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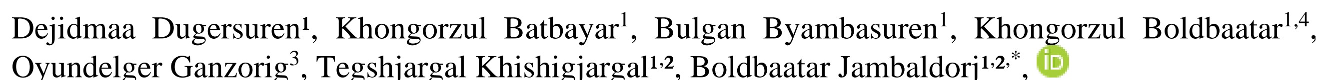
Article

Determining the Optimal Conditions for Extracting Keratin Protein from Chicken Feathers by Hydrolysis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Abstract
Chicken feathers, a byproduct of poultry farming, consist of approximately 90% keratin protein and are a protein-rich raw material [1]. Keratin is highly resistant to chemical and physical influences and degrades slowly in nature, causing environmental pollution when discarded. Currently, there are no industries or businesses in Mongolia processing waste chicken feathers. To extract keratin protein hydrolysates from chicken feathers, hydrolysis using sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), and a combination of NaOH and H_2O_2 was employed. The feather microstructure was analyzed by scanning electron microscopy (SEM), protein yield by Kjeldahl and gravimetric methods, molecular weight by SDS-PAGE electrophoresis, structural analysis by Fourier-transform infrared spectroscopy (FT-IR), and thermal properties by thermogravimetric analysis (TG/DTA). The study found that protein yield varied with hydrolysate composition and temperature. A 4% NaOH hydrolysate resulted in the highest yield of 74.8%, while a 2M H_2O_2 + NaOH hydrolysate produced a 61.5% yield. High-temperature, short-duration experiments produced gel-like protein hydrolysates. This research determined some optimal conditions for preparing high-yield keratin hydrolysates from discarded chicken feathers in Mongolia. These findings can serve as a foundation for future industrial applications and further research.
Keywords: Chicken feathers, keratin, hydrolysis

1 Introduction

Due to increasing global food demand, poultry farming has expanded, resulting in an estimated 8.5 billion tons of chicken feather waste annually. Such waste is typically disposed of by burning or landfilling [2]. However, feathers are an inexpensive, eco-friendly, natural protein (keratin) source and are widely used in cosmetics, biomedicine, and other industries. Keratin is a protein found in the skin, horns, hair, feathers, and scales of mammals, reptiles, birds, and fish, playing a structural role [3]. Feathers are composed of about 91% keratin protein, 1.3% fat, and 7.7% water. Keratin is made up of 18–20 amino acids linked by peptide bonds. It also contains covalent disulfide, ionic, and hydrogen bonds [4]. Feather keratin is rich in glycine, alanine, serine, cystine, and valine, but low in lysine, methionine, and tryptophan [5]. Due to its chemical and physical stability and slow biodegradation, keratin has the potential to be developed into various hybrid materials for medicine, agriculture, and electronics [6].

Multiple methods have been employed to extract keratin from waste biomass, including alkaline hydrolysis, oxidative treatments, enzymatic digestion, and ionic liquid dissolution [7]-[9]. Among these, alkaline hydrolysis using sodium hydroxide (NaOH) remains the most accessible and scalable approach, especially for industrial use in developing countries due to its low cost and simplicity [10]. Recent studies have optimized keratin extraction from diverse raw materials such as sheep wool, goat hair, and chicken feathers, producing hydrolysates with varying molecular weights and solubility [11] [12].

In Mongolia, imported chicken breeds adapted to the local climate undergo seasonal changes in feather structure, as noted by poultry farmers. We previously identified optimal conditions for extracting keratin from Mongolian sheep wool. This study aims to apply those methods to chicken feathers, identifying optimal conditions for producing water-soluble and insoluble keratin hydrolysates from waste feathers and laying the groundwork for new protein-based materials.

2 Materials and Methods

2.1 Sample Preparation

Chicken feathers from the "Ross-308" breed were obtained from Bayalag Takhia LLC. Feathers were washed 2–3 times with surfactant solution, rinsed with clean water, soaked in 50% ethanol for 24 hours to remove fats, rinsed again, air-dried, and cut into small pieces.

