vapour permeability of protein-based packaging films limits their use in food packaging. The WVP amounts of fish gelatin-based films containing different levels of TA and CA are given in Fig. 3. Incorporating phenolic acids as cross-linking agents into gelatin films indicated a significant effect on the WVP of films ($p < 0.05$). The highest WVP value was obtained in

the control film (8.65 g/cm s Pa). Since the permeability to water vapor depends on the hydrophilic and hydrophobic nature of the films [40] and the transfer of water vapor is done by the hydrophilic parts of the films [41], the establishment of cross-linking by phenolic acid (TA and CA) in the structure of fish gelatin, by reducing the number of polar groups of the films, reduced the WVP. Increasing the levels of these phenolic acids in the gelatin films also significantly reduced the WVP values ($p < 0.05$). So that in both films cross-linked with TA and CA, the lowest WVP was observed at the level of 5% of these phenolic acids (5.87 and 7.09 g/cm s Pa, respectively). TA Due to the higher degree of cross-linking in gels containing TA than CA [42], the WVP of the films containing TA was lower than films containing CA. Research has confirmed that incorporating the phenolic compounds into the gelatin-based films results in hydrogen bonds between the hydroxyl groups of the phenolic compounds and the gelatin polypeptide chains. In this way, the structure of the gelatin films becomes compacter, and the empty spaces in it are reduced, thus reducing the WVP of the films [43]. Consistent with the present study results, Araghi et al. [22] also found that in the fish gelatin-based films containing caffeic acid, increasing the levels of this phenolic acid reduced the WAC values. Similarly, a significant reduction in the WVP value was also demonstrated in the casein-based films with tannic acid [12], the gelatin film with protocatechuic acid [44], the soy protein-based films with rutin [45], the wheat gluten-based films with gallic acid [46], the silver carp myofibrillar protein-based films with tannins [47], and the gelatin-based films with green tea extract [48].

Oxygen permeability (OP) of the gelatin films

Oxygen permeability is one of the important features of biopolymer-based films used for food packaging because oxygen is a major and critical factor in the occurrence of lipid oxidation reactions and the growth of microorganisms. The chemical nature of macromolecules, the accumulation of molecules, and the amount of cross-linking are the most important factors affecting oxygen permeability. Generally, the OP of fish gelatin-based films is lower than that of mammalian gelatin-based films [49]. The lower OP of fish gelatin-based films is due to lower amounts of amino acids like proline and hydroxyproline in this gelatin, reducing the amount of OP. The OP values of the fish gelatin-based edible films containing different levels of TA and CA are given in Fig. 4. The results showed a significant decrease in OP values of films by adding TA and CA to the formulation of gelatin films and increasing their levels from 1 to 5% ($p < 0.05$). The control had the highest OP value (15.79 cc-mil/m^2.day), and the establishment of cross-linking with TA and CA reduced the OP values of the films ($p < 0.05$).

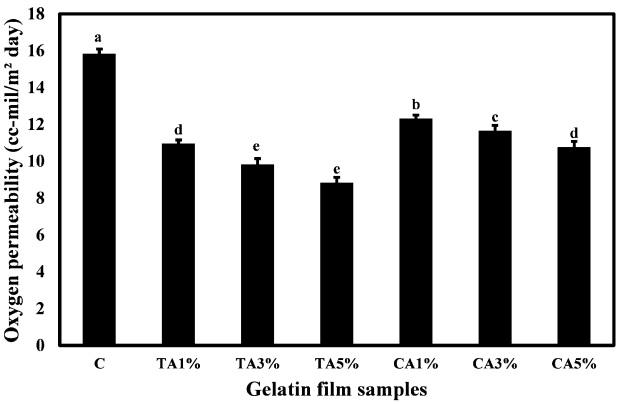

Fig. 4 The oxygen permeability (cc-mil/m^2.day) of the cold-water fish gelatin-based films contain different TA and CA levels. Bars represent mean (n = 3) ± SD. Different letters on the bars indicate significant differences at 5% level of probability among films. TA and CA represent tannic acid and caffeic acid, respectively

