Effects of Ferulic Acid Crosslinking Treatment on Gelatin Properties (Postprint)

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Abstract

The water adsorption isotherm of cross-linked gelatin at 25°C was determined using the constant weight method, while the gel strength and viscosity of ferulic acid cross-linked gelatin solutions at various concentrations were measured using a texture analyzer and an Ubbelohde viscometer, respectively, to evaluate the effects of ferulic acid cross-linking treatment on the water adsorption, gelation, and viscous properties of gelatin. The results indicated that: under identical water activity conditions, the equilibrium moisture content of cross-linked gelatin was slightly lower than that of gelatin at low water activity, but slightly higher at high water activity; the water adsorption isotherm data could be well fitted by the GAB model; the critical mass concentration for gel formation of cross-linked gelatin solution was 0.7% g/mL, which was close to that of gelatin; above the critical concentration, the gel strength of cross-linked gelatin increased with solution concentration, but was significantly lower than that of gelatin at the same concentration; the intrinsic viscosity of cross-linked gelatin (354.38 mL/g) was higher than that of gelatin (85.80 mL/g).

Full Text

Effects of Ferulic Acid Cross-linking Treatment on Gelatin Properties

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Abstract

Cross-linked gelatin was prepared using ferulic acid as a cross-linking agent in a previous study. The moisture sorption isotherm of the cross-linked gelatin was determined gravimetrically at 25°C. The gel strength and viscosity of solutions with different concentrations of cross-linked gelatin were measured using a texture analyzer and Ubbelohde viscometer, respectively. The results show that for solutions with the same water activity, the equilibrium moisture content of cross-linked gelatin was slightly lower than that of non-cross-linked gelatin at low water activity, but slightly higher at high water activity. The moisture sorption isotherm data were mathematically fitted to the GAB model. The critical concentration for cross-linked gelatin solution to form a gel was 0.7% g/mL, which was similar to that of non-cross-linked gelatin solution. Above the critical concentration, the gel strength of cross-linked gelatin increased with increasing concentration, but was obviously lower than that of non-cross-linked gelatin at the same concentration. The intrinsic viscosity of cross-linked gelatin (354.38 mL/g) was higher than that of non-cross-linked gelatin (85.80 mL/g).

KEY WORDS: organic polymer materials, cross-linked gelatin, ferulic acid, moisture sorption isotherm, gel strength, viscosity

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1. Introduction

Gelatin is a protein obtained through partial hydrolysis of collagen from animal skin, tendons, bones, and other tissues. As an important polymer material, gelatin is widely used due to its non-toxicity, low cost, good film-forming properties, and biocompatibility [1-7]. However, its applications are significantly limited by its high moisture absorption, susceptibility to degradation, and water solubility at elevated temperatures, particularly in the medical field. Cross-linking gelatin with appropriate agents can improve its functional properties [8-13].

Aldehydes such as formaldehyde [14], glutaraldehyde [15-17], glyoxal [14, 18-20], and glycolaldehyde [18] are commonly used as gelatin cross-linkers. Although these compounds react rapidly with proteins, they exhibit considerable toxicity. Enzymes like transglutaminase [21-26] and natural cross-linkers such as genipin

[27-29] have also been employed, but their high cost remains a drawback. Plant phenolic acids are abundant, safe, and non-toxic, and have been investigated as protein cross-linkers in recent years. Strauss et al. [30] used phenolic acids including caffeic acid, chlorogenic acid, and ferulic acid as cross-linking agents for gelatin and proposed a reaction mechanism. Cao et al. [31] prepared ferulic acid and tannic acid cross-linked gelatin films, examining the effects of cross-linker concentration and pH on mechanical properties, swelling, and water vapor permeability. Jiang et al. [32] investigated the effects of ferulic acid cross-linking on the antioxidant capacity, water vapor permeability, and water solubility of tilapia skin gelatin. In previous work, the authors prepared cross-linked gelatin using ferulic acid as a cross-linking agent with horseradish peroxidase catalysis under hydrogen peroxide conditions, and examined the effects of temperature, pH, and cross-linker concentration on the degree of cross-linking [8]. This study characterizes the properties of gelatin cross-linked with ferulic acid.