2.2 Preparation of Keratin Hydrolysate

In the alkaline hydrolysis method, 1 g of finely chopped chicken feathers was treated with 20 mL of NaOH solution at concentrations of 1%, 2%, and 4%. The reaction was carried out at 50–60 ^{0}C for 10, 20, and 30 minutes under continuous magnetic stirring. Following hydrolysis, the mixture was filtered to remove insoluble residues, and the filtrate was subjected to dialysis for 72 hours using a 0.22 μm dialysis membrane (Jiele PU, China). After dialysis, the pH of the solution was adjusted to 7, 5, and 2 using 2 M HCl. The resulting hydrolysate was then vacuum-filtered and dried. For the oxidative-alkaline hydrolysis method, 20 mL of H_2O_2 solution (1 M, 2 M, or 4 M) was combined with 2 M NaOH to achieve an alkaline pH of approximately 11. Subsequently, 1 g of feathers was added, and the mixture was stirred at 50–60 ^{0}C for 30 minutes. After filtration and cooling, the solution underwent dialysis for 72 hours using a 0.22 μm dialysis membrane (Jiele PU, China). The pH was then adjusted to 7, 5, and 2 with 2 M HCl, followed by vacuum filtration and drying. A schematic representation of the overall process is presented in Figure 1.[13].

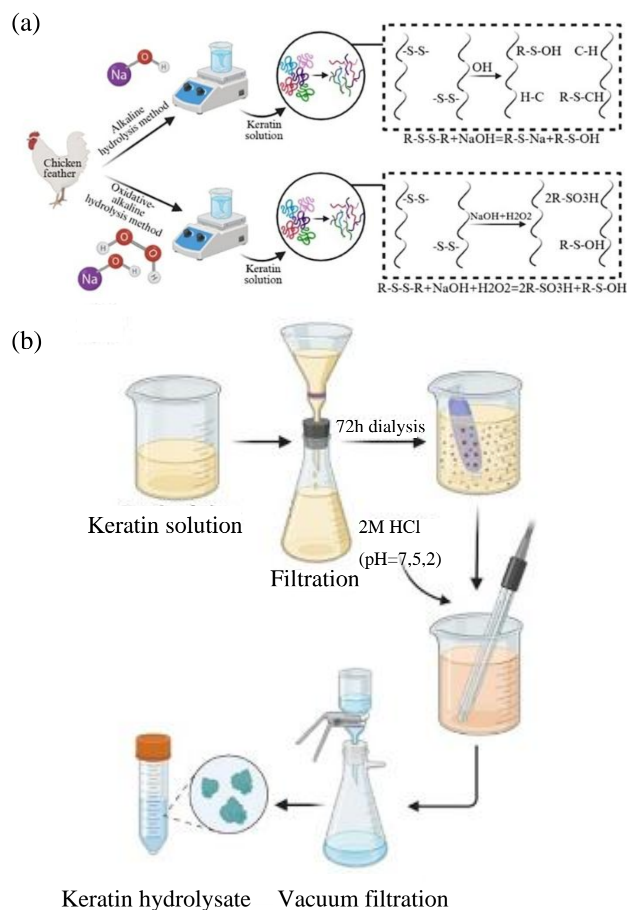

Fig 1. (a) Alkaline and oxidative-alkaline hydrolysis routes for keratin extraction from chicken feathers. (b) Purification process of the keratin hydrolysate.

2.3 Determination of Protein Yield and Molecular Weight

Protein yield was determined by comparing the dry mass of the recovered keratin hydrolysate with the initial mass of raw feathers. The protein content of both untreated feathers and the obtained hydrolysates was quantified using the Kjeldahl method, with cow milk used as a reference standard [14]. All experiments were performed in triplicate (n = 3), and the results are expressed as mean ± standard deviation. The molecular weight distribution of the extracted proteins was analyzed by SDS-PAGE. Electrophoresis was conducted using a 5% stacking gel and a 12% resolving gel, with 20 μg of protein loaded into each well. A protein marker ranging from 14.4 to 116 kDa was used as a reference. The electrophoresis was performed at 80–150 V for approximately 2 hours until clear band separation was achieved. The gels were then stained with Coomassie Brilliant Blue R-250, destained twice using a standard destaining solution, and imaged for further analysis [15].

