Toward a Circular Economy: Transforming Wool Waste from Leather Industry into Keratin with a Reductive Natural Deep Eutectic Solvent

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Toward a Circular Economy: Transforming Wool Waste from Leather Industry into Keratin with a Reductive Natural Deep

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Eutectic Solvent

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Abstract

The leather processing industry generates significant animal fiber waste, while current ineffective disposal of animal fiber waste (incineration and landfilling) results in pollution, which obstructs the leather industry’s path to sustainable development. This study reports a green and efficient strategy for the valorization of wool waste using a reductive natural deep eutectic solvent (NADES) system composed of citric acid (CA) and L-cysteine (L-Cys). The experimental evaluations assessed wool dissolution efficiency, keratin yield, and the properties of regenerated keratin. Notably, a 2:1 molar ratio of CA to L-Cys enabled complete wool dissolution at 90 °C within 3 hours, yielding ~76% keratin, comprising 58% water insoluble keratin (WIK) and 18% water soluble keratin (WSK). SDS-PAGE analysis showed a broad molecular weight distribution for WIK, whereas WSK was primarily below 20 kDa. X-ray diffraction results confirmed a pronounced reduction in the á-helix content of WSK relative to natural wool and WIK. Fourier-transform infrared spectroscopy, differential scanning calorimetry, and elemental analysis indicated trace CA and L-Cys residues in WSK, which nonetheless exhibited good cytocompatibility, highlighting its potential for applications in biomedical and material sciences. This approach not only addresses waste management challenges but also opens avenues for production of high value-added

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products, thereby enhancing the environmental and economic sustainability of the leather industry.

Key Words : Reductive natural deep eutectic solvent, citric acid, L-Cysteine, wool waste, keratin

1. Introduction

Keratin, a cysteine-rich fibrous protein, is abundant in natural sources such as wool, feathers, hair, and horn. It holds significant promise as a sustainable substitute for fossil-based materials.1-3 With the growth of the global economy, the leather processing industry generates considerable quantities of waste wool, presenting an abundant and underutilized resource for keratin extraction.4 Recently, keratin derived from wool has attracted increasing interest due to its unique properties, such as high strength, good biocompatibility, and biodegradability.5, 6 These attributes make it a valuable material for applications in biomedicine, cosmetics, and materials science.7-9 However, the extraction of keratin from wool remains challenging, owing to the extensive strong intermolecular and intramolecular disulfide and hydrogen bonds that stabilize the á-helix and â-sheet structures of the native keratin.10, 11

Deep eutectic solvents (DESs) have recently emerged as a promising class of green solvents formed through hydrogen bonding interactions between a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD).12

DESs offer advantages such as low toxicity, biodegradability, and facile preparation.13 Natural deep eutectic solvents (NADESs), composed of natural components such as sugars, amino acids, and organic acids, further embody green chemistry principles.14 In this study, we develop a reductive NADES composed of citric acid (CA) and L-Cysteine (L-Cys), demonstrating its efficacy for keratin extraction from wool. CA is also a green reducing regent, preventing the oxidation of L-Cys. We systematically investigate the effects of varying molar ratios of CA to L-Cys on the yields and molecular weight distribution of the regenerated water-insoluble keratin (WIK) and water-soluble keratin (WSK). By comprehensively characterizing the chemical structure, morphology, and physical properties of the extracted keratin, we demonstrate the potential of the reactive NADES system as a

green and efficient approach for wool keratin extraction.

Figure 1 : Preparation and characterizations of the NADES. (a) The formulations and preparation process of reductive NADES. (b) The DSC curves of NADES with different molar ratios of CA to L-Cys. (c) The viscosities of NADES with different molar ratios of CA to L-Cys.

2. Results and discussion

2.1 The preparation and characterizations of NADES

To synthesize the reactive NADES, CA (hydrogen bonding donor) and L-Cys (hydrogen bonding acceptor) were mixed at three molar ratios (1:1, 2:1, and 3:1) and transferred to a pressure vessel, respectively. An additional 20% (wt/wt) of water, relative to the total mass of CA and L-Cys, was added. The reaction was conducted at 85 °C for approximately one hour, yielding uniform solutions. The preparation of the reactive NADES is illustrated in Fig. 1a. Following synthesis, the NADES solutions were characterized using DSC to analyze their thermal properties, as shown in Fig. 1b. The CA and LCys have melting points of 153 °C and 240 °C, respectively. As shown in Fig. 1c, the viscosities of CA:LCys mixtures at ratios of 2:1 and 3:1 were similar and lower than that of the 1:1 mixture.

2.2 Regeneration of wool keratin

The regeneration process is illustrated in Fig. 2a. At 900

C for 3 h, the NADES systems exhibited distinct dissolution efficiencies, as shown in Fig. 2b. The keratin solutions derived from the CA:L-Cys/2:1 ratio was clearer compared to those obtained from the CA:L-Cys/1:1 and CA:L-Cys/3:1 systems. To further quantify the dissolution efficiency, the mass balance after the dissolution was calculated (Fig. 2c). The protein content of natural wool, determined by Kjeldahl method, was approximately 98.9%.

