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Enhancing the properties of fish gelatin edible film by proanthocyanidin-titanium coordination crosslinking effect †

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Abstract: Poor mechanical of the fish gelatin film has severely limited its application in food industry. To solve this problem, the titanium (Ti^{4+}) was incorporated with proanthocyanidin (PA) polymer, dimer and monomer respectively, then applied as crosslinkers to improve the quality of the gelatin film. Results showed that, film crosslinked by PA incorporated with Ti^{4+} showed significantly better mechanical properties than crosslinked by PA or Ti^{4+} along, which indicated coordination effects during crosslinking. Furthermore, polymerization degree (PD) of the PA were shown to have positive impact on the crosslinking reactions, the higher the PD of the PA, the better the properties were shown by films. To explain these observed coordination effects, ATR-FTIR, X-ray diffraction spectrum and fluorescence spectrometry were used to investigate the crosslinking reaction, and PA-Ti^{4+} chelating reaction were found to responsible for the coordination. Results from high-performance size exclusion chromatography- evaporative light scattering detector (HPSEC-ELSD) and zeta sizer analysis indicated the coordination crosslinking effects were positively related with the PD of the PA. The antioxidant ability, hydrophobicity and morphrology were also improved by the PA-Ti^{4+} coordination effect. The results will provide useful information for developing high-quality gelatin edible film.

Keywords: edible film; cross-link; proanthocyanidin; tannin; titanium

1. Introduction

Edible film, a potential candidate to replace the plastic package, has been discovered for quite a long time. But some poor properties, especially poor mechanical properties, need to be improved before expending applications of the edible films.

Proanthocyanidin (PA), also known as condensed tannin, is widely distributed in fruit and vegetables. Researchers have discovered the PAs [1,2] or titanium [3] can be individually applied as edible film crosslinkers. In fact, the PAs are also found highly reactive to metal ions (such as Fe^{3+}) and able to aggregate and form polymer networks through chelating bonds[4]. But how about the PA-Ti^{4+} chelating reaction? Can it provide coordination crosslinking effects and further improve the properties of edible films?

In current study, PA and titanium (Ti^{4+}) salt were used as crosslink agents, and the coordination crosslinking effect was investigated through attenuated total internal reflectance Fourier transform infrared spectroscopy (ATR-FTIR), X-ray diffractometer (XRD), high-performance size exclusion chromatography- evaporative light scattering detector (HPSEC-ELSD) and zeta sizer analysis. The morphology and hydrophobicity of film surface were tested through scanning electron microscope (SEM) and contact angle analysis.

2. Materials and Methods (Heading 1)

2.1. Chemicals and Reagents

Proanthocyanidin polymer were extracted from hops and purified in accordance with Kennedy's report[5]. Proanthocyanidin dimer and monomer were purchased from Aladdin Chemical (Shanghai) Co., Ltd. (Shanghai, China), Fish skin gelatin (from cold water fish) was purchased from Sigma Aldrich (Shanghai, China), other chemicals were all analytical grade and obtained from Aladdin Chemical (Shanghai) Co., Ltd.

2.2. Preparation of films

The fish skin gelatin films were prepared according to the method from previous research [6] but modified as following, fish skin gelatin was dissolved in milli-Q water and heat at 40 ℃ for 2 h, then titanium sulfate solution, proanthocyanidin solution and glycerol were added. Consequently, pH of the solution was adjusted to 4.0 with NaOH then volume of the solution was adjusted to 100 mL with milli-Q water to obtain the crosslinked gelatin solution (200g/L gelatin, 7 g/L proanthocyanidin, 4 g/L Ti^{4+}, 5 g/L glycerol, pH=4.0). After sonication for 30 min, 3 mL crosslinked gelatin solution were transferred into a 6-well Teflon plate (2 cm diameter), and dried at 50 ℃ for 48 h, then gelatin film was obtained.

2.3. Mechanical property evaluation

The mechanical properties, including tensile strength (TS) and elongation at break (EAB) were measured according to the method of Arfat et al[6].

2.4. ATR-FTIR

ATR-FTIR analysis were provided on a 200SXV inferred spectrum (Nicolet, USA) to obtain the spectrum of the films.

2.5. XRD

XRD spectrum were obtained from an D8 Advance X-ray diffractometer (Bruker, USA) based on published method[7].

2.6. HPSEC-ELSD

A RID-20A HPSEC system (Shimadzu, Tokyo) was used to measure the molecular weight distribution of the crosslinked gelatin particle, the absorbance was obtained with an ELSD (Shimadzu, Japan).

2.7. Particle size

Particle size of the crosslinked gelatin were tested on a Zetasizer nano serious instrument (Malvern, England).

2.8. SEM

The surface of the film was analyzed with a Gemini 300 field emission scanning electron microscope (ZEISS, Germany).

2.9. Contact angle

Contact angle of the films were tested with a CA100D contact angle tester (Yingnuo, China).

2.10. Anti-oxidant activity

1.0 g film was dissolved in 50 mL milli-Q water, samples were taken after 8 h and the antioxidant ability of the solution was tested in accordance with a previous report [8].

