Extraction and Characterization of Keratin and Keratin Hydrogels from Wasted Rabbit Hair

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Abstract. Fabrication and characterization of keratin hydrogel extracted from waste rabbit hair with the application of L-cysteine as reducing agent, using heating-and-cooling method at different pH and heating temperature, are the mainly focus of this study. The structural, morphological and chemical characteristic of different keratin samples were compared. It is concluded that the recovery rate of keratin of waste rabbit hair had reached 61%, and the hydrogel was fabricated in the condition that the concentration of keratin powder was above 125 mg/ml and the temperature was above 55 ℃. Furthermore, rabbit hair keratin hydrogel could be potential applied in a diverse range of food, feed and wound dressing fields.

1. Introduction
China has a long history of nearly 100 years in Rabbit Hair (RH) textile manufacturing, and being a leadership in the annual yield, high quality products, and producing technology [1]. In recent years, under the guidance of the national policy of stimulating domestic demand, the domestic market of rabbit hair products develops actively, and the processing technology of RH fiber and textiles including carding, sliver mixing and yarn spinning has made great progress [2]. RH fiber can improve comfort and flexibility obviously, increases the additional value of clothing, and has the high commercial value. Due to the surface morphology of unidirectional scale structure, some properties cause low spinnability of RH fiber, such as low tensile strength, week abrasion resistance, small crimp elasticity and bad cohesiveness [3], which result in producing a large amount of waste materials including staple yarn, kemp, coarse wool and heterotypical fibers. These waste materials are always discarded and incinerated directly, which not only cause the waste of resources, but also pollutes the environment. According to statistics, tons of RH fiber waste are discarded every year in China. RH fiber belongs to protein fiber containing a variety of proteins. Among them, keratin content is more than 90% [4], which is a kind of protein resources with high commercial value. Therefore, the current research on RH fiber has gone depth into the protein molecular level [5], and how to recycle the waste and use it rationally has become a hot point in scientific research.

Keratin is abundant in nature and widely exists in hair, nails, feathers and horns of animals [6]. It has the characteristics of good biodegradability, biocompatibility, cell adhesion performance, strong polarity, and high chemical activity [7], so it is always commonly used in medical dressing and cosmetics. Keratin solution can be used for electrosprinning to prepare environment friendly and high biocompatible fibers [8]. It can also be used to manufacture hydrogel, applying in livestock feed to provide essential elements and trace elements for animal growth; in adsorption material to treat waste
water due to hydrogen bonds or hydrophobic bonds formed between keratin molecules and dye ions and as well as heavy metal ions \cite{9}, in fabric coating treatment to improve the performances such as anti-wrinkle, anti-shrinkage and pilling resistance of fabric, in textile finishing treatment to improve the dyeing ability of textile because a large number of polar groups such as amino groups, hydroxyl groups are introduced and they can connect with dye ions \cite{11}. Up to now, there is little study on the extraction of keratin and the preparation of hydrogel materials. Tasaki Ken \cite{12} extracted keratin from pig hair by the method of thermal hydrolysis. Alahyaribeik Samira \cite{13} extracted keratin from chicken feathers using different reducing agents. Wang L \cite{14} extracted keratin from wool by melting. Although there have some studies on keratin extracting, the research about extraction of waste RH and the application of keratin in different field is rarely touched, so more work to be done in future is proposed. In this paper, we extracted keratin from waste RH by reduction method and prepared biomass hydrogel by heating and cooling method in order to make full use of waste materials produced by textile production and expand the application field of keratin.

2. Material and methodology

2.1. Materials
Rabbit hair (Shandong) used to extract keratin. Urea, NaOH, L-cysteine, Ethanol, KBr and Polyethylene glycol 20000 were purchased from Tianjin kermel chemical reagent Co. Ltd..

2.2. Extraction of keratin from rabbit hair waste
Extraction of keratin was carried out using the reduction method, where l-cysteine is used as a reducing agent to extract keratin powder from waste rabbit hair. 20g waste rabbit hair was cleaned by ethanol prior to be conditioned at 30min, then oven dried for 12 hours at 80°C. Subsequently, rabbit hairs were cut into 1cm pieces and put in 400ml of aqueous solution containing urea(8M), L-cysteine (0.165M), adjusted pH to 11 by NaOH, and kept stirring for 5h at 85°C. After that, it was filtrated and dialyzed in distilled water in a dialysis tube (molecular cut off 12,000 - 14,000) for 3 days at room temperature, by changing the distilled water every 4 hours until the keratin solution is white color. Furtherly, the keratin solution was concentrated in a solution of 18% polyethylene glycol 20000 for 24 hours. Then, the concentrated solution is freeze-dried in a vacuum lyophilized for 3 days. Finally, the completely dried keratin solution was ground in a high-throughput tissue grinder for 4 minutes and gained rabbit hair keratin powder.