2.4 Surface Characterization and Spectroscopic Analysis

Surface morphology and degradation behavior of feather samples were characterized using scanning electron microscopy (SEM). Three types of samples were analyzed: untreated feathers, feathers hydrolyzed with NaOH for 10 minutes, and feathers treated with a combined H_2O_2–NaOH system for 10 minutes. Prior to imaging, all samples were sputter-coated with a thin gold layer (~5–10 nm) using a vacuum coater operating at approximately 0.05–0.1 mbar to ensure adequate surface conductivity. SEM images were acquired using a NeoScope JCM-6000 benchtop SEM under high-vacuum mode, with an accelerating voltage of 10–15 kV and magnifications of 600× and 2400× [16].

Chemical structural changes were investigated by Fourier transform infrared (FT-IR) spectroscopy. Spectra were recorded using a Shimadzu IR Prestige-21 spectrometer equipped with a DLATGS detector. Air-dried samples were finely ground, mixed with spectroscopic-grade KBr, and pressed into pellets prior to analysis. The FT-IR spectra were collected over the range of 4000–1000 cm^{-1}, with a resolution of 4 cm^{-1} and 32 scans per sample to improve the signal-to-noise ratio [17]. All measurements were conducted at the Institute of Physics and Technology, Mongolian Academy of Sciences.

2.5 Thermal Decomposition Analysis

Thermal properties of raw feathers and keratin-like proteins from the two methods were analyzed using a TG-DTA8122 system from Japan. Approximately 5 g of the sample was placed in an aluminum holder and heated from room temperature to 500^0C at 6^0C/min under a nitrogen flow rate of 10 ml/min [18].

3 Results and discussion

3.1 Evaluation of Protein Yield

Protein yield from chicken feathers was strongly influenced by hydrolysis conditions. As shown in Figure 2, hydrolysis with NaOH alone demonstrated a direct relationship between concentration and protein recovery: 1% NaOH for 30 minutes yielded 42.3% protein, 2% NaOH resulted in 56.7%, and 4% NaOH produced the highest yield of 74.8%. Extending hydrolysis beyond 30 minutes slightly decreased the yield, indicating that 30 minutes was the optimal time. To recover protein fragments, the pH of the hydrolysate was adjusted to 7, 5, and 2, and precipitation was monitored at 10, 20, and 30 minutes. Maximum precipitation occurred at pH 5 for 1% NaOH and at pH 2 for 2% NaOH, highlighting the importance of both NaOH concentration and pH in protein recovery.

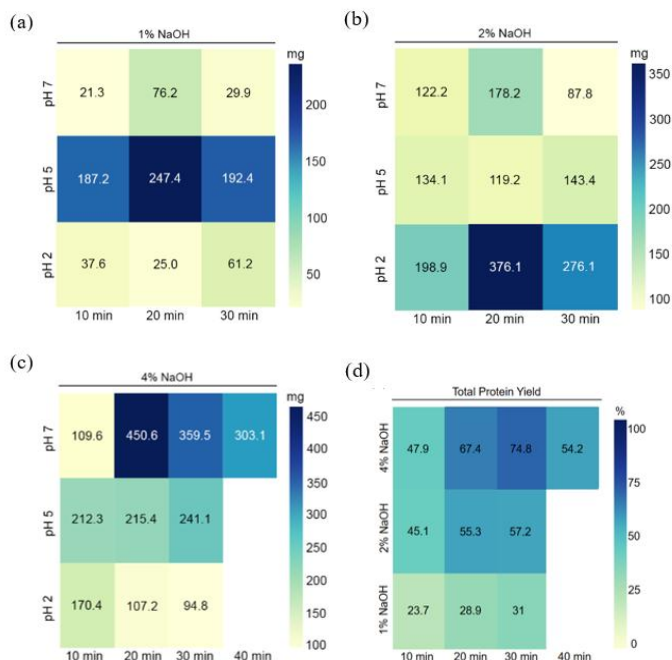

Fig 2. Effect of NaOH concentration, pH, and time on protein yield during alkaline hydrolysis. (a) 1% NaOH; (b) 2% NaOH; (c) 4% NaOH; heat maps show protein yield (mg) at pH 2, 5, and 7 over time (10–40 min); (d) Total protein yield (%) as a function of NaOH concentration (1–4%) and time.