The OP values of the gelatin films containing TA and CA ranged from 8.80–10.91 cc-mil/m^2.day and 10.73–12.28 cc-mil/m^2.day, respectively. The gelatin reaction with CA and TA increased the formation of hydrogen bonds, made the gelatin-based film structure denser, and reduced the free spaces or discontinuities in the gelatin polymer matrix, thus improving the oxygen barrier of the films. Chiou et al. [50] also agreed that cross-linking in the gelatin structure of Pollack fish by glutaraldehyde reduces the OP. At the same time, this cross-linker increases the OP of pink salmon gelatin. In research conducted by Araghi et al. [22], increasing the levels of caffeic acid and ferulic acid in the fish gelatin-based films resulted in a significant decrease in the OP of the films. Improvement of oxygen barrier properties of gelatin and chitosan films due to the incorporation of phenolic acids has also been reported by Halim et al. [51], Aljawish et al. [52], and Zhang et al. [2].

Color indexes of the gelatin films

The color of the packaging films is one of the important optical parameters that play a significant role in consumers' acceptance of packaged food. Generally, the optical properties of the packaging films depend on the solvent and additives and the condition of the film preparation [53]. Table 1 shows the colour indexes of the fish gelatin-based films containing different levels of TA and CA. The results demonstrated that the addition of TA and CA to the gelatin-based films significantly reduced the lightness (from 81.59 in the control sample to 56.53 in the films containing 5% TA) and increased the yellowness intensity of the film samples (from 53.71 in the control sample to 55.21 in the films containing 1% CA), and changed the color of the films from redness to greenness ($p < 0.05$).

Table 1 The color indexes of fish gelatin film samples containing different concentrations of TA and CA

Gelatin films	L*	a*	b*	ΔE
Control	81.59 ± 0.91 a	0.18 ± 0.65 a	53.71 ± 0.46 f	–
1% TA	67.03 ± 0.62 d	− 3.55 ± 0.24 c	57.85 ± 0.61 c	15.59 ± 0.40 c
3% TA	61.13 ± 0.67 e	− 4.04 ± 0.38 c	58.92 ± 0.45 b	21.89 ± 0.72 b
5% TA	56.53 ± 1.07 f	− 4.12 ± 0.42 c	60.25 ± 0.47 a	26.25 ± 0.39 a
1% CA	72.59 ± 0.71 b	− 2.56 ± 0.23 b	55.21 ± 0.35 e	9.53 ± 0.54 f
3% CA	70.99 ± 0.62 c	− 2.96 ± 0.34 b	56.20 ± 0.57 d	11.33 ± 0.65 e
5% CA	68.26 ± 0.78 d	− 3.18 ± 0.53 bc	57.74 ± 0.62 c	14.33 ± 0.51 d

The values are mean ± SE (n = 3). Different letters show significant differences at 5% level of probability between values in the same columns. TA and CA represent the tannic acid and caffeic acid, respectively

Increasing the levels of these phenolic acids also caused the color of the produced films to darken. The darkening of the gelatin film color is probably due to the reaction between phenolics and gelatin in the presence of oxygen and in alkaline conditions, which leads to the oxidation of the structure of phenolic compounds and the Quinone compound formation [54]. The color of the gelatin films containing TA was darker than the color of the film samples containing CA. With increasing levels of TA and CA, total color change (ΔE) also increased significantly ($p < 0.05$). The higher the ΔE value, the more colored the films [55]. Lin et al. [56] also reported a darker color of the sodium caseinate-based films cross-linked with *genipin* than the control film. Zhang et al. [20], in a study of the effect of caffeic acid on the color of the bovine gelatin-based edible film and accordance with the results of the present study, demonstrated that the addition of this phenolic acid to the films changed the color of film samples from yellow-pale to dark brown. Araghi et al. [22], and Ge et al. [38] also reported that the gelatin-based films darkened due to phenolic acids.