2.3. Production of rabbit hair keratin hydrogel
The keratin powder was dissolved in 10ml distilled water with addition of 0.3g L-cysteine, and heated at a constant temperature for 30 min under stirring, then cooled down to room temperature.

The first set of experiments was designed to investigate the performance of different weight of keratin powder of 1.5g, 1.75g and 2.0g. The second set of experiments was designed to investigate the performance of different temperature of 55°C, 65°C and 75°C.

3. Analysis and characterization

3.1. The recovery rate of keratin
The undissolved rabbit hair fiber was placed in a glass weighing dish, dried at 80°C to a constant weight, and weighed every 30 min until the mass undulation within ±0.01 g. It was denoted as m1, the recovery rate of keratin was calculated according to the Eq.1.

\[ Q = \frac{m_0 - m_1}{m_0} \]
Q is the recovery rate of RH keratin (%); $m_1$ is the mass of residual precipitate (g); $m_0$ is the mass of rabbit hair fiber after pretreatment (g).

3.2. **SEM**
The keratin hydrogel produced at different concentrations and temperatures were freeze-dried. The microstructure of the hydrogel was observed by Scanning Electron Microscope (SEM) (Phenom, Shanghai) pictures randomly collected from the samples.

3.3. **Fourier transform infrared (FT-IR) analysis of keratin hydrogel**
FI-IR spectra were gained on a Thermo scientific Nicolet iS50 model spectrometer with Smart Orbit-Diamond model ATR on transmittance mode. The spectrum data is in the range of 4000-400 cm$^{-1}$ with a 0.4 cm$^{-1}$ spectral resolution. The number of scanning times is 20. The samples were mixed by 0.12 mg KBr powder and 0.01 mg freeze-dried keratin hydrogel powder, then grinding and pressure.

3.4. **UV–vis spectrophotometer analysis**
An Ultraviolet-Visible Infrared spectrophotometer (Thermo-EVOLUTION201) was employed for determining the samples’ absorbance and draw the absorption spectrum.

4. Results and discussion

4.1. **Recovery rate**
The mass of RH before extraction is $m_0 = 20$ g, and the mass of residual precipitate is showed in Table 1.

|   | 1   | 2   | 3   | 4   | 5   | mean |
|---|-----|-----|-----|-----|-----|------|
| $m_1$ | 7.79 | 7.83 | 7.75 | 7.83 | 7.80 | 7.80 |

The recovery rate is calculated as 61%.

4.2. **SEM**
The morphologies of the keratin powder and hydrogel materials were investigated and the SEM of samples are shown in Figure 1.
Figure 1. SEM images of the keratin powder and hydrogel. (a) Keratin powder; (b) Keratin hydrogel; (c-e) Keratin hydrogel with different concentrations; (f-h) Keratin hydrogel with different temperatures.

Compare picture (a) with (b), the microstructure of keratin powder is lamellar and irregular and the microstructure of keratin hydrogel is non-uniform and porous. It can be inferred that the molecules in the keratin hydrogel were rearranged to form covalent bonds, presenting a spatial network structure. Good connectivity, air permeability and water retention of the material is expected due to the pores.

As shown in (c-e), the porous structure of the gel becomes denser with a higher concentration of keratin, while the porous structure of the gel becomes looser with a lower concentration of keratin. Moreover, with the increase of keratin concentration, the pore size of each pore in the porous structure also decreased significantly, indicating that the higher the concentration of keratin, the closer the cross-linking between molecules in the gel prepared.

As shown in (f-h), the higher the temperature of the preparation of keratin gel, the smaller the aperture of the pore in its three-dimensional porous structure and the closer the structure. High temperature can make keratin molecules move more violently and react more completely, and the more closely the cross-linking between molecules in the prepared hydrogel.

4.3. FT-IR analysis
The chemical composition of the keratin powder and hydrogel was determined through FT-IR analysis. Figure 2 shows FT-IR spectrums of the extracted keratin productions according to two different existences.

