COMMUNICATIONS IN SCIENCE AND TECHNOLOGY
Homepage: cst.kipmi.or.id

The effect of ultrasonication on the quality of keratin extraction based on ionic liquid from duck feather

Senja Salzanabila Putri Perdana[a], Nita Kusumawati[a,*], Maria Monica Sianita Basukiwardojo[a], Pirim Setiarso[a], Gina Noor Djalilah[b], Khofifatul Rahmawati[a]

[a]*Department of Chemistry, Universitas Negeri Surabaya, Surabaya, 60231, Indonesia*
[b]*Department of Medical Education, Universitas Muhammadiyah Surabaya, Surabaya, 60113, Indonesia*

Article history:
Received: 17 September 2025 / Received in revised form: 11 November 2025 / Accepted: 17 November 2025

Abstract

This study investigates the effect of ultrasonic-assisted extraction (UAE) and solvent extraction (SE) on keratin recovery from duck feathers using sodium sulfide-based ionic liquids under different pH conditions. The results showed that SE at acidic pH (pH=3) achieved the highest yield (92%), whereas UAE showed lower recovery (28%) under mildly acidic conditions (pH=5). Spectroscopic and electrophoretic analyses using FTIR confirmed the β-sheet structure with characteristic peaks at 3400 cm^{-1} (O–H and N–H stretching) and 1660 cm^{-1} (C=O stretching). Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis analysis (SDS-PAGE) revealed protein bands in the 15–25 kDa range, typical of β-keratin, with higher intensity in SE. Morphological analysis using SEM revealed finer and more homogeneous particles for UAE, while SE produced denser aggregates. Thermal analysis revealed two main degradation stages, occurring at 0–100 °C and 250–500 °C, with UAE samples exhibiting lower residual mass (5.46%) than SE (8.65%). Particle size analysis showed UAE samples had larger but more uniformly distributed particles. XRD results confirmed semi-crystalline structures, with UAE increasing amorphous content and SE maintaining crystallinity. These findings highlight the complementary advantages for tailoring keratin properties toward diverse applications.

Keywords: Keratin extraction; duck feather; solvent extraction; ultrasonic-assisted extraction; pH variation

1. Introduction

Global poultry production has increased steadily, with poultry meat consumption including major poultry species such as ducks, geese, and chickens projected to reach 154 million tons by 2031 [1]. The European Union, as one of the largest producers and exporters, reported an annual production of 13.4 million tons [2]. This growth generates large amounts of by-products such as feathers, bones, skin, and fat, most of which remain underutilized. In the United States, for instance, untreated poultry feather waste is estimated at approximately 4 billion pounds annually [3].

Indonesia, as a leading duck producer in Southeast Asia, maintains a population of approximately 47.8 million ducks (285,000 tons meat and generating 95,000–110,000 tons of feather waste annually) in 2023, mainly in Central Java, East Java, West Java, and South Sulawesi [4,5,6]. Improper disposal of waste can cause groundwater contamination, soil pollution, and odor problems that impact local communities [7]. Given the high keratin content of duck feathers, effective waste management and environmentally friendly technologies are required to convert this biomass into valuable products.

Duck feathers represent a promising renewable biomass resource as they contain approximately 91% keratin, 8% water, and 1% lipids [1,5]. Keratin is a fibrous protein rich in cysteine residues and stabilized by covalent disulfide bonds; this contributes mechanical strength and resistance to degradation [8]. It occurs mainly as α-keratin in mammals and β-keratin in poultry feathers, which form a dense sheet structure (3–4 nm, 10–22 kDa), conferring rigidity and resistance [1,9].

Various keratin extraction techniques exist, including, ionic liquid (IL) [10,11], deep eutectic solvents [12,13], enzymatic methods [14,15], and ultrasound-assisted extraction (UAE) [16,17]. Ionic liquids are promising green technologies, as they selectively dissolve keratin while maintaining protein integrity. They are also non-volatile, thermally stable, and recyclable [11]. Ionic liquids are organic salts composed entirely of ions and can disrupt hydrogen bonds and disulfide cross-links [18], while DES exhibit comparable physicochemical properties and are considered greener alternatives [19]. Enzymatic methods operate under mild conditions and produce high-quality peptides and amino acids, but the cost and industrial scalability remain challenges[15].

* Corresponding author.
Email: nitakusumawati@unesa.ac.id
https://doi.org/10.21924/cst.10.2.2025.1785

UAE improves extraction efficiency through acoustic cavitation, which enhances mass transfer, reduces time, and increases solvent penetration into the keratin matrix [20,21]. Previous studies have shown that ultrasonic treatment accelerates keratin dissolution in ionic liquids through cavitation-induced high-temperature and high-pressure microzones, thereby improving extraction efficiency under milder conditions [22,23].

Keratin extraction from chicken feathers has been studied extensively, whereas studies on duck feathers using IL systems remain limited, particularly with respect to keratin yield and quality. Duck feathers differ from chicken feathers in composition, fiber diameter, and protein structure, which influence their interactions with IL and response to ultrasonic treatments [24,25]. The high β-sheet content and amino acid composition of duck feathers require the selection of specific ionic liquids to achieve efficient and optimal extraction.