Mechanical properties of the gelatin films

The mechanical properties of the packaging films indicate the film's integrity under shear force conditions that this shear or stress may occur during handling, processing, and storage (Park, 2001). The mechanical properties of the fish gelatin-based edible films, including tensile strength, Young's modulus, and elongation at break, were measured by a texture analyzer. The results are presented in Table 2. The maximum tensile strength that a film can withstand is tensile strength (TS) [57]. TS is an important parameter that greatly impacts the resistance and protection of food packaging. Higher TS is more desirable for food packaging because it can improve and guarantee the quality and safety of products [58]. Elongation at break (EB) of the film refers to the maximum changes of film's length against the applied force until it breaks, which indicates the flexibility of the films and their resistance to shape changes without cracking [57]. Young's modulus (YM) or elasticity modulus is the stress-to-strain ratio in the linear region and is directly related to the strength of films [59]. The lowest tensile strength (28.64 MPa) and Young's modulus (8.33 MPa), and the highest elongation at the breakpoint (10.41%) were observed in the control sample, and the addition of TA and CA increased significantly the TS and YM and reduced the EB of gelatin-based films ($p < 0.05$). Compared to the control sample, the highest increase in TS (44.91% increase) and YM (41.34% increase) were observed in the films containing 3% TA. The films containing 3%, and 5% TA (6.21% and 5.85%, respectively) also had the lowest EB and no significant difference was observed between these two samples. The effect of TA on improving the mechanical strength of

Table 2 The mechanical properties of fish gelatin film samples containing different concentrations of TA and CA

Gelatin films	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)
Control	28.64 ± 1.02 f	8.33 ± 0.56 f	10.41 ± 0.47 a
1% TA	47.95 ± 0.93 c	13.00 ± 0.39 b	7.09 ± 0.35 e
3% TA	51.99 ± 0.74 a	14.20 ± 0.33 a	6.21 ± 0.39 f
5% TA	50.36 ± 0.69 b	13.98 ± 0.34 a	5.85 ± 0.28 f
1% CA	35.83 ± 0.95 e	9.34 ± 0.40 e	9.41 ± 0.39 b
3% CA	40.66 ± 1.06 d	10.36 ± 0.42 d	8.49 ± 0.41 c
5% CA	46.24 ± 0.99 c	11.38 ± 0.38 c	7.78 ± 0.24 d

The values are mean ± SE (n = 3). Different letters show significant differences at 5% level of probability between values in the same columns. TA and CA represent the tannic acid and caffeic acid, respectively

gelatin films was greater than that of CA. Improving the mechanical strength of TA and CA films is probably because cross-linking agents stabilize the film matrix. As a result of the reaction of cross-linking agents with gelatin, new reactions are stronger than the reactions between the gelatin chains. There are four binding reactions between the cross-linking agent (phenolic acid) and film polymer, including ionic, hydrogen, hydrophobic, and covalent [16]. The occurrence of these bonding reactions in the cross-linked films can reduce voids, increase the compactness of film structure, and reduce the mobility of molecules [60]. This is a clear reason to increase the mechanical strength of films and reduce their flexibility due to the incorporation of cross-linking agents. In line with the present study results, Leite et al. [61] reported that the addition of tannic acid to the gelatin films increased the tensile strength of the film samples by 79%.

Similarly, Zhang et al. [2] demonstrated that with the addition of tannic acid to the chitosan/gelatin-based films from 0 to 1%, the film samples' tensile strength increased significantly. Halim et al. [51] also observed an increase in the tensile strength of the gelatin-based films due to the incorporation of tannic acid. Improving the mechanical strength of the protein-based films by adding phenolic acids was also reported by Menezes et al. [19], Tammineni et al. [34], Cao et al. [33], and Kim et al. [62].

Conclusion

In this study, the effect of cross-linking by TA and CA at different concentrations on the physicochemical and mechanical properties of the cold-water fish gelatin-based film. Modifying cold water fish gelatin by natural crosslinking agents such as TA or CA could improve the physicochemical, barrier, and mechanical properties of produced films based on fish gelatin. Both phenolic acids studied in this research enhanced the properties of fish gelatin films. However, the ability of TA was significantly higher than CA. Considering the desirable properties of the fish gelatin films modified by TA and CA, biodegradable and food packaging can be used.