2. Materials and Methods

2.1 Materials

- Gelatin (Type B), food grade
- Horseradish peroxidase (RZ 2.67, activity 220 U/mg)
- Ferulic acid, analytical grade
- Hydrogen peroxide, analytical grade
- Deionized water
- Sodium hydroxide, analytical grade
- Anhydrous ethanol, analytical grade

2.2 Preparation of Cross-linked Gelatin

Cross-linked gelatin was prepared under optimized conditions from literature [8]. Appropriate amounts of gelatin were placed in 250 mL conical flasks, dissolved in deionized water with heating at 40°C to prepare 9% g/mL gelatin solution. A ferulic acid stock solution (200 mmol/L) was added to achieve a final concentration of 40 mmol/L, followed by 1 mol/L H_2O_2 solution at a 1:1 molar ratio with ferulic acid. After mixing, the pH was adjusted to 8.0 using 0.05 mol/L NaOH solution. Horseradish peroxidase solution (0.2 mg/mL) was then added to achieve an enzyme activity of [missing value] at 40°C for 24 h. The product was freeze-dried, pulverized, and passed through a 100-mesh sieve. The product was repeatedly washed with anhydrous ethanol to remove residual ferulic acid, then dried at 80°C to remove ethanol, yielding cross-linked gelatin with a cross-linking degree of 10%.

2.3 Characterization of Cross-linked Gelatin

UV Spectroscopy: UV absorption spectra of gelatin (0.25 mg/mL), ferulic acid (0.01 mg/mL), and cross-linked gelatin (0.25 mg/mL) were measured using a UV-2401PC spectrophotometer with distilled water as blank over 190-400 nm.

FTIR Spectroscopy: FTIR spectra of gelatin and cross-linked gelatin were analyzed using an AVATAR360 spectrometer with KBr pellet method (resolution: 4 cm^{-1}, scans: 32, range: 400-4000 cm^{-1}).

Moisture Sorption Isotherm: Moisture sorption isotherms of gelatin and cross-linked gelatin at 25°C were determined gravimetrically [14]. Accurately weighed 1.0-1.5 g samples were placed in pre-weighed weighing bottles and stored over saturated salt solutions in sealed desiccators. Water activities (a_w) of saturated salt solutions are listed in . After reaching constant weight, samples were weighed to calculate moisture content (m), and a_w was measured using an Aqualab Series 3TE water activity meter. Data were fitted to the GAB (Guggenheim-Anderson-de Boer) model using Matlab R2009b software. The GAB model equation is:

$$m = \frac{m_0 c k a_w}{(1 - k a_w)(1 - k a_w + c k a_w)}$$

where m is equilibrium moisture content, a_w is water activity, c and k are constants, and m_0 is monolayer water content.

Gel Strength: Powdered, dried gelatin and cross-linked gelatin were soaked in distilled water at room temperature for 30 min, then dissolved at 40°C to prepare solutions of 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, and 1.0% g/mL. Fifteen mL of each solution was placed in a 25 mL beaker and gelled at 10°C for 16-18 h. Gel strength was measured using a TA-XT Plus texture analyzer with SMS P/0.5 probe (descending rate: 1 mm/s, compression distance: 4 mm). Gel strength was defined as the maximum stress required to compress the gel by 4 mm.

Viscosity: Gelatin solutions were prepared at concentrations of 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, and 0.008 g/mL. Cross-linked gelatin solutions were prepared at 0.0003, 0.0004, 0.0005, 0.0006, 0.0008, 0.0010, 0.0012, and 0.0016 g/mL. Viscosity was measured at 40°C ($±$0.1°C) using an Ubbelohde viscometer. Flow time through the capillary was measured with an electronic stopwatch (precision: 0.01 s). Each measurement was repeated at least 5 times with differences $ $0.2 s between measurements, and the average was taken.

3. Results and Discussion

3.1 UV Spectra

[Figure 1: see original paper] shows the UV absorption spectra of gelatin, ferulic acid, and cross-linked gelatin. Gelatin exhibited a single absorption peak at 215.5 nm, arising from the $\pi \rightarrow \pi^*$ transition of C=O groups. Ferulic acid showed three peaks at 311.5 nm, 288.5 nm, and 215.5 nm, all from $\pi \rightarrow \pi^*$ transitions of the benzene ring. Cross-linked gelatin displayed two peaks at 285.5 nm and 213.5 nm. The new peak at 285.5 nm in cross-linked gelatin persisted after repeated ethanol washing, indicating that the spectrum was not simply a superposition of gelatin and ferulic acid spectra, but rather resulted from chemical cross-linking between gelatin and ferulic acid.