When hydrogen peroxide (H_2O_2) was used in combination with NaOH, protein yield was strongly dependent on peroxide concentration (Figure 3). Hydrolysis with 1 M H_2O_2 produced a low yield of 7.6%, indicating incomplete keratin breakdown. Treatment with 2 M H_2O_2 provided the highest yield of 61.5%, showing optimal solubilization of keratin, whereas 4 M H_2O_2 reduced the yield to 17%. At this higher concentration, the solution became viscous and gel-like, likely due to excessive oxidation and aggregation of protein fragments, which impeded efficient extraction. These results demonstrate that moderate H_2O_2 concentration is crucial for effective protein recovery.

The protein content of untreated feathers and hydrolysates, determined by Kjeldahl analysis, is summarized in Figure 2 (f). Raw chicken feathers contained 10.98% nitrogen, corresponding to 68.6% protein. Hydrolysis with 2 M H_2O_2 produced soluble keratin with 61.8% protein, consistent with the measured yield. The residue from 4 M H_2O_2 treatment contained only 40.83% protein, reflecting incomplete solubilization and aggregation at higher peroxide concentration.

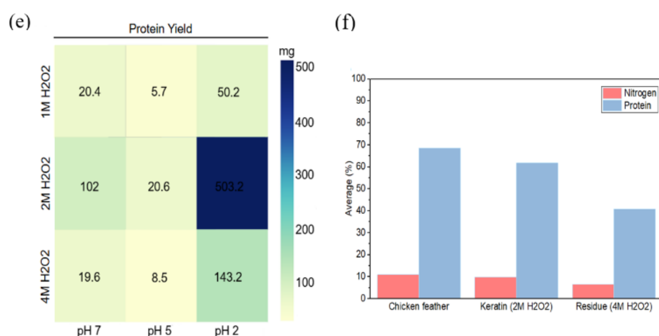

Fig 3. Heat maps showing (e) the effect of H_2O_2 concentration (1–4 M) and pH on protein yield, and (f) protein and nitrogen contents (%) in raw feathers, extracted keratin, and residual material.

Overall, the highest yields of soluble protein hydrolysates were achieved either by hydrolysis with 4% NaOH for 30 minutes or by treatment with 2 M H_2O_2 combined with NaOH. The precipitation behavior under different pH conditions and the Kjeldahl results further support the effectiveness of these hydrolysis methods. These materials should be considered keratin-like protein hydrolysates rather than fully confirmed keratin, pending further molecular characterization.

3.2 Analysis of Molecular Weight Distribution

The molecular weight distribution of the feather-derived protein hydrolysates was analyzed by SDS-PAGE. A 5% stacking gel and a 12% separating gel were used. A protein marker ranging from 14.4 to 116 kDa was run in the first lane. Lane 2 contained the protein sample hydrolyzed with 4% sodium hydroxide, lane 3 contained the sample hydrolyzed with 2 M hydrogen peroxide, and lane 4 contained the insoluble residue obtained after 4 M hydrogen peroxide treatment.

The electropherogram showed prominent bands primarily around 25 kDa in all hydrolyzed samples, along with faint bands near the 14.4 kDa marker. No distinct bands were observed below 14.4 kDa, as the marker used does not allow reliable estimation in that range. The residue from the 4 M H_2O_2 treatment, despite forming a gel-like structure, displayed a banding pattern very similar to the soluble hydrolysates, with the main band still centered around 25 kDa and a faint lower band close to 14.4 kDa. Kjeldahl analysis showed that this residue contained 6.53% nitrogen, corresponding to approximately 40.83% protein content. This confirms that a significant portion of protein remained in the aggregated gel rather than being fully solubilized or degraded into very small peptides.