Sodium sulfide-based ionic liquids (Na_2S) systems are commonly applied in protein extraction due to their strong solubility and selectivity toward keratin [17]. The choice of ionic liquid strongly influences extraction yield, molecular weight distribution, secondary structure, and functional properties of keratin. Similarly, Cordova et al., (2025) reported that UAE significantly enhanced bioactive recovery, yielding the highest anthocyanin content within 5 minutes from winemaking by-products [21]. Basukiwardojo et al., (2025) demonstrated that IL-UAE under optimal conditions achieved an 82% keratin yield [17]. Furthermore, UAE represents a promising and sustainable approach for keratin utilization.

Ultrasonication parameters critically influence keratin extraction by improving mass transfer through cavitation, whereas excessive intensity may induce protein degradation [16,26]. This study investigates the effects of UAE on keratin extraction from duck feathers within an IL system, focusing on extraction yield, molecular weight distribution, structural integrity, and functional properties. Comprehensive physicochemical characterization was performed using FTIR, SDS-PAGE, SEM, XRD, DTA/TGA, and PSA. The findings provide insight into how UAE parameters influence keratin quality and provide a foundation for efficient, sustainable technologies to valorize duck feather waste into high-value industrial products.

2. Materials and Methods

2.1. Materials

Duck feathers were obtained from a local poultry farm in Bangkalan, Indonesia. The chemical used for feather preparation included anhydrous Sodium Sulfide (Na_2S) (≥99% purity; Merck, Singapore), Ethanol (≥96% purity; Merck, Singapore), Sodium Hydroxide (≥96% purity; Merck, Singapore), Hydrochloric Acid (HCl) (≥37% purity; Fluka, Austria), and distilled water.

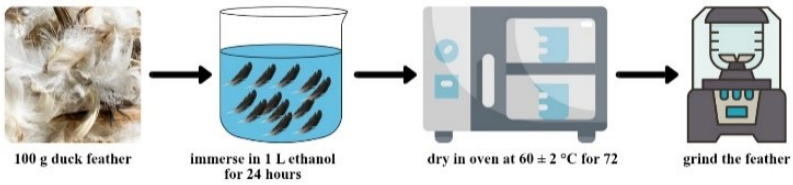

Fig. 1. Pre-treatment process to remove contaminants

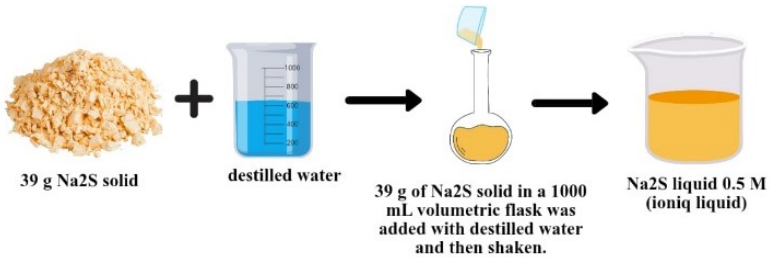

Fig. 2. Preparation of ionic liquid solvent for extraction

2.2. Method

The keratin extraction procedure followed the methods reported by Basukiwardojo et al. and Pourjavaheri et al. [17,27], using sodium sulfide as the solvent. Keratin extraction using reducing agents, such as sodium sulfide, is one of the most widely applied approaches [10] and generally involves four main steps: pre-treatment, extraction, precipitation, and purification.

2.2.1. Pre-treatment of duck feathers

Contaminants such as dirt, blood, oils, and pathogens were removed from the duck feathers following the ethanol extract purification method by Meko et al. [28]. A total of 100 g of duck feathers were immersed in 1000 mL of ethanol for 24 h. The samples were subsequently dried at 60 ± 2 °C for 72 h. The dried duck feathers were ground into fine particles using a grinder. Fig. 1. shows the pre-treatment process.

2.2.2. Ionic liquid (IL) solvent preparation

Chemical compounds composed exclusively of cations and anions may exist in the liquid phase at room temperature or temperatures below 100 °C. Accordingly, sodium sulfide (Na_2S), as an ionic compound, dissolves in water to form an ionic medium that functions as an IL-based solvent (Fig. 2.).

2.2.3. Extraction of keratin

Keratin was extracted from duck feathers (10 g) using two methods: ultrasonic-assisted extraction (UAE) and Solvent Extraction (SE) (Fig. 3.). In both methods, duck feathers were immersed in 200 mL of 0.5 M sodium sulfide (Na_2S) solution at a material-to-solvent ratio of 1:20 (w/v), and the pH was adjusted to 10.5 using 0.05 M sodium hydroxide (NaOH). In the SE method, the mixture was heated to 40 °C and stirred at 100 rpm using a magnetic stirrer for 6 hours. In the UAE method, the pH adjustment followed the procedure described by Basukiwardojo et al. [17], and extraction was conducted at 40 °C using an Elmasonic S 80 H ultrasonic bath for an hour. [29].