Funding This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

Data availability The data that support the findings of this study are available from the corresponding author, upon reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interest.

Ethical approval This study does not involve any human or animal testing.

References

1. S. Jafarzadeh, A. Salehabadi, A. Mohammadi Nafchi, N. Oladzadabbasabadi, S.M. Jafari, Cheese packaging by edible coatings and biodegradable nanocomposites; improvement in shelf life, physicochemical and sensory properties. Trends Food Sci. Technol. **116**, 218–231 (2021). https://doi.org/10.1016/j.tifs.2021.07.021
2. C. Zhang, Z. Yang, J. Shi, X. Zou, X. Zhai, X. Huang, Z. Li, M. Holmes, M. Daglia, J. Xiao, Physical properties and bioactivities of chitosan/gelatin-based films loaded with tannic acid and its application on the preservation of fresh-cut apples. LWT Food Sci. Technol. **144**, 111223 (2021). https://doi.org/10.1016/j.lwt.2021.111223
3. N. Tamimi, A. Mohammadi Nafchi, H. Hashemi-Moghaddam, H. Baghaie, The effects of nano-zinc oxide morphology on functional and antibacterial properties of tapioca starch bionanocomposite. Food Sci. Nutr. **9**(8), 4497–4508 (2021). https://doi.org/10.1002/fsn3.2426
4. A. Mohammadi Nafchi, A.K. Alias, Mechanical, barrier, physicochemical, and heat seal properties of starch films filled with nanoparticles. J. Nano Res. **25**, 90–100 (2013). https://doi.org/10.4028/www.scientific.net/JNanoR.25.90
5. A. Abedinia, A. Mohammadi Nafchi, M. Sharifi, P. Ghalambor, N. Oladzadabbasabadi, F. Ariffin, N. Huda, Poultry gelatin: characteristics, developments, challenges, and future outlooks as a sustainable alternative for mammalian gelatin. Trends Food Sci. Technol. **104**, 14–26 (2020). https://doi.org/10.1016/j.tifs.2020.08.001
6. T.U. Rashid, S. Sharmeen, S. Biswas, T. Ahmed, A.K. Mallik, M. Shahruzzaman, M.N. Sakib, P. Haque, M.M. Rahman, *Gelatin-Based Hydrogels* (Springer, Cham, 2018), pp. 1601–1641
7. J. Skopinska-Wisniewska, M. Tuszynska, E. Olewnik-Kruszkowska, Comparative study of gelatin hydrogels modified by various cross-linking agents. Materials **14**(2), 396 (2021). https://doi.org/10.3390/ma14020396
8. P. Taheri, R. Jahanmardi, M. Koosha, S. Abdi, Physical, mechanical and wound healing properties of chitosan/gelatin blend films containing tannic acid and/or bacterial nanocellulose. Int. J. Biol. Macromol. **154**, 421–432 (2020). https://doi.org/10.1016/j.ijbiomac.2020.03.114
9. N.A. Azlim, A. Mohammadi Nafchi, N. Oladzadabbasabadi, F. Ariffin, P. Ghalambor, S. Jafarzadeh, A.A. Al-Hassan, Fabrication and characterization of a pH-sensitive intelligent film incorporating dragon fruit skin extract. Food Sci. Nutr. **10**(2), 597–608 (2022). https://doi.org/10.1002/fsn3.2680
10. J. Park, J. Nam, H. Yun, H.-J. Jin, H.W. Kwak, Aquatic polymer-based edible films of fish gelatin crosslinked with alginate dialdehyde having enhanced physicochemical properties. Carbohydr. Polym. **254**, 117317 (2021). https://doi.org/10.1016/j.carbpol.2020.117317
11. A.A. Al-Hassan, A.M. Abdel-Salam, F. Al Nasiri, H.M. Mousa, A. Mohammadi Nafchi, Extraction and characterization of gelatin developed from camel bones. J. Food Meas. Charact. **15**, 4542–4551 (2021). https://doi.org/10.1007/s11694-021-01029-y
12. M.L. Picchio, Y.G. Linck, G.A. Monti, L.M. Gugliotta, R.J. Minari, C.I. Alvarez Igarzabal, Casein films crosslinked by tannic acid for food packaging applications. Food Hydrocolloids **84**, 424–434 (2018). https://doi.org/10.1016/j.foodhyd.2018.06.028