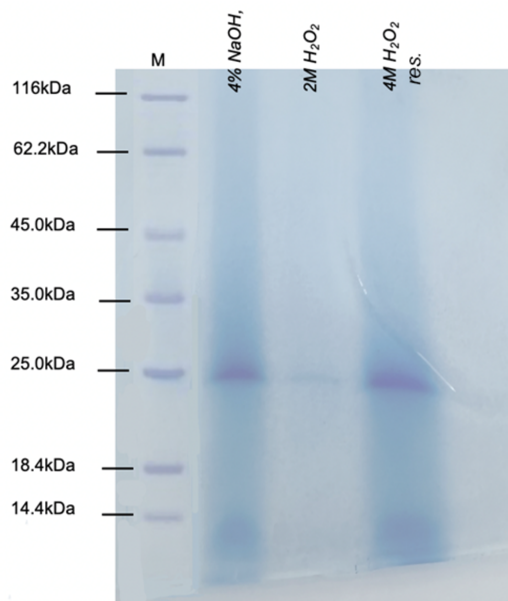

Fig 4. SDS–PAGE analysis of keratin like protein samples extracted under different chemical conditions. M: molecular weight marker; lanes show keratin extracts prepared using 4% NaOH, 2 M H_2O_2, and 4 M H_2O_2.

These results indicate that the extracted proteins are keratin-like proteins derived from chicken feathers. Because the study did not perform amino acid sequencing or comparison with standard purified keratin, the obtained products are best described as feather-derived protein hydrolysates (keratin-like proteins) rather than pure keratin. The similar molecular weight distribution between the soluble hydrolysates and the 4 M H_2O_2 residue suggests that excessive hydrogen peroxide concentration primarily induces protein aggregation and gel formation rather than extensive peptide bond cleavage. The core structural proteins of the feather (likely including α-keratin and possibly minor amounts of other structural proteins) appear to resist complete solubilization under strong oxidative conditions, yet their overall polypeptide size distribution remains comparable to that of the soluble fractions. This observation provides useful insights for the potential valorization of the insoluble residue as a protein-rich biomaterial.

3.3 Morphological Characterization

The microstructure of native and treated chicken feathers was examined using scanning electron microscopy (SEM).

As shown in Figure 4, the untreated feather (a) displayed a well-organized, intact surface with tightly packed cuticle cells along the barbs. After 4% NaOH treatment (b), partial disruption of the cuticle layer was observed, indicating degradation of the keratinous structure. In contrast, the feather treated with 4 M H_2O_2 (c) exhibited extensive surface damage and fragmentation, suggesting stronger oxidative cleavage of disulfide bonds and protein chains. These morphological changes confirm that chemical hydrolysis disrupts the fibrous architecture of feathers, facilitating keratin-like protein extraction.

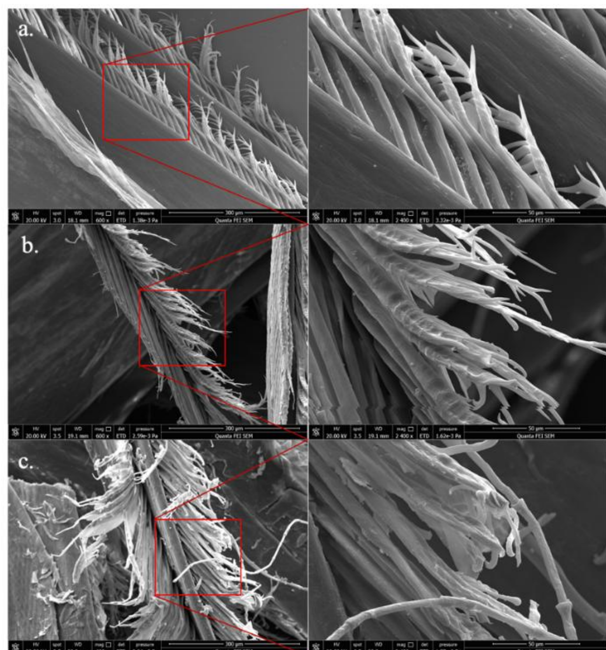

Fig 5. SEM image of (a) untreated feather, (b) after 4% NaOH treatment, (c) the feather treated with 4 M H_2O_2

