Fabrication and characterization of microfibrillated cellulose and collagen composite films

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ABSTRACT

Microfibrillated cellulose (MFC)-collagen composite films were prepared with a dispersion of acid swollen collagen fibers and carboxylated MFC at different ratios in an alkaline homogenous system. The surface topographic results obtained from SEM analyses indicated that the MFC entangled uniformly with collagen in the film and formed a closely interwoven network to reinforce the film structure. However, the MFC addition decreased the smoothness and light transparency of the films due to the aggregation of MFC. Compared to the film prepared with pure collagen, the hybrid composite film showed a higher strength and Young’s modulus but lower elongation. The swelling of the composite film in water increased with the increase of the MFC ratio in the film matrix. DSC and TG analyses demonstrated that adding MFC to collagen benefited the thermal stability of the films, due to the conformational and crystal changes in the MFC/collagen structure indicated by the FT-IR and XRD results. The MFC/collagen composite film can potentially be used as an edible material in the food and packaging industry, in particular for meat products.

Key words: microfibrillated cellulose; collagen, hybrid film; mechanical properties; water barrier; thermal properties

1. INTRODUCTION

Edible films such as sausage casing function as a protection barrier to extend the shelf life and preserve the qualities of meat food. These film materials are commonly made with collagen. The mechanical and barrier properties of these collagen films can be improved by blending collagen with biopolymers such as chitosan, protein and gelatin films. Due to its superior biocompatibility, weak antigenicity, accepted edibility, strength, and abundance, collagen has been fabricated into forms such as gels, sponges, fibers, and films, and been widely used in the pharmaceutical, food, healthcare, and cosmetic industries. Generally, collagen, a super family with 28 different members identified to date, is the most abundant protein of animal origin, wherein type I collagen is the dominant and wildly occurring main structural building blocks of connective tissues, conferring unique multi-scale organization, mechanical properties and biological functionality. Thus, the typically strong, rigid nature of skins, tendons and bones are attributed to the basic structure formed by many of these cross-linked collagen fibrils. The primary reason for the usefulness of collagen in biomedical application is that collagen can form fibers with extra strength and stability through its self-aggregation and cross-linking.

It is well known that hydration of native collagen causes swelling and seemingly dissolves completely in acidic solutions. Swollen collagen can dehydrate to insoluble collagen again when pH is made near neutral, classifying it as a reversible treatment. Hydration of collagen has a well-known phenomenon being studied using different pH, which is obedient to the Hofmeister rule which is for a pH < pI (where the anions are counter-ions). The repulsive double layer forces increase in the following order, NaSCN < NaI < NaCl, while at higher pH > pI, the forces increase in this order, NaCl < NaI < NaSCN. Originally, it was thought that the influence an ion has on macromolecular properties was in part, a consequence of the “making” or “breaking” bulk water structure. Recent studies of water molecules in salt solutions have however demonstrated that the bulk water structure is not central to the Hofmeister effect. Based on the swelling of collagen fiber due to hydration, modifications of electrostatic interactions through alteration in pH and ionic strength are quite effective approaches to modulate collagen structure and film functionality, wherein the co-extruded collagen casings with high elasticity and tensile strength is one of them.

To improve the mechanical and barrier properties of the collagen films, biopolymers such as cellulose can be added as a reinforcing component. Cellulose is a renewable and biodegradable material of high strength and low density. It is a natural and abundant biopolymer extracted from plants, and has been used as building materials, paper, textiles, and food additives. This wide
range of applications for cellulose nanofibrils (MFC) from native cellulose has sparked an increase in interest and attention. This biopolymer naturally exists as a semi-crystalline polysaccharide in the form of fiber occurring in wood, cotton, hemp and other plant-based materials with little existing in algae, tunicates, and some bacteria, has a width ranging from 5 to 20 μm and length in the range of 0.5 up to several millimetres and serves as the dominant reinforcing phase in plant structures. The fiber is structured by a linear chain of ringed glucose molecules and has a flat ribbon-like conformation. The repeat unit is comprised of two anhydroglucose rings \((\text{C}_6\text{H}_{10}\text{O}_5)_n\); \(n = 10,000\) to 15,000, where \(n\) is depended on the cellulose source material) linked together through an oxygen covalently bonded to \(\text{C}_1\) of one glucose ring and \(\text{C}_4\) of the adjoining ring (1 - 4 linkage), called the α-1-4 glucosidic bond. The intra chain hydrogen bonding between hydroxy groups and oxygen of the adjoining ring molecules stabilizes the linkage and results in the linear configuration of the cellulose chain. Within these cellulose fibrils there are regions where the cellulose chains are arranged in a highly ordered (crystalline) structure, containing amorphous or disordered regions.