3. Results

3.1. Mechanical property

The fish skin gelatin films crosslinked with different PA provided different mechanical properties. First, crosslinked films showed higher TS and EAB than film without crosslink treatments (Table 1). Meanwhile, the films crosslinked by PA-Ti^{4+} showed

higher TS and EAB than the films crosslinked with single crosslinker (PA or Ti^{4+}). TS of PA-Ti^{4+} crosslinked films showed positively related with the PD of the PA.

Table 1. the mechanical properties of the gelatin films.

Crosslinker	Thickness	TS (MPa)	EAB (%)
PA polymer-Ti^{4+}	0.29 ±0.03	7.51 ±0.93	31.27 ±1.25
PA polymer	0.31 ±0.04	6.35 ±0.82	21.33 ±0.44
PA dimer- Ti^{4+}	0.36 ±0.04	2.43 ±0.28	30.12 ±2.96
PA dimer	0.30 ±0.03	1.71 ±0.11	24.12 ±3.15
PA monomer-Ti^{4+}	0.30 ±0.04	1.73 ±0.15	28.11 ±1.33
PA monomer	0.31 ±0.03	1.35 ±0.14	20.43 ±3.11
Ti^{4+}	0.36 ±0.02	1.11 ±0.77	21.42 ±4.33
None	0.27 ±0.04	0.09 ±0.02	10.33 ±1.27

3.2. Corrdination crosslinking effect

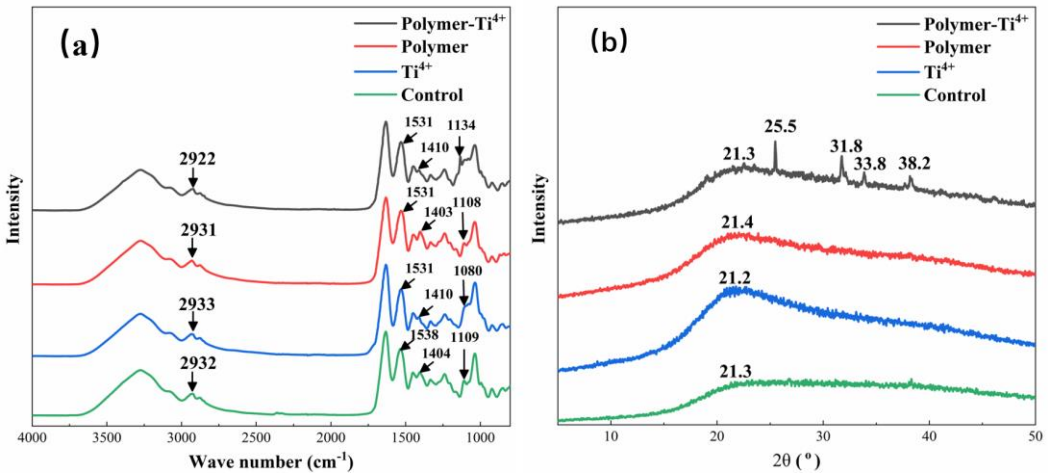

Figure 1. ATR-FTIR (a) and XRD (b) spectrum of the gelatin film crosslinked by PA polymer and Ti^{4+}.

Absorption bands shifted at 2922-2932 cm^{-1}, 1531-1538 cm^{-1}, 1403-1410 cm^{-1}, 1403-1410 cm^{-1}, 1134-1080 cm^{-1} after crosslinking treatment. It indicated the crosslink reaction within gelatin molecules (Figure 1a). New peaks were identified after crosslinked by PA- Ti^{4+}, represent the chelating reaction between PA and Ti^{4+} within gelatin film.

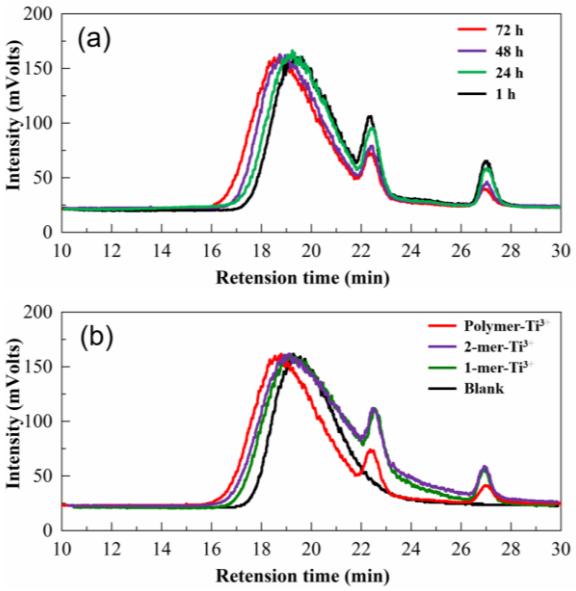

Figure 2. HPSEC-ELSD chromatogram of gelatin crosslinked by PA-Ti^{4+} across 72 h (a), molecular weight distribution of gelatin crosslinked by Ti^{4+} combined with PA monomer, dimer and polymer respectively (b).