3.4 Fourier-Transform Infrared (FT-IR) Spectroscopy Results

The chemical bond changes in protein hydrolysate derived from feathers were studied using FT-IR spectroscopy (Figure 5). The results showed several distinct peaks between 500-4000 cm^{-1}, which correspond to the main protein bonds, such as -O-H, COOH, $-CH_2$, -S-S, and -NH. The main protein bands observed were Amide I (1641-1651 cm_{-1}), Amide II (1533-1536 cm^{-1}), Amide III (1222-1252 cm^{-1}), and -CH- (2917-2927 cm^{-1}) and ($-CH_{2-}$) (2915-2940 cm^{-1}, 2840-2870 cm^{-1}), which were characteristic of the keratin molecule. A second peak around 1630 cm^{-1} was observed for both feathers and keratin-like protein, influenced by the carboxyl group carbonyl and amide groups. The -S-S- disulfide bond absorption occurred between 500-650 cm^{-1}, showing a broad peak that narrowed and decreased in absorption. Water-soluble keratin exhibited poor absorption, with narrow and short peaks, indicating its solubility and interaction with other organic compounds.

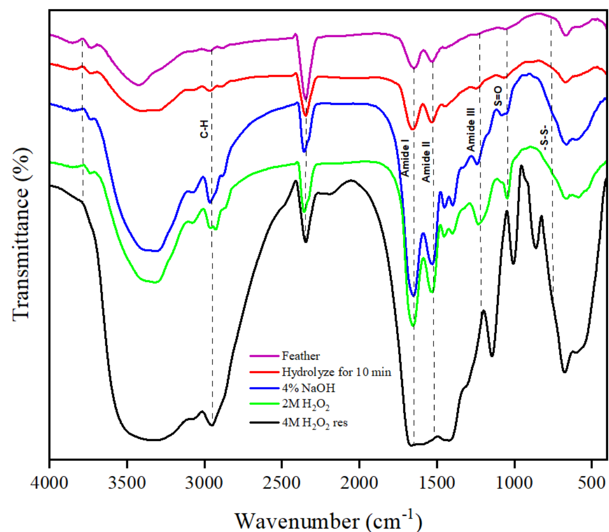

Fig 6. FT-IR spectra of raw feather and keratin-like protein samples obtained under different extraction conditions.

3.5 Thermogravimetric Analysis (TGA) Results

Thermal analysis was performed on four different samples: As shown in Figure 6, feather sample (black line), keratin like protein hydrolyzed with 4% sodium hydroxide (red line), keratin like protein hydrolyzed using a mixture of 2 M hydrogen peroxide and sodium hydroxide (blue line), and the residual keratin gel obtained after hydrolysis with a 4 M hydrogen peroxide and sodium hydroxide mixture (green line). Keratin-like protein degradation occurs in two stages, with decomposition taking place between 61^0C and 267^0C. A significant weight loss was observed between 200^0C and 400^0C, which is associated with the denaturation of the helical structure and the breakdown of peptide chains. Thermal degradation starts at around 61^0C and proceeds more rapidly above 158^0C.