Since cellulose has prodigious hydroxyl groups at the surface, surface modification based on polymer grafting, coupling agents, acetylation and cationic modification was performed in order to improve compatibility and homogeneous dispersion within the polymer matrices. Others have resorted to TEMED mediated oxidation of HCl derived nanocrystals to convert alcohol groups to carboxylic acid moieties for better dispersibility. The properties and structure of swollen carboxylated cellulose fibers, which have been studied, states that these fibers can homogenously and effectively swell in aqueous solution.

The derivative properties of collagen and cellulose themselves carry many specific advantages such as bovine hide collagen-cellulose derived biodegradable hybrid films, cellulose nanocrystal reinforced collagen composite films, bacterial cellulose collagen composites, bacterial cellulose-cellulose nanocomposite for bone tissue engineering and collagen-cellulose composite thin films that mimic soft-tissue and allow stem cell orientation. In one instance, cross-linked fibrous composites were prepared based on cellulose nanofibers and collagen with in situ pH induced fibrillation. Until now, little research has been conducted in food packaging field.

In this study, a 2-step process was developed based on the amphiprotic properties of collagen, to address the issue of the electrostatic interaction between the \(-\text{NH}_2\) groups of amino acids of collagen and the \(-\text{COOH}\) groups of MFC, which will inevitably leads to co-precipitation of the acid-swollen collagen fibers and MFC when they are mixed together. The co-precipitation causes inhomogeneous morphology and subsequently inferior mechanical performance of the resulting films. In current study, the collagen fibers were first swollen in an acidic medium followed by anionization in an alkaline medium, and then mixed with MFC to form a homogeneous mixture. The mechanical properties of films were evaluated based on tensile strength, Young’s modulus and elongation at break under dry and wet state, as well as swelling kinetics and residue rate in different conditions. DSC and TGA were used to investigate the thermal behaviors of the films, and the morphological properties were observed by SEM. FTIR and XRD were employed to characterize the interactions between collagen and MFC in the composite films. The thickness, surface roughness, and transparency of the composite films were also determined.

2. EXPERIMENTAL

A sample of bovine split skin (about 4 cm × 4 cm), pretreated by 10% (wt) lime (based on the wet skin) for 30 days was donated by Longbao Collagen casing Co., Ltd. (Zibo, China). A 1% (wt) carboxylated microfibrillated cellulose (MFC) dispersion was obtained from Tianjin Haojia Cellulose Co., Ltd. (Tianjin, China), prepared from the hardwood sulfite-based dissolving pulp based on a carboxylated modification and homogenization under high pressure. The MFC was 10-20 nm wide, less than 1 μm in length and had substitution degree of roughly 0.1. Glycerol (99.5 %) was obtained from Sigma-Aldrich (Shanghai, China). Other reagents were all analytical grade and used without further purification.

2.1. Preparation of the blend of collagen and MFC

Limed bovine split was immersed in distilled water repeatedly every 7 hours until pH was near 7. The washed skin was treated with 0.05 mol/L hydrochloric acid solution at a 1:3 skin to acid ratio and at a temperature of 20°C for 24 h. Collagen fibers were peeled carefully from the swollen skin with a rake tweezer carefully without damage, and mixed with distilled water to form a homogenous 1.0% (wt) collagen slurry.

2.2. Preparation of the MFC-collagen films

To prepare collagen-MFC blends, the acidic collagen solution was adjusted to pH 11 by adding 2 mol/L NaOH at 4°C, and the MFC dispersion was then mixed with collagen solutions of various ratios between 100:0, 75:25, 50:50, 25:75, and 0:100, with 10% (wt) glycerol as plasticizer (based on the total weight of collagen and MFC). The blend was homogeneously mixed for 2 h then casted onto pre-leveled acrylic plates (13 cm × 13 cm) followed by 24 h incubation at 25°C with ventilation to initiate formation of film. The films were then stored in an environmental chamber (20 ± 0.5°C, 50 ± 5% RH) for 7 d before analysis.

2.3. Characterization

2.3.1. Mechanical properties

Tensile strength (TS) and elongation at break (EAB) of dry and wet films samples were measured using a TA XT
plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK). Film strips (6.0 cm × 2.54 cm) of each sample were carefully cut and clamped between the tensile grips probes followed by stretching at the crosshead rate of 1 mm/s with an initial distance of 30 mm in a 500g-load cell. The wet strength was measured upon a 12 h immersion of the films in distilled water, and after the surface was dried with a tissue paper. For dry strength detection, the films were taken out from an environmental chamber without further treatment. At least ten specimens selected from each film were tested.

