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Research Article

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Preparation and characterization of collagen food packaging film

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ABSTRACT

Collagen was extracted from the skin of pigs, and blended with sodium alginate, starch, and sodium carboxymethyl cellulose. Food packaging films were obtained by crosslinking the blend with glutaraldehyde. Fourier transform infrared spectroscopy (FT-IR), SEM and TGA analyses were used to characterize the resulting films. A strong hydrogen bonding was detected between the composite film molecules. Mechanical measurements show that the elongation at break and tensile strength of the films are significantly improved after addition of sodium alginate. A network structure is formed due to crosslinking of collagen molecules by glutaraldehyde. Addition of sodium alginate enhances the interaction between molecules in the film. On the other hand, collagen is well compatible with sodium carboxymethyl cellulose, sodium alginate and starch. The surface morphology of the film appears more homogeneous after crosslinking. The combined effects of these factors allowed to improve the mechanical properties of the collagen-based films.

Keywords: Collagen; Sodium Alginate; Glutaraldehyde; Food packaging

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INTRODUCTION

With the rapid development of the national economy and improvement of the quality of life, a great deal of packaged food has entered in our everyday life. A large variety of food packaging materials are currently available, with plastic packaging being the most widely used [1]. The environmental pollution due to accumulation of plastic wastes is increasingly considered as a major problem of our modern society. Therefore, the development of naturally degradable biopolymers has attracted much attention in the field of food packaging. Such biopolymers include polysaccharides, proteins, composites of polysaccharides or proteins with fatty acids such as starch films, modified cellulose films, collagen films, chitosan films, etc.

Food packaging film should exhibit a good mechanical strength, and also prevent the migration of water, oxygen, fatty acids and other aromatic components to ensure the food quality and to extend the shelf life [2].

Collagen is widely used in the field of food industry due to its outstanding properties including good film forming ability, resistance to organic solvents, biocompatibility, stability, etc. For example, collagen fiber films present good antioxidant property, and are used for the packaging of meat, fish, etc. Artificial sausage casing made from collagen presents good taste and high transparency. However, the greatest disadvantage of food packaging made from collagen is the poor mechanical strength. To solve this problem, we prepared a novel food packaging film using collagen as the main component and sodium alginate as a reinforcing agent. Glutaraldehyde was used as crosslinking agent to improve the strength of the collagen film.

EXPERIMENTAL SECTION

2.1 Materials

Collagen (laboratory prepared); soluble starch (AR); glutaraldehyde (AR); glycerol (AR); sodium carboxymethyl

cellulose (CP); sodium alginate (AR); Na_2CO_3（AR）; absolute ethanol (AR); acetic acid (AR). Na_2HPO_4 (AR); NaH_2PO_4 (AR).

2.2 Methods

2.2.1 Extraction of collagen

Residual fat was removed from fresh pig skin. The pig skin was chopped into pellets, and placed in a flask. 10% Na_2CO_3 solution was then added in the flask until immersion of the pellets. Degreasing was realized in a water bath 45℃, followed by filtration. The degreased pellets were introduced in another flask filled with distilled water. The pH of the solution was adjusted to 8 before addition of enzyme. Hydrolysis proceeded at 40℃ for 2 h. The solution was filtrated to remove the pellets, and then centrifuged. The upper layer of grease and the insoluble fraction at the bottom were removed. The resulting solution was finally freeze dried to yield a white collagen powder [3].

2.2.2 Preparation of collagen food packaging film

Novel collagen-based food packaging films are prepared from a mixture of collagen, starch, sodium carboxymethyl cellulose, and sodium alginate, using glutaraldehyde as cross-linking agent and glycerol as humectant, according to the following procedure:

(1) 0.25 g of sodium carboxymethyl cellulose was added in 4 ml of water, and stirred to yield a transparent gel.

(2) 0.1 g of starch was added in 0.4 ml of water, and heated at 70℃ for 4 min to yield a paste.

(3) 0.2 g of collagen powder was dissolved in 1 ml of water at 40℃. 2 ml of 50% glutaraldehyde and 2 ml of glycerol were then added. The mixture was stirred to yield a homogeneous solution.

(4) The starch solution and carboxymethyl cellulose gel were added in the collagen mixture, and homogenized at 40℃ under stirring. Sodium alginate (0 - 0.5 g) was then added. The mixture was stirred 15 min, yielding a liquid paste.

(5) After degassing, the liquid paste was poured on a template, and freeze-dried for 24 h (temperature: -15℃～-10℃, vacuum: 1 Pa). Finally the films were removed from the template.

2.2.3 Mechanical properties of the composite film

Rectangular samples with dimension of 30 × 10 mm^2 were cut from the collagen film. The mechanical properties of the films were determined as reported in literature [4]. The tensile strength corresponding to the maximum tensile force on a unit cross-sectional area is calculated using the following equation:

$$TS=F / S$$

where TS is the tensile strength (KPa), F the maximum tension at break (N), and S the sectional area of the sample (mm^2).