13. P. Hazaveh, A. Mohammadi Nafchi, H. Abbaspour, The effects of sugars on moisture sorption isotherm and functional properties of cold water fish gelatin films. Int. J. Biol. Macromol. **79**, 370–376 (2015). https://doi.org/10.1016/j.ijbiomac.2015.05.008
14. B. Kaczmarek, O. Mazur, Collagen-based materials modified by phenolic acids—a review. Materials **13**(16), 3641 (2020). https://doi.org/10.3390/ma13163641
15. D. Huang, Z. Zhang, Q. Quan, Y. Zheng, Tannic acid: a versatile and effective modifier for gelatin/zein composite films. Food Packag. Shelf Life **23**, 100440 (2020). https://doi.org/10.1016/j.fpsl.2019.100440
16. I. Choi, S.E. Lee, Y. Chang, M. Lacroix, J. Han, Effect of oxidized phenolic compounds on cross-linking and properties of biodegradable active packaging film composed of turmeric and gelatin. LWT Food Sci. Technol. **93**, 427–433 (2018). https://doi.org/10.1016/j.lwt.2018.03.065
17. A. Erge, Ö. Eren, Chicken gelatin modification by caffeic acid: a response surface methodology investigation. Food Chem. **351**, 129269 (2021). https://doi.org/10.1016/j.foodchem.2021.129269
18. A.L. Girard, S.R. Bean, M. Tilley, S.L. Adrianos, J.M. Awika, Interaction mechanisms of condensed tannins (proanthocyanidins) with wheat gluten proteins. Food Chem. **245**, 1154–1162 (2018). https://doi.org/10.1016/j.foodchem.2017.11.054
19. M.D.L.L.R. Menezes, N.D.R. Pires, P.L.R. da Cunha, M. de Freitas Rosa, B.W.S. de Souza, J.P.D.A. Feitosa, M.D.S.M.D. Souza Filho, Effect of tannic acid as crosslinking agent on fish skin gelatin-silver nanocomposite film. Food Packag. Shelf Life **19**, 7–15 (2019). https://doi.org/10.1016/j.fpsl.2018.11.005
20. X. Zhang, M.D. Do, P. Casey, A. Sulistio, G.G. Qiao, L. Lundin, P. Lillford, S. Kosaraju, Chemical modification of gelatin by a natural phenolic cross-linker, tannic acid. J. Agric. Food Chem. **58**(11), 6809–6815 (2010). https://doi.org/10.1021/jf1004226
21. M.A. Ortiz-Zarama, A.R. Jiménez-Aparicio, J. Solorza-Feria, Obtainment and partial characterization of biodegradable gelatin films with tannic acid, bentonite and glycerol. J. Sci. Food Agric. **96**(10), 3424–3431 (2016). https://doi.org/10.1002/jsfa.7524
22. M. Araghi, Z. Moslehi, A. Mohammadi Nafchi, A. Mostahsan, N. Salamat, A. Daraei Garmakhany, Cold water fish gelatin modification by a natural phenolic cross-linker (ferulic acid and caffeic acid). Food Sci. Nutr. **3**(5), 370–375 (2015). https://doi.org/10.1002/fsn3.230
23. G. Strauss, S.M. Gibson, Plant phenolics as cross-linkers of gelatin gels and gelatin-based coacervates for use as food ingredients. Food Hydrocolloids **18**(1), 81–89 (2004). https://doi.org/10.1016/S0268-005X(03)00045-6
24. M.N. Abdorreza, L.H. Cheng, A.A. Karim, Effects of plasticizers on thermal properties and heat sealability of sago starch films. Food Hydrocolloids **25**(1), 56–60 (2011). https://doi.org/10.1016/j.foodhyd.2010.05.005
25. A. Vejdan, S. Ojagh, A. Adeli, M. Abdollahi, Preparation and characterization of the physical and mechanical properties of agar/fish gelatin bilayer films for food packaging. Fish. Sci. Technol. **4**(3), 133–147 (2015)
26. D.Y. Yi, B.M. Siddique, J.C. Lai, Development of biopolymer film with different ratios of Gelatine to Chitosan reinforced with zinc oxide nanoparticles for food covering/preservation. IOP Conf. Ser.: Mater. Sci. Eng. **429**, 012039 (2018). https://doi.org/10.1088/1757-899x/429/1/012039
27. ASTM. Standard Test Methods for Water Vapor Transmission of Materials-ASTM E96/E96M-16. Annual Book of ASTM Standards. Philadelphia, PA2016.
28. ASTM. Standard Test Method for Oxygen Gas Transmission Rate Through Plastic Film and Sheeting Using a Coulometric Sensor-ASTM D3985-17. Annual Book of ASTM Standards. Philadelphia, PA2017.