2.3.2. Swelling kinetics
Swelling capacity of the collagen films were calculated gravimetrically by measuring the mass of the samples before and after incubation in distilled water at 25 °C for 1, 2, 3, 6, 12 and 24 hours, and at various pH (4, 5, 6, 7, 8, 9) as described in the literature.

2.3.3. Thermal properties
DSC and TGA of casing samples were carried on using a simultaneous thermal analyzer (SDT Q600 TGA/DSC, TA Instrument). The samples weighed around 5-10 mg, were heated from room temperature to 600 °C at a heating rate of 10 °C/min under an inert atmosphere of N₂ at a flow rate of 100 ml/min. The data were analyzed with universal analysis 2000 software. The MFC sample was prepared through air drying of 10ml of 2% (wt) MFC dispersion. All film samples were detected without further treatment.

2.3.4. Morphological analyses
The surface morphology of the film samples were examined using a scanning electron microscope (SEM, JEOL JSM-6400) at 15 kV. The samples were coated with an ultra-thin (5-10 nm) layer of gold prior to SEM examination.

2.3.5. UV-Visible wavelength scanning
The films were measured using a UV-visible spectrometer (Perkin-Elmer, Lambda-25) by scanning over the wavelength range of 200 to 700 nm.

3. RESULTS AND DISCUSSION
3.1. Morphological properties of collagen fibers, MFC, and their composites.

Figure 1 SEM images of collagen fibers (top), and MFC fibers (bottom) at various magnifications.
The surface morphology of collagen fibers and cellulose fibers using scanning electron microscopy is shown in Figure 1. Under the same enlarged view (Scale bar, 100 μm, Figure 1a and Figure 1c), unwound, fine and soft collagen fiber structure and inflexible, thick and strong cellulose fibers were observed.

As clearly shown in Figure 2a and Figure 2c, the surface of the pure collagen film was smooth and homogenous and the pure cellulose film was rough due to bundles of aggregated MFC. In the MFC-collagen composite, collagen was homogenously cross-linked with MFC to form an interwoven net due to the similar basic charge conditions of the biomaterials. Moreover, Figure 2A-2C presented rather different microstructures in the cross section of the films, specifically the extent of their wrinkles in order of: pure cellulose film > MFC-collagen composites > pure collagen film.

### 3.2. Mechanical property

<table>
<thead>
<tr>
<th>MFC: CL</th>
<th>Thickness (µm)</th>
<th>Young’s modulus (MPa)</th>
<th>Tensile strength (MPa)</th>
<th>Elongation at break (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>45±2</td>
<td>1051.79±4.66</td>
<td>35.71±0.98</td>
<td>3.40±0.11</td>
</tr>
<tr>
<td>75:25</td>
<td>42±4</td>
<td>966.98±7.76</td>
<td>38.17±0.75</td>
<td>3.95±0.17</td>
</tr>
<tr>
<td>50:50</td>
<td>46±3</td>
<td>623.40±9.73</td>
<td>41.79±0.93</td>
<td>6.70±0.20</td>
</tr>
<tr>
<td>25:75</td>
<td>60±5</td>
<td>415.42±7.32</td>
<td>35.02±0.11</td>
<td>8.43±0.07</td>
</tr>
<tr>
<td>0</td>
<td>80±5</td>
<td>236.27±3.11</td>
<td>30.03±0.13</td>
<td>12.71±0.23</td>
</tr>
</tbody>
</table>

From Table 1, the pure MFC showed the highest Young’s modulus value (1051.79 MPa), followed by MFC-collagen composites films and the pure collagen film (236.27 MPa), indicating MFC quantities had an important role on Young’s modulus. The addition of MFC also increased the thickness of collagen films ranging from 45±2 µm to 80±5 µm as MFC dosage decreased from 100% (wt) to 0% (wt), which can be attributed to density and aggregation of MFC.

In Table 1, the tensile strength values changed as the MFC ratio varied. Among them, the MFC-collagen composite film of 50:50 ratio had the highest tensile strength (41.79 ± 0.93 MPa), which was attributed to the interaction of collagen and MFC and the stiffness of MFC. However, the MFC reinforced collagen films showed a significant decrease in the elongation at break. The decrease can be attributed to a decreased polymer matrix mobility, which is important for the flexibility of edible film. The higher tensile strength and lower elongation at break of the MFC-collagen films demonstrated their superiority over pure collagen films and pure MFC films.
3.3. Thermogravimetric analysis

Generally, the thermogravimetric (TG) curve of the biopolymer based film presents three stages, involving the loss of physically absorbed water, structured water, and decomposition of biomolecules. As depicted in Figure 3a, the weight loss of each sample went up to 10% below 150 °C, which was mainly due to the evaporation of free water and other volatile substances. Moreover, collagen films and MFC-collagen composites were stable at about 230 °C (residue of more than 85%), while stable temperature of MFC film was about 250 °C.

