Supporting Information (SI)

Sustainable Recovery of Keratin from Chicken Feather Waste and its Processing for Biomedical Applications

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Extended Introduction

Compared with other sources, such as wool and human hair [1–8], feathers are 2.5 times more abundant globally than wool and more easily controlled in their native form than human hair [5,9], making them particularly attractive for large-scale valorisation. Importantly, the development of effective methods for the valorisation of chicken feather waste aligns with the circular economy framework and the UN Sustainable Development Goals (SDGs) [10–14].

Commonly reported methods have negative impacts on keratin structure and properties. For example, elevated temperatures during chemical hydrolysis led to amino acid degradation, and chemical compounds such as thiols and 2-mercaptoethanol pose environmental risks. Additionally, most conventional methods are energy-intensive and time-consuming, underscoring the need to develop more sustainable and effective processes at an industrial scale [9,15,16]. Compared with conventional methods, IL-based processes not only achieve high protein recovery yields but also preserve protein properties for biomedical applications. Nevertheless, process performance, solvent cost, and solvent recovery and reuse remain important considerations for large-scale implementation [14,17].

Table S1. Common methods proposed for keratin recovery and their most relevant characteristics.

Method	Characteristics		Ref.
Alkaline hydrolysis	-	Long dissolution time and potential protein degradation	18
	+	Simple process	
Acid hydrolysis	-	Long dissolution time and use of hazardous solvents	19
	+	Simple process	
Oxidation	-	Potential protein degradation and low keratin recovery yield	20
	+	Simple process	
Reduction	-	Use of hazardous and flammable solvents	21
	+	High protein recovery yield	
Microwave	-	High industrial application costs	22
	+	Short dissolution time	
Ultrasound	-	High industrial application costs	23
	+	Short dissolution time	
Dissolution with Ionic liquids	-	High cost and hazardous, if not properly designed	2,14,24
	+	High protein dissolution and recovery yield, if properly designed	
Dissolution with Deep eutectic solvents	-	High cost and hazardous, if not properly designed	25
	+	High protein dissolution and recovery yield, if properly designed	

Extended keratin characterisation

In the FTIR analysis (Figure 2a), we identified the characteristic stretching vibrations of O–H and N–H (amide A), C═O stretching (amide I), N–H bending and C–H stretching (amide II), and amide III. These findings were in accordance with the chicken feather sample. TGA analysis (Figure 2b) revealed a two-step degradation process. The first step, occurring up to 100 °C, corresponds to water loss, while the second one, beginning at 190 °C, corresponds to the degradation of the secondary structure of keratin, aligning with the feathers' properties. SDS-PAGE analysis (Figure 2c) determined the molecular weight of keratin samples, showing a band around 5-12 kDa, which is consistent with the expected molecular weight of feather-derived keratin (approximately 10 kDa, β-keratin). Regarding X-ray (Figure 2d), diffraction peaks between 5° - 14° (α-protein) and 15° - 25° (β-protein) confirmed the structural integrity of the recovered keratin. Elemental analysis (Figure 2e) provided insights into the composition of keratin, which contained 49.39 wt% carbon, 7.29 wt% hydrogen, 14.90 wt% nitrogen, and 1.23 wt% sulphur. The nitrogen content is attributed to peptide bonds, while the sulphur content is linked to the cysteine amino acids present in keratin [9,14].

Extended keratin recovery at an industrial scale

A process simulation was performed in Aspen Plus V11, integrating the Aspen Economics package, to estimate the equipment purchase and plant's installation costs.
The IL feed (1000 $kg \cdot h^{-1}$) with feathers at a loading of 5 % was considered. A stream of 1450 $kg \cdot h^{-1}$ with 20.25 wt% of ethanol in water was introduced, cooled with a refrigerant, and allowed to settle for 5 h. Multiple-effect evaporators were considered for IL regeneration.

Extended keratin-based materials

The strong interactions between keratin and the polymers (cellulose and chitin) are due to the robust hydrogen bonding network among their functional groups (CH_3, CONH, NH_2, and OH) [26]. To understand the impact of blend composition on film properties, we processed films made from keratin-cellulose (75:25 and 50:50 w/w) and keratin-chitin (75:25 and 50:50 w/w). These were characterised using physicochemical (FTIR, TGA, and contact angle),

mechanical (tensile strength), and morphological analyses (scanning electron microscopy (SEM)) [27].

The FTIR spectra (Figure 4b) displayed the characteristic bands of keratin-based films, confirming the presence of keratin (amide A, I, II, and III) and the biopolymers used for the blends (cellulose and chitin), revealing effective blending. TGA analysis (Figure 4c) showed that all keratin film samples were stable up to 215 °C, while the blends were stable only up to 160 °C. Blend decomposition occurred between 160 °C and 360 °C, with a slow degradation, and was divided into steps, suggesting physical rather than chemical interactions between the biopolymers [27]. SEM analysis (Figure 4d) revealed smooth surfaces for all keratin-based films except those prepared at pH 12 or with 10 wt% glycerol. The roughness observed at pH 12 was due to the use of sodium hydroxide (NaOH) for pH adjustment, while glycerol-induced roughness resulted from solution destabilisation during casting and drying. Regarding the blends, the films appeared homogeneous, suggesting an appropriate blending; however, some irregularities indicated the presence of biopolymer particles on the surface, likely due to different coagulation rates [27].

The wettability of keratin-based films, assessed by contact angle measurements (Figure 4e), demonstrated their hydrophilic nature (contact angle lower than 90 °) due to the presence of hydrogen-bond donor and acceptor groups, such as amino, carboxylic, and hydroxyl groups [27]. The tensile strength of keratin-based films (Figure 4e) significantly increased by creating blends, rising from 14 ± 2 MPa to 66 ± 1 MPa for keratin-chitin films. This demonstrates the benefit of adding chitin due to its more promising mechanical properties [27].

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