29. A. Esfahani, A. MohammadiNafchi, H. Baghaei, L. Nouri, Fabrication and characterization of a smart film based on cassava starch and pomegranate peel powder for monitoring lamb meat freshness. Food Sci. Nutr. (2022). https://doi.org/10.1002/fsn3.2918 (**In Press**)
30. ASTM. Standard Test Method for Tensile Properties of Thin Plastic Sheeting D882–18. Annual book of ASTM standards. Philadelphia, PA2018.
31. W.Z. Siburian, E. Rochima, Y. Andriani, D. Praseptiangga, Fish gelatin (definition, manufacture, analysis of quality characteristics, and application): a review. Int. J. Fish. Aquatic Stud. **8**(4), 90–95 (2020)
32. H.M. Rawel, J. Kroll, U.C. Hohl, Model studies on reactions of plant phenols with whey proteins. Food / Nahrung **45**(2), 72–81 (2001)
33. N. Cao, Y. Fu, J. He, Mechanical properties of gelatin films cross-linked, respectively, by ferulic acid and tannin acid. Food Hydrocolloids **21**(4), 575–584 (2007). https://doi.org/10.1016/j.foodhyd.2006.07.001
34. N. Tammineni, B. Rasco, J. Powers, C. Nindo, G. Ünlü, Bovine and fish gelatin coatings incorporating tannins: effect on physical properties and oxidative stability of salmon fillets. J. Food Chem. Nutr. **2**(2), 93–102 (2014)
35. A. Sionkowska, B. Kaczmarek, K. Lewandowska, Modification of collagen and chitosan mixtures by the addition of tannic acid. J. Mol. Liq. **199**, 318–323 (2014). https://doi.org/10.1016/j.molliq.2014.09.028
36. L. Ge, X. Li, R. Zhang, T. Yang, X. Ye, D. Li, C. Mu, Development and characterization of dialdehyde xanthan gum crosslinked gelatin based edible films incorporated with amino-functionalized montmorillonite. Food Hydrocolloids **51**, 129–135 (2015). https://doi.org/10.1016/j.foodhyd.2015.04.029
37. C. Mu, J. Guo, X. Li, W. Lin, D. Li, Preparation and properties of dialdehyde carboxymethyl cellulose crosslinked gelatin edible films. Food Hydrocolloids **27**(1), 22–29 (2012). https://doi.org/10.1016/j.foodhyd.2011.09.005
38. L. Ge, M. Zhu, X. Li, Y. Xu, X. Ma, R. Shi, D. Li, C. Mu, Development of active rosmarinic acid-gelatin biodegradable films with antioxidant and long-term antibacterial activities. Food Hydrocolloids **83**, 308–316 (2018). https://doi.org/10.1016/j.foodhyd.2018.04.052
39. H.C. Voon, R. Bhat, A.M. Easa, M.T. Liong, A.A. Karim, Effect of addition of halloysite nanoclay and SiO_2 nanoparticles on barrier and mechanical properties of bovine gelatin films. Food Bioprocess. Technol. **5**(5), 1766–1774 (2012). https://doi.org/10.1007/s11947-010-0461-y
40. S. Ekramian, H. Abbaspour, B. Roudi, L. Amjad, A. Mohammadi Nafchi, An experimental study on characteristics of sago starch film treated with methanol extract from *Artemisia sieberi* Besser. J. Food Meas. Charact. **15**(4), 3298–3306 (2021). https://doi.org/10.1007/s11694-021-00895-w
41. M.C. Gómez-Guillén, M. Ihl, V. Bifani, A. Silva, P. Montero, Edible films made from tuna-fish gelatin with antioxidant extracts of two different murta ecotypes leaves (*Ugni molinae* Turcz). Food Hydrocolloids **21**(7), 1133–1143 (2007). https://doi.org/10.1016/j.foodhyd.2006.08.006
42. E. Parsaie, A. Mohammadi Nafchi, L. Nouri, The effects of caffeic acid and tannic acid on physicochemical, morphological and hydrogel properties of cold-water fish gelatin. Food Sci. Technol. **18**(111), 267–277 (2021)
43. X. Zhang, L. Ma, Y. Yu, H. Zhou, T. Guo, H. Dai, Y. Zhang, Physico-mechanical and antioxidant properties of gelatin film from rabbit skin incorporated with rosemary acid. Food Packag. Shelf Life **19**, 121–130 (2019). https://doi.org/10.1016/j.fpsl.2018.12.006