3.2 FTIR Spectra

[Figure 2: see original paper] presents FTIR spectra of gelatin before and after ferulic acid cross-linking. In native gelatin, peaks at 1654.06, 1549.88, and 1241.10 cm^{-1} correspond to C=O stretching (Amide I), N-H bending (Amide II), and C-N stretching (Amide III) of peptide bonds, respectively [33-35]. The broad, strong peak at 3330.65 cm^{-1} is attributed to N-H and O-H stretching vibrations. After cross-linking, the introduction of -OH groups from ferulic acid enhanced O-H stretching, resulting in a similar peak at 3343.94 cm^{-1}. The peak at 2953.79 cm^{-1} from C-H stretching shifted to 2966.74 cm^{-1} due to =C-H stretching from ferulic acid [36]. The low cross-linking degree (10%) caused minimal changes to the gelatin molecular structure, resulting in only minor spectral differences.

3.3 Moisture Sorption Isotherms

[Figure 3: see original paper] shows moisture sorption isotherms for gelatin and cross-linked gelatin fitted by the GAB model, with fitting parameters listed in . At low water activity (<0.85), gelatin exhibited slightly higher equilibrium moisture content than cross-linked gelatin, indicating greater water adsorption capacity. This is attributed to the introduction of hydrophobic aromatic rings and reduction of hydrophilic amino groups during cross-linking, which decreased hydrogen bonding and water absorption. However, the low cross-linking degree introduced few ferulic acid residues, resulting in minimal difference between the two materials. At high water activity (>0.85), cross-linked gelatin showed higher equilibrium moisture content, likely due to exposure of additional hydrophilic sites during swelling. These characteristics are consistent with previous studies [14, 22, 37].

3.4 Gel Strength

[Figure 4: see original paper] shows gel strength for gelatin and cross-linked gelatin at various concentrations. At concentrations of 0.1%-0.5% g/mL, both materials showed equal, concentration-independent gel strength, as these low concentrations were insufficient to form a complete gel network; resistance to the probe primarily came from the sol fraction. At 0.6% for gelatin and 0.7% for cross-linked gelatin, gel strength increased sharply, indicating network formation. Above these critical concentrations, gel strength increased progressively with concentration due to denser molecular entanglement, increased junction points, and stronger intermolecular forces. However, at equivalent concentrations above the gel point, cross-linked gelatin exhibited lower gel strength than gelatin, with the difference increasing at higher concentrations. Gel networks form primarily through intermolecular hydrogen bonds, influenced by electrostatic repulsion and hydrophobic interactions. Reduced gel strength after cross-linking may result from diminished hydrogen bonding capacity due to cross-linking between polar groups on protein side chains and ferulic acid [38]. Additionally, cross-linked gelatin solutions had higher pH values than gelatin solutions at the same concentration (e.g., pH 6.5 for 1.0% gelatin vs. pH 8.4 for cross-linked gelatin), resulting in greater deviation from the isoelectric point, stronger electrostatic repulsion, and consequently weaker gels. As concentration increased, the pH deviation became more pronounced for cross-linked gelatin, further increasing electrostatic repulsion and limiting the magnitude of gel strength enhancement compared to gelatin. These trends align with findings by Shao et al. [38].

3.5 Viscosity

[Figure 5: see original paper] shows the reduced viscosity of gelatin and cross-linked gelatin solutions. Both exhibited decreasing reduced viscosity with increasing concentration, but the slope was steeper for cross-linked gelatin. Calculations from the regression equations revealed that at equivalent concentrations, cross-linked gelatin solutions had higher viscosity than gelatin solutions [39]. The linear equations yielded intrinsic viscosities of 85.80 mL/g for gelatin and 354.38 mL/g for cross-linked gelatin. Since measurements were performed under identical temperature and solvent conditions, these results suggest that cross-linked gelatin has a higher relative molecular mass than gelatin, confirming successful cross-linking.

4. Conclusion

Ferulic acid cross-linked gelatin exhibited slightly lower water adsorption capacity than gelatin at low relative humidity, but higher adsorption at high relative humidity. The critical concentration for gel formation in cross-linked gelatin solutions was 0.7% g/mL, similar to that of gelatin. Above this concentration, gel strength increased with concentration but remained significantly lower than that of gelatin at equivalent concentrations. The higher intrinsic viscosity of cross-linked gelatin suggests a greater relative molecular mass, providing further evidence of successful cross-linking.

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