The elongation at break is calculated as follows:

$$E =(L-L_0) / L_0 \times 100\%$$

where E is the elongation at break (%), L the film length at break (mm), L_0 the original sample length (mm).

2.2.4 Measurements

Ultraviolet (UV) measurements were realized using a 2802 UV/VIS spectrophotometer (Shanghai UNICO).

Gel permeation chromatography (GPC) was realized using 1100/1200 HPLC (Agilent Technologies Inc. USA) equipped with 18 angle laser light scattering (Wyatt Technology Company USA) and Optilab T-rEX differential refractive index detectors, 100 000 aqueous phase column (Japan Shodex OHpak SB-803 HQ), and a guard column (Japan Shodex OHpak SB-G). A pH = 6.0 phosphate buffer of 25mM was used as the eluent. Measurements were realized at a flow rate of 0.5 mL/min at 35°C.

Thermal gravimetric analysis (TGA) was performed using a Q500 instrument (TA Co., USA). Measurements were made at a heating rate of 10℃/min under nitrogen protection.

Fourier transform infrared (FTIR) spectroscopy was performed using a VERTEX70 spectrometer (Brook Co., Germany).

Scanning electron microscopy (SEM) was realized using Quanta250 microscope (FEI Co., USA). The films were previously sputter coated with SBC-12 ion sputtering instrument (KYKY Technology Co., Ltd.).

RESULTS AND DISCUSSION

3.1 Characterization of collagen

Collagen powder was dissolved in 0.5 M acetic acid to yield a 0.05% solution. The UV absorption was obtained using 0.5 M acetic acid as the control. Figure 1 shows the UV spectrum of collagen. A strong absorption is observed at 218nm. According to the literature [5], collagen presents a strong absorption in the 200 ~ 230 nm range, while other proteins are located in the range of 250 ~ 290 nm. Therefore, UV measurements confirm that pure collagen was obtained in this work.

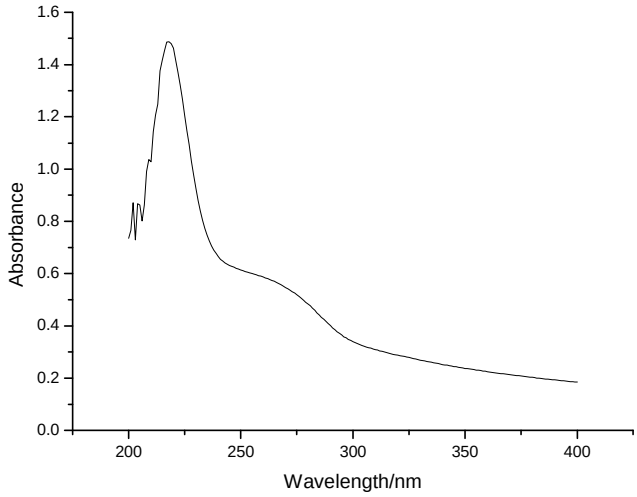

Figure 1 UV-Vis spectrum of collagen

The molecular weights of collagen were determined by GPC. The weight-average molecular weight (Mw) and number-average molecular weight (Mn) were 7930 and 7400, respectively. And the polydispersity index (Ip = M_w/M_n) is 1.07.

3.2 Structure-properties of collagen composite films

Various amounts of sodium alginate (0 - 0.5 g) were used for the preparation of collagen-based food packaging film according to the above mentioned process. The surface morphology and mechanical properties of the films were determined to evaluate the influence of the amount of sodium alginate on film properties (Table 1). Table 1 shows that the amount of added sodium alginate strongly influences on the surface properties of collagen films. With the amount of sodium alginate below 0.2 g, the films are soft and sticky, and cannot be used for packaging. In contrast, films obtained with higher amounts of sodium alginate (≥ 0.3 g) can be easily removed from the template.

Table 1 Effect of sodium alginate addition on the surface morphology of collagen films

M*/g	Surface properties
0	Hardly removable, rather smooth, very soft, very sticky, rather flexible, light yellow
0.1	Hardly removable, smooth, very soft, rather sticky, rather flexible, light yellow
0.2	Hardly removable, smooth, very soft, rather sticky, rather flexible, light yellow
0.3	Easily removable, smooth, dry surface, flexible, yellow
0.4	Easily removable, rather smooth, dry surface, flexible, yellow
0.5	Easily removable, rather smooth, dry surface, flexible, yellow

** The amount of sodium alginate*

Figure 2 presents the changes of the tensile strength as a function of the amount of sodium alginate. It appears that the tensile strength increases almost linearly with the amount of sodium alginate, from 24 KPa without sodium alginate to 287 KPa with 0.5 g of sodium alginate. In contrast, the elongation at break initially increases with addition of sodium alginate, reaches a maximum (76%) with 0.3 g of sodium alginate, and then decreases (Figure 3). In fact, addition of sodium alginate leads to viscosity increase of the liquid, thus disfavors the degassing of the system. In consequence, the resulting films may be heterogeneous and present poor properties. Taking all factors

into consideration, the optimal amount of added sodium alginate is 0.3 g.