From the derivative thermogravimetry (DTG) curves (In Figure 3b), it was demonstrated that the maximum heat decomposition temperature of collagen films and MFC films were 291.58 °C and 279.74 °C respectively. However, the composite films presented a more complex decomposition behavior, with the thermal decomposition temperature of the main functional group of the material ranging from 190 °C to 380 °C, reflecting the transition of the solid state and loss of small molecular weight fragments.

3.4. Differential scanning calorimetry (DSC)

The Tm (melting temperature) values of the pure collagen films, pure cellulose and the MFC-collagen films were illustrated in Figure 4, showing some differences among pure MFC films (116.73 °C), pure collagen films (80.29 °C), and the three composite films with the values of 114.69 °C, 107.89 °C and 106.32 °C, respectively. The increase of the Tm of the collagen-MFC composite films with increasing MFC ration may be explained by the cross-linking of the MFC fibrils with collagen fibers, forming multiple intermolecular hydrogen bonding between the -COOH groups of carboxylated MFC and the -NH2 groups of collagen fibers. These interactions resulted in the fracture of hydrogen bonds within the collagen and rearrangement of the triple helix structure in the process of heat absorption of DSC. Similarly, the increased cross-links were observed in malonic acid cross-linked chitosan and collagen 3D scaffolds.

3.5. Swelling Kinetics

In Figure 5, the swelling capacity of the films presented the greatest change in the first three hours, and the curve is also the steepest in this period. In addition, the swelling capacity of the composite film containing 25%
MFC is lower than the pure collagen film, while the other composite film samples were however higher than the pure film. The swelling capacity of composite films is related to the hydrophilic potential of the material, primarily dependent on the functional groups and the density of effective cross-linking. Generally, cross-linking of collagen molecules promote a reduction in the swelling of the composite. At a lower MFC ratio, the cross-linking was increased between the two biopolymers, consequently reducing swelling of the films. However, at a higher ratio, more MFC did not benefit the formation of more cross-linkages, and instead interfered with the net structure of the collagen matrix. Meanwhile, the swelling rate of the composite films increased as the MFC ratio increased due to the increase of the hydrophilic groups from MFC.

3.6. Crystallization properties

As described in Figure 6, all the film samples had a sharp crystallization peak appearing at \( 2\theta = 22.5^\circ \). A peak of the pure collagen film appeared at \( 2\theta = 7.5^\circ \), which is a characteristic peak of collagen. However, two peaks (15.8° and 22.5°) were observed in the MFC samples and composite samples, which represented anhydrous crystallization peaks. For the composite films, the disappearance of the characteristic diffraction peaks (7.5°) of collagen, and the appearance of characteristic peaks for MFC at \( 2\theta = 15.8^\circ \) and strong at \( 2\theta = 22.5^\circ \), indicated strong interaction of MFC and collagen in the composite films.

3.7. FT-IR analysis

Figure 7 illustrates the FT-IR spectral details of MFC-collagen composite films, pure collagen films and pure MFC films. A broad, strong absorption in the region of 3,200 - 3,500 cm\(^{-1}\) and 2,850 - 3,000 cm\(^{-1}\) were observed for all samples, caused by the -OH and -C-H bonds respectively. The other characteristic peaks of pure collagen film and composite membrane appeared at around 1,630 cm\(^{-1}\), 1,550 cm\(^{-1}\) and 1,240 cm\(^{-1}\), which represented -C=O of amide-I band, -NH and -CN of amide-II band and -CN and -NH of amide-III band, respectively.

A sharp intense absorption peak was observed at 1,316 cm\(^{-1}\), caused by the derivatives of the main chain of the cellulose. These FT-IR results suggested that MFC was ionically cross-linked with collagen in the composite films.

4. CONCLUSIONS

High performance composite films were successfully fabricated with MFC and collagen fibers at various weight ratios: 100:0, 75:25, 50:50, 25:75 and 0:100. A relatively higher tensile strength was observed for the MFC-collagen films of 50/50 ratio. Adding MFC to collagen benefited the thermal stability of the composite films in terms of TGA and DSC, and reduced their swelling capacity to some extents. FT-IR and XRD results supported the hypothesis that there was a strong interaction in the composite films between the MFC and collagen fibers, which disrupted their crystallization.

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