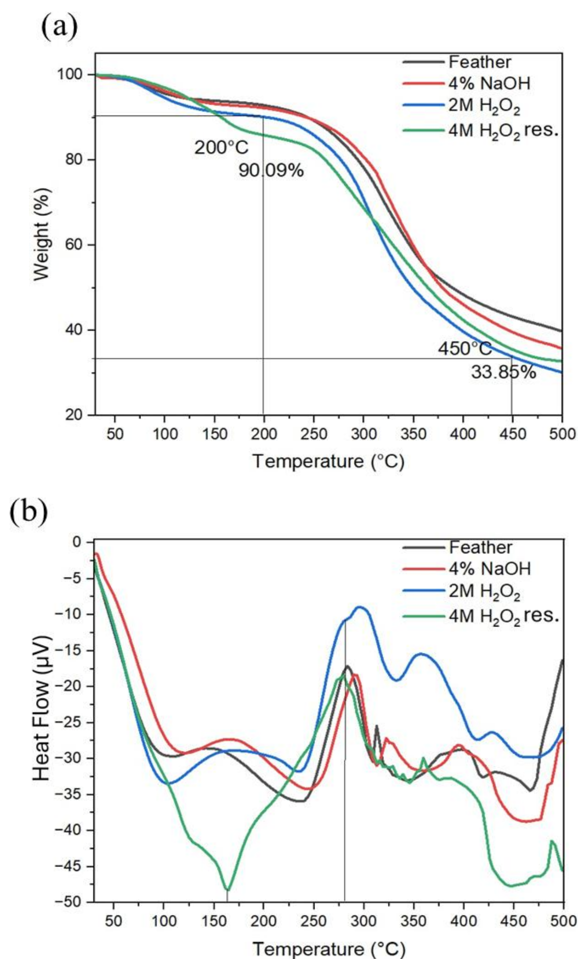

Fig 7. Simultaneous thermogravimetric analysis and differential thermal analysis results for keratin like proteins.

4 Conclusions

Alkaline hydrolysis using sodium hydroxide remains a widely used and cost-effective approach for extracting protein hydrolysates from keratin-rich sources. In our previous study, keratin-like protein was extracted from Mongolian sheep wool using 1% sodium hydroxide solution, yielding 71.3%. In the present study, hydrolysis of chicken feathers with 1% sodium hydroxide did not achieve complete dissolution, whereas using 4% sodium hydroxide allowed full dissolution and a 74.8% yield of protein hydrolysate derived from feathers (keratin-like protein). These results indicate that feather-derived protein hydrolysates may require stronger alkaline conditions than wool-derived protein to achieve effective extraction. Other reported methods, such as sulfide reduction (Na_2S), have achieved up to 65.8% yield from chicken feathers, suggesting that adjusting reagent concentration can influence the extraction efficiency [19].

Additionally, a combination of hydrogen peroxide and sodium hydroxide was tested to obtain soluble protein hydrolysates. Using 2 M hydrogen peroxide, yields from sheep wool and chicken feathers were 68% and 61.5%, respectively. Kjeldahl analysis showed that raw chicken feathers contained 68.6% protein, while the extracted hydrolysates contained 61.5% protein, indicating that the hydrolysates include not only protein but also other organic compounds. The molecular weight distribution observed in SDS-PAGE (with the limitations of the 14.4–116 kDa marker used) shows low-molecular-weight bands consistent with chemical hydrolysis of feather protein, producing peptide fragments typical of β-keratin-like sequences. FTIR analysis confirmed that the extracted protein hydrolysates mainly consist of uniform, low-molecular-weight fragments with preserved amide structures. Thermal analysis demonstrated that the hydrolysates have reasonable heat resistance, whereas residues generated under oxidative conditions underwent rapid thermal degradation.

These findings suggest that chicken feather-derived protein hydrolysates hold potential for applications in heat-resistant and functional biomaterials. It is important to note that the extracts were not compared with standard keratin or fully characterized by amino acid sequencing. Therefore, these hydrolysates are described as protein hydrolysates derived from feathers, keratin-like protein rather than purified keratin. All experimental procedures, including hydrolysis and analytical measurements, were conducted with specified conditions, but no formal economic assessment was performed in this study.

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Author Contributions

T.S., K.F., O.A and N.H. designed the fieldwork and analyses for water chemistry, sequential extraction and XRD. T.S., K.F., O.A., A.A. and D.D. took samples and field measurements. Y.T. performed XAFS analysis. T.S., A.A. and G.B. conducted analyses for water chemistry, sequential extraction, and XRD. T.S. and K.F. wrote the paper.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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