After crosslinking by PA-Ti^{4+}, gradually decreased retention times of the gelatin were observed by HPSEC-ELSD chromatogram. It indicated a gradually increasing gelatin molecular weight after crosslinking treatment. Furthermore, the molecular weight of the gelatin was also affected by the type of the crosslinker, the higher the PD of the PA, the higher the gelatin molecular weight were observed.

Table 2. Particle size, contact angle and DPPH scavenging ability.

Crosslinker	Average size (nm)	Contact angle (°)	DPPH scavenging (%)
Polymer-Ti^{4+}	447.2 ± 18.32	119.9 ± 10.9	72.3 ± 1.26
Polymer	16.42 ± 1.04	53.1 ± 1.6	92.5 ± 3.11
Dimer- Ti^{4+}	17.33 ± 3.11	130.3 ± 1.4	61.3 ± 1.82
Dimer	7.66 ± 1.21	53.2 ± 1.7	92.4 ± 1.66
Monomer-Ti^{4+}	16.83 ± 1.44	111.0 ± 3.9	85.4 ± 3.11
Monomer	7.42 ± 0.73	58.1 ± 4.2	90.1 ± 4.33
Ti^{4+}	14.66 ± 1.32	107.3 ± 5.6	0
None	5.15 ± 0.26	39.4 ± 3.2	0

Particle size of the gelatin were also affected by the crosslinker. Apparently, after crosslink treatment, gelatin particles showed bigger size. Gelatin crosslinked by PA-Ti^{4+} also showed bigger size than crosslinked by PA or Ti^{4+}. These result implied the coordination crosslinking effect from while PA and Ti^{4+} were simutanously provided as crosslinker.

3.3. Surface mophology and hydrophobicity

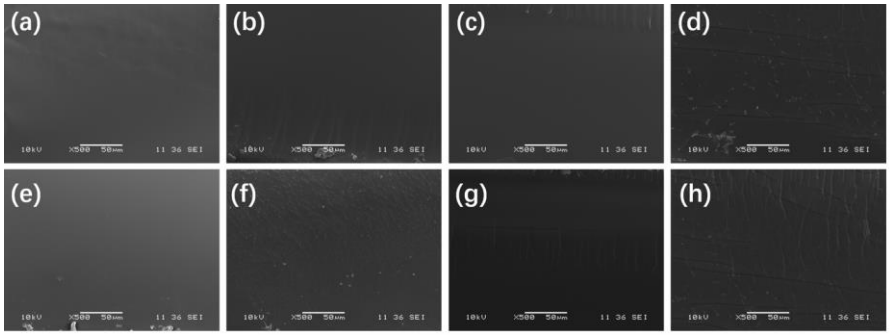

Figure 3 SEM image of the surface of the gelatin film crosslinked by (a) none, PA (b) monomer, (c) dimer, (d) polymer, (e) Ti^{4+}, (f) monomer- Ti^{4+}, (g) dimer- Ti^{4+}, (h) polymer- Ti^{4+}.

The gelatin film basically showed relatively smooth surface morphology (Figure 3), but their surface showed different hydrophobicity after crosslink treatments (Table 2). As for the film crosslinked by PA-Ti^{4+}, the surface showed higher contact angles, indicated more hydrophobic surfaces. But for the films without crosslinked by Ti^{4+}, lower contact angles were observed. Films crosslinked by PA and PA-Ti^{4+} presented DPPH scavenging abilities. But for the films crosslinked by Ti^{4+} or without crosslinking treatment, poor DPPH scavenging abilities were shown (Table 2).

4. Discussion

PAs are composed of flavon-3-ol as subunits which are rich in catechol moieties. It was founded that the PAs can react with some metal ions through chelating reaction. In current research, the PA-Ti^{4+} chelating reaction was introduced to fish skin gelatin film crosslinking process and aimed to improve the properties of the gelatin film.

Gelatin film showed higher mechanical properties if the PA and Ti^{4+} were simultaneously provided as crosslinker. This result indicated a coordination effect provided by PA-Ti^{4+} chelating reaction. Band shifting and new peaks from ATR-FTIR and XRD spectrum proved the chelating reaction within gelatin films. Molecular weight distribution and particle size analysis proofed the molecular weight and colloidal sizes of gelatin were further increased by the PA-Ti^{4+} coordination crosslinking effect.

5. Conclusion

PA and Ti^{4+} were applied to crosslink fish skin gelatin based edible films, the coordination crosslink effects were observed on the aspect of mechanical properties, gelatin particle size and molecular weight. The bigger the PD of the PA, the better the coordination effect were observed. Furthermore, the films provided fine surface morphology and hydrophobicity and antioxidant activity. These results not only in agree with the phenomenon observed in leather industry, which applied the PA-metal ion chelating to improve the quality of leather, it also implied other polyphenols (such as hydrolysable tannin[9]) and non-toxic metal ions could also have this coordination effect on edible film crosslinking.

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