44. C. Zhong, P.-F. Hou, Y.-X. Li, W.-Y. Yang, M. Shu, G.-P. Wu, Characterization, antioxidant and antibacterial activities of gelatin film incorporated with protocatechuic acid and its application on beef preservation. LWT Food Sci. Technol. **151**, 112154 (2021). https://doi.org/10.1016/j.lwt.2021.112154
45. K. Friesen, C. Chang, M. Nickerson, Incorporation of phenolic compounds, rutin and epicatechin, into soy protein isolate films: mechanical, barrier and cross-linking properties. Food Chem **172**, 18–23 (2015). https://doi.org/10.1016/j.foodchem.2014.08.128
46. A.-S. Hager, K.J. Vallons, E.K. Arendt, Influence of gallic acid and tannic acid on the mechanical and barrier properties of wheat gluten films. J. Agric. Food Chem. **60**(24), 6157–6163 (2012). https://doi.org/10.1021/jf300983m
47. X. Nie, L. Zhao, N. Wang, X. Meng, Phenolics-protein interaction involved in silver carp myofibrilliar protein films with hydrolysable and condensed tannins. LWT Food Sci. Technol. **81**, 258–264 (2017). https://doi.org/10.1016/j.lwt.2017.04.011
48. J. Wu, S. Chen, S. Ge, J. Miao, J. Li, Q. Zhang, Preparation, properties and antioxidant activity of an active film from silver carp (Hypophthalmichthys molitrix) skin gelatin incorporated with green tea extract. Food Hydrocolloids **32**(1), 42–51 (2013). https://doi.org/10.1016/j.foodhyd.2012.11.029
49. S. Javidi, A. Mohammadi Nafchi, H.H. Moghadam, Synergistic effect of nano-ZnO and *Mentha piperita* essential oil on the moisture sorption isotherm, antibacterial activity, physicochemical, mechanical, and barrier properties of gelatin film. J. Food Meas. Charact. **16**(2), 964–974 (2022). https://doi.org/10.1007/s11694-021-01217-w
50. B.-S. Chiou, R.J. Avena-Bustillos, P.J. Bechtel, H. Jafri, R. Narayan, S.H. Imam, G.M. Glenn, W.J. Orts, Cold water fish gelatin films: effects of cross-linking on thermal, mechanical, barrier, and biodegradation properties. Eur. Polym. J. **44**(11), 3748–3753 (2008). https://doi.org/10.1016/j.eurpolymj.2008.08.011
51. A.L.A. Halim, A. Kamari, E. Phillip, Chitosan, gelatin and methylcellulose films incorporated with tannic acid for food packaging. Int. J. Biol. Macromol. **120**, 1119–1126 (2018). https://doi.org/10.1016/j.ijbiomac.2018.08.169
52. A. Aljawish, L. Muniglia, A. Klouj, J. Jasniewski, J. Scher, S. Desobry, Characterization of films based on enzymatically modified chitosan derivatives with phenol compounds. Food Hydrocolloids **60**, 551–558 (2016). https://doi.org/10.1016/j.foodhyd.2016.04.032