Figure 2. FT-IR spectrums of the extracted keratin productions (a: powder, b: hydrogel)
As seen in the figures, characteristic absorption of rabbit hair keratin peaks show assigned mainly to the peptide bonds with the characteristics of amide I - III vibrations. The absorption peak of C-H in \(-\text{CH}_3\) in keratin molecule is 2938cm\(^{-1}\), the peak of the C-S and S-S bonds of cystine residues are 930cm\(^{-1}\) and 588cm\(^{-1}\), respectively, and it is obvious that the extracted keratin powder shows a more intensive C-S adsorption. The amid I peak is mainly connected with the C=O stretching vibration and it occurs around 1666cm\(^{-1}\) while the amid II, which falls around 1529cm\(^{-1}\) is related to N-H bending and C-H stretching vibration. The amid III peak occurs around 1236cm\(^{-1}\) and it results from in phase combination of C-N stretching and in N-H in plane bending, with some contribution from C-C stretching and C-O bending vibrations.

By contrast, the infrared spectra of keratin powder and hydrogel is basically identical. The amid I peak of keratin powder is 1673cm\(^{-1}\), and the peak moves to 1666cm\(^{-1}\) of hydrogel. The mainly reason for this change is that the hydrogen bonds between the chains of keratin molecules reduce and forming of covalent bonds with water during the processing of hydrogel formation. The blue shift of amid I peak occurs under the action of covalent bonds. The amid II peak at 1539cm\(^{-1}\) indicates that structure of keratin is alpha helix, at 1508cm\(^{-1}\) is beta fold. Figure 2 shows the amid II peak of keratin powder is 1536cm\(^{-1}\), and hydrogel is 1529cm\(^{-1}\), the displacement changes -7, indicating the alpha helix structure in keratin decreased, transferred to irregular crimp and beta fold structure. In conclusion, the secondary structure between keratin powder and hydrogel has changed.

4.4. UV–vis spectrophotometer analysis

UV absorbance of keratin was tested and showed in Fig. 3, and a maximum absorption peak at 216nm with intensity of 2.42 is observed. The strong absorption band at 200–250nm symbolize the presence of a K band, inferring a conjugated system with a double bond, which may attribute to the existing of peptide bonds and amino acids inside. The benzene ring of the R side chain of the aromatic amino acids such as tyrosine, phenylalanine, tryptophan, etc. inside keratin has an unsaturated conjugated double bond system, while transferring from \(\pi\) to \(\pi^*\) of electrons in the \(\pi\) orbital of the double bond happens when they absorb ultraviolet light, thus producing near-ultraviolet light absorption. Additional, conjugation effect between the double bonds will greatly enhance the ultraviolet absorption intensity. Moreover, RH keratin contains non-aromatic amino acids such as unsaturated bond C=O and C=N, and the group in its side group produces \(n \rightarrow \pi^*\) transition for electrons exposed to ultraviolet light.

Figure 3. Ultraviolet spectrum of keratin

Figure 4 shows the absorbance peak increases with the increase of keratin concentration in the hydrogel. The maximum absorption peak is at 207nm and the absorbance is 3.46. Figure 5 shows the relationship between absorbance and reaction temperature that the absorbance increases with the rising
of reaction temperature. The maximum appears at 207nm with the absorbance of 3.30. Therefore, the absorbance of keratin hydrogel is higher than that of keratin, implying a stronger absorption capacity of ultraviolet light. Rising temperature or increasing the concentration of keratin can make the structure more closely and thus increase UV absorbance good.

![Ultraviolet spectrum of different concentrations of keratin hydrogel](image1)

**Figure 4.** Ultraviolet spectrum of different concentrations of keratin hydrogel (a:125mg/ml, b:150mg/ml, c:175mg/ml)

![Ultraviolet spectrum of different temperature of keratin hydrogel](image2)

**Figure 5.** Ultraviolet spectrum of different temperature of keratin hydrogel (a:55℃, b:65℃, c:75℃)

### 5. Conclusions

The morphology of keratin hydrogels was observed by scanning electron microscopy (SEM), showing an obvious porous structure with a multi-layer reticular section. It belongs to heterogeneous hydrogel for each pore has different pore size. Moreover, by observing the morphology of keratin hydrogel, it can be found that the higher the temperature and concentration, the higher degree of cross-linking between molecules chains and the smaller the pore size. The hydrogels were characterized by FT-IR and UV spectrum analyses, and it was found that the content of alpha helix structure decreases accompanied with the increase of the content of irregular curly structure and beta - folding structure as the transformation of keratin powder into hydrogels. The angle of protein secondary structure has changed, implying a higher ability to absorb ultraviolet light.
The keratin gel prepared in this study belongs to natural hydrogel. It has a three-dimensional, cross-linked network microstructure with good tissue adaptability and degradability in vivo. It can be used as a carrier for drug delivery in biomedical field. It has a good UV absorption function, and can be used to further determine the UV protection performance and prepare the sunscreen gel.

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