Figure 2 : The dissolution of natural wool. (a) The dissolution of wool and regeneration of keratin. (b) The pictures of wool dissolution in the NADES systems with different molar ratios of CA to L-Cys. (c) The composition of raw and mass balance of regenerated keratin. (d) SDS-PAGE pattern of the protein standard (left) and the regenerated keratin.

2.3 Structures and properties of natural wool and regenerated keratin

The WIK and WSK obtained from different NADES systems exhibited different structures, leading to variations in their properties. The XRD profile of the natural wool displayed two strong diffraction peaks at

2θ

The CA:L-Cys/2:1 system achieved complete dissolution (100%), outperforming CA:L-Cys/3:1 (92.6%) and CA:LCys/1:1 (86.45%). The total keratin yield for the CA:LCys/2:1 system (75.43%) surpassed that of CA:L-Cys/ 3:1 (68.53%) and CA:L-Cys/1:1 (64.23%). The molecular weight distribution of WIK and WSK obtained from the different NADES systems were analyzed using SDSPAGE (Fig. 2d). The molecular weight distribution of WIK was relatively wide (3~60 kDa). In contrast, the molecular weight distribution of WSK was primarily concentrated below 20 kDa.

α-helix β-sheet

of 9° and 19°, corresponding to and structure, respectively (Fig.3a). Post-dissolution, the

α-helix β-sheet

peak at 9° weakened in WIK, while the peak at 19° intensified and shifted leftward, indicating increased crystal plane spacing (Fig. 3b). For WSK, the 9° peak diminished further, disappearing entirely in the 1:1-WSK sample, reflecting reduced crystallinity.

Figure 3 : The structures and properties of raw keratin in natural wool and regenerated keratin. The XRD patterns of (a) WIK and (b)WSK. The FTIR patterns of (c) WIK and (d) WSK. The DSC curves of (e) WIK and (f) WSK. The TGA curves of (g) WIK and (h) WSK. (i) The C/S ratios of raw and regenerated keratin.

The chemical structures of natural wool, WIK, and WSK were investigated by ATR-FTIR (Fig. 3c-d). Characteristic absorption bands, including amide A (3280 cm^{-1}), amide I (1630 cm^{-1}), amide II (1520 cm^{-1}), and amide III (1230 cm^{-1}), were present in all samples, indicating structural preservation. The thermal properties of the raw keratin in natural wool and regenerated keratin were investigated by DSC and TGA (Fig. 3e-f). Both raw keratin and regenerated keratin exhibited an initial endothermic peak at 75-900 C, corresponding to water loss. A weak peak at 1900 Cin the regenerated WSK was attributed to the CA decomposition. The TGA results revealed two weight loss stages (Fig. 3g and Fig. 3h). The first stage, occurring below 1000 C, was on account for the loss of water in

samples. The second stage, from 240

to 400 °C, is associated with the breakage of disulfide bonds and the â-sheet structure and skeletal degradation. The maximum degradation temperatures (Tmax) for WIK and WSK were slightly higher than that of raw keratin, indicating enhanced thermal stability for WIK and WSK. The elemental analysis of all samples was determined by an elemental analyzer for the content of C, H, N, and S. Compared with the raw keratin, the S content in the regenerated WSK was obviously higher. Based on these findings, the C/S ratio of each sample was calculated, as illustrated in Fig. 3i. Compared with the natural wool, the C/S ratios of regenerated WIK were slightly higher, while that of regenerated WSK were lower. Additionally, the C/S ratios of regenerated WSK increased with the increase in the ratio of L-Cys in NADES. This result

indicated that residual L-Cys may be present in WSK. In addition, the cytotoxicity of the regenerated WIK and WSK obtained using the CA:L-Cys/2:1 system was assessed. From the results, the cell viabilities of regenerated 2:1WIKand 2:1-WSK were all more than 70% after 3 days of culture (Fig. 4), indicating the 2:1-WIK and 2:1-WSK had excellent cytocompatibility, even with trace amounts of residual CA and L-Cys in regenerated 2:1-WSK.

Figure 4 : The cytotoxicity test of regenerated (a) 2:1-WSK and (b) 2:1-WIK.

3. Conclusions

In this study, CA and L-Cys were utilized to develop a novel reductive NADES for wool dissolution. Under mild conditions (90 !, 3 h), the system achieved complete wool dissolution, yielding approximately 58% WIK and 18% WSK. These yields are comparable to, or even surpass, those reported for other solvent systems. Structural analysis via XRD indicated a reduction in á-helix content in WIK, with the structure nearly disappearing in WSK. Furthermore, the thermal stability of the regenerated keratin, assessed by TGA, was comparable to that of natural wool. FTIR, DSC, and elemental analyses confirmed trace amounts of residual CA and L-Cys in WSK, which has no adverse influence on cytocompatibility. These findings highlight the potential of using reductive NADES composed of CA and L-Cys as a green and efficient method for extracting wool keratin.

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