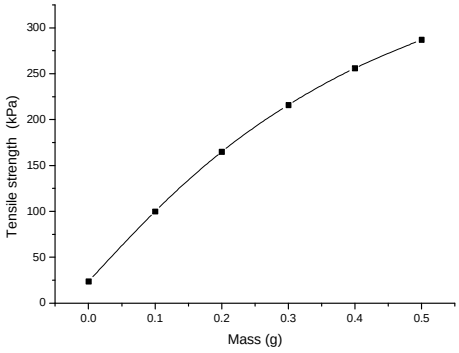

Figure 2 Effect of the amount of sodium alginate on the tensile strength of collagen films

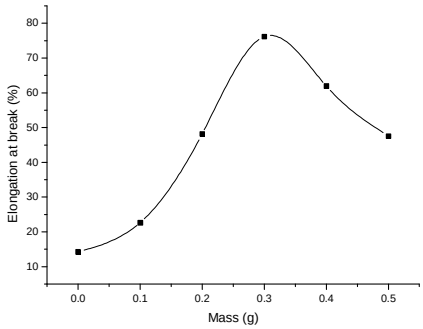

Figure 3 Effect of the amount of sodium alginate on the elongation at break of collagen films

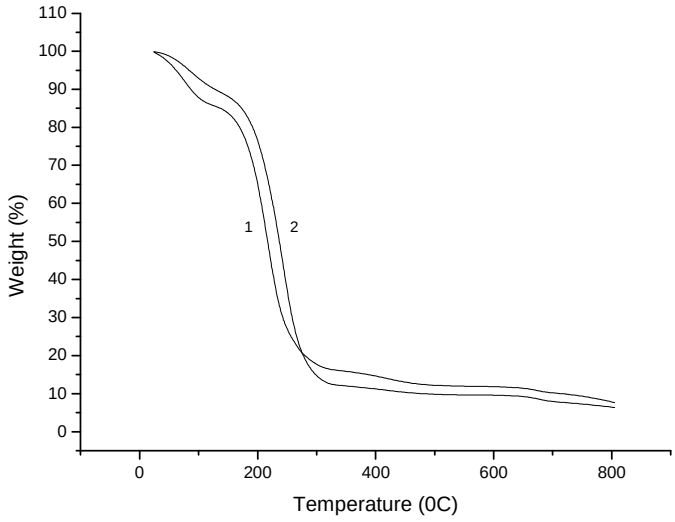

Figure 4 TGA curves of collagen films: 1 – with addition of sodium alginate; 2 – without addition of sodium alginate

TGA was used to evaluate the thermo-stability of the collagen composite films. Figure 4 presents the TGA curves of two collagen composite films with or without addition of sodium alginate. Similar profiles are obtained in both cases. The whole weight loss process can be divided into three phases. The first phase consists in a dehydration of the films (54 ~ 110℃). Both films exhibit a significant weight loss of about 15% due to the loss of free water and crystal water. The second phase results from the decomposition of the collagen film. Rapid weight loss is observed in the temperature range of 185-260°C due to chain cleavage of the organic components. The maximum weight loss rate is detected at 217℃ for film 1 with addition of sodium alginate and at 243°C for film 2 without addition of sodium alginate. This finding indicates that addition of sodium alginate leads to structural changes due to interactions between sodium alginate and other components, thus resulting in a decrease of the temperature with maximum weight loss rate. The third phase ranges from 670 to 800°C. Only small weight loss is detected in this phase [6].

Figure 5 shows the FTIR spectra of original collagen and collagen composite film. Characteristic bands of collagen are observed. The bands at 3421, 2925, 1641, 1562, 1411, 1245, 1051, and 644 cm^{-1} which belong to stretching vibration of –N-H bonds, stretching vibration of –C-H bonds, stretching vibration of amide III –C=O bonds, bending vibration of amide III –N-H bonds, in-plane bending vibration of –C-H bonds, stretching vibration of amide III –C-N bonds, asymmetric stretching vibration of –C-O-C bonds, and out-plane bending vibration of amide IV –C-N bonds, respectively (Fig. 5b)[7].