53. N. Oladzadabbasabadi, A. MohammadiNafchi, F. Ariffin, A.A. Karim, Chapter 11—plant extracts as packaging aids, in *Plant Extracts: Applications in the Food Industry*. ed. by S.A. Mir, A. Manickavasagan, M.A. Shah (Academic Press, New York, 2022), pp. 225–268
54. X. Zhang, M.D. Do, P. Casey, A. Sulistio, G.G. Qiao, L. Lundin, P. Lillford, S. Kosaraju, Chemical cross-linking gelatin with natural phenolic compounds as studied by high-resolution NMR spectroscopy. Biomacromol **11**(4), 1125–1132 (2010). https://doi.org/10.1021/bm1001284
55. L. Wang, Q. Wang, J. Tong, J. Zhou, Physicochemical properties of chitosan films incorporated with honeysuckle flower extract for active food packaging. J. Food Process. Eng. **40**(1), e12305 (2017). https://doi.org/10.1111/jfpe.12305
56. H.-C. Lin, B.-J. Wang, Y.-M. Weng, Development and characterization of sodium caseinate edible films cross-linked with genipin. LWT Food Sci. Technol. **118**, 108813 (2020). https://doi.org/10.1016/j.lwt.2019.108813
57. M. Pereda, A.G. Ponce, N.E. Marcovich, R.A. Ruseckaite, J.F. Martucci, Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity. Food Hydrocolloids **25**(5), 1372–1381 (2011). https://doi.org/10.1016/j.foodhyd.2011.01.001
58. N.S. Said, N.M. Sarbon, Physical and mechanical characteristics of gelatin-based films as a potential food packaging material: a review. Membranes **12**(5), 442 (2022). https://doi.org/10.3390/membranes12050442
59. A. Heydari, F. Samimi Hashjin, A. Mohammadi Nafchi, Study of the functional and antimicrobial properties of combined cassava starch and bovine gelatin films reinforced with nano titanium dioxide. Innov. Food Technol. **5**(3), 373–384 (2018). https://doi.org/10.22104/jift.2017.2228.1515
60. G.L.A. Makishi, R.S. Lacerda, A.M.Q.B. Bittante, H.N.M. Chambi, P.A. Costa, C.A. Gomide, R.A. Carvalho, P.J.A. Sobral, Films based on castor bean (*Ricinus communis* L.) proteins crosslinked with glutaraldehyde and glyoxal. Ind. Crops Prod. **50**, 375–382 (2013). https://doi.org/10.1016/j.indcrop.2013.07.025
61. L.S.F. Leite, C. Pham, S. Bilatto, H.M.C. Azeredo, E.D. Cranston, F.K. Moreira, L.H.C. Mattoso, J. Bras, Effect of tannic acid and cellulose nanocrystals on antioxidant and antimicrobial properties of gelatin films. ACS Sustain. Chem. Eng. **9**(25), 8539–8549 (2021). https://doi.org/10.1021/acssuschemeng.1c01774
62. S. Kim, M.E. Nimni, Z. Yang, B. Han, Chitosan/gelatin–based films crosslinked by proanthocyanidin. J. Biomed. Mater. Res. Part B **75B**(2), 442–450 (2005). https://doi.org/10.1002/jbm.b.30324