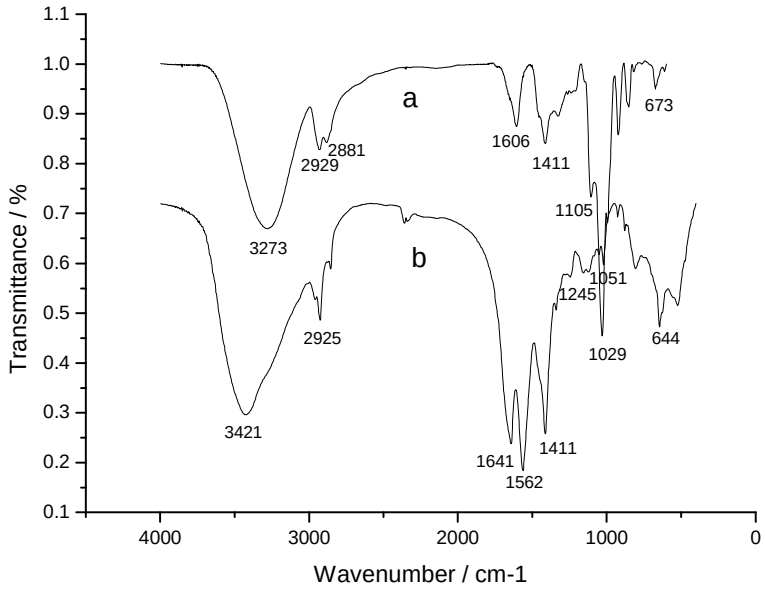

Figure 5 FTIR spectra of: (a) collagen composite film, and(b) collagen

In the spectrum of collagen composite film (Figure 5a), the band at 3273 cm^{-1} is assigned to -OH stretching vibration and –N-H stretching vibration. Compared to the spectrum of collagen (Figure 5b), the absorption band shifts to lower wavenumber zones. Similarly, the bands at 2929 and 2881 cm^{-1} are assigned to –C-H stretching vibration. The bands at 1105 and 1029 cm^{-1} with high intensity belong to –C-N stretching vibration and –C-O-C asymmetric stretching vibration, respectively. In fact, the crosslinking reaction of aldehyde group of glutaraldehyde with the amine groups and side chain hydroxyl groups in collagen generate many –C-N and –C-O bonds. Thus the two bands exhibit high intensity, especially the –C-O band at 1029 cm^{-1}.

Therefore, the presence of amine and hydroxyl groups in the collagen film leads to intermolecular hydrogen bonding. A network structure is formed due to cross-linking of collagen with glutaraldehyde, enhancing inter- and intramolecular interactions. This will help to improve the mechanical and moisture barrier properties.

Figure 6 shows SEM images of collagen composite film obtained with or without crosslinking agent. It appears that the surface of the composite film with crosslinking agent is more homogenous, suggesting good compatibility of collagen protein with sodium carboxymethyl cellulose, sodium alginate, and starch. This is beneficial for the improvement of the mechanical properties.

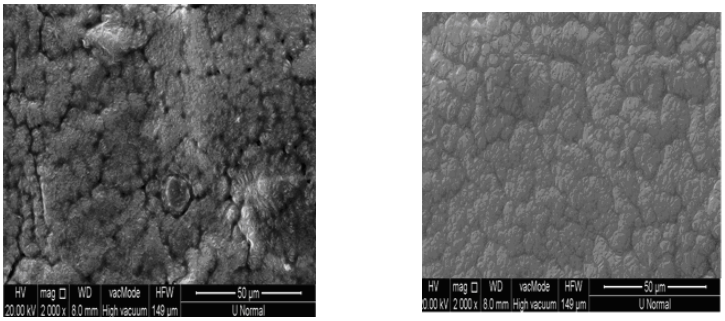

Figure 6 SEM images of collagen composite films: (a) with crosslinking agent, (b) without crosslinking agent

CONCLUSION

This work studied the preparation of collagen composite film as potential food packaging material. The influence of the amount of sodium alginate on the surface properties, tensile strength and elongation at break was considered. The optimum amount of sodium alginate was obtained. Addition of sodium alginate was found to greatly improve the tensile strength and elongation at break of the collagen composite films.

FTIR, SEM and TGA analyses show that there is a strong hydrogen bonding between the molecules in the composite film. A network structure is formed by using glutaraldehyde as a crosslinker. A good compatibility is obtained between collagen and sodium carboxymethyl cellulose, sodium alginate, and starch. Addition of sodium alginate enhances the interactions between the molecules, and leads to more homogeneous morphology. The combined effects of these factors greatly improved the mechanical properties of the collagen composite film.

The novel collagen film prepared in this work appears uniform, transparent and smooth, without bubbles. The film presents a good flexibility, mechanical properties and thermal stability. Taking into account its biodegradability and bio-based origin, collagen composite film can be considered as an ideal alternative to traditional packaging materials.

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