2.2.4. Precipitation

The solution was centrifuged at 10,000 rpm for 20 min at 10 °C. The supernatant was collected, and the pH was adjusted to the desired value (1, 3, or 5) using 5% HCl solution. The mixture was left for 2 h to allow precipitation.

2.2.5. Purification

The precipitate was separated by filtration, washed three to four times with distilled water, and dried at 60 °C for 48 hours in a Memmert UN55 oven.

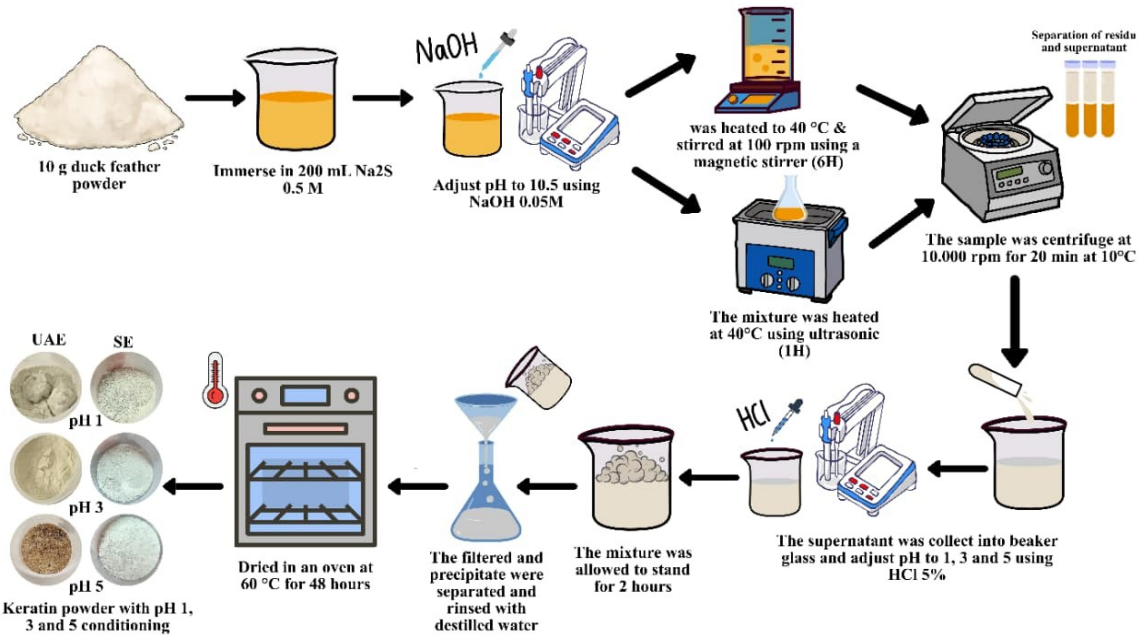

Fig. 3. Keratin extraction process step using IL-UAE and Solvent Extraction method

2.3. Yield

Yield and weight loss were determined according to Eq. (1-3).

$$\%yield = \frac{W_{fk}}{W_{ik}} \times 100 \quad (1)$$

Furthermore, weight loss is calculated using formula (2-3) as follows:

$$\%loss = \frac{W_{ik}-W_{fk}}{W_{ik}} \times 100 \quad (2)$$

$$\%total\ weight\ loss = \frac{W_{i}-W_{fk}}{W_{i}} \times 100 \quad (3)$$

where W_{ik} and W_{fk} refer to the initial (wet) and final (dry) keratin weights, respectively, while W_i represents the initial weight of duck feathers.

2.4. Fourier transform infrared (FTIR) analysis

Keratin extracted from duck feathers was analyzed using FTIR spectroscopy to identify functional groups within its molecular structure [5]. The detected peaks were compared with characteristic keratin peaks reported by Basukiwardojo et al. and Alvarez et al. [5,17] . The analysis was performed using a Perkin Elmer Spectrum Two FTIR spectrometer, with spectra recorded over the range of 4000–500 cm^{-1} at a resolution of 4 cm^{-1}.

2.5. Sodium dodecyl sulfate Polyacrylamide gel electrophoresis (SDS-page) analysis

SDS-PAGE was employed to separate proteins based on their molecular weight. The method applies an electric current to move proteins through a polyacrylamide gel matrix, which acts as a molecular sieve. Resolving gel (15% acrylamide/bis-acrylamide) and stacking gel (6% acrylamide/bis-acrylamide) were prepared. A 20 µL sample was mixed with 20 µL loading buffer containing Tris-HCl, SDS, glycerol, bromophenol blue, and dithiothreitol (DTT), then heated at 95 °C for 3–5 min. Subsequently, 10 µL of the sample and 8 µL of protein marker (a mixture of 12 purified proteins, 4.6–300 kDa) were loaded into the gel wells. Electrophoresis was performed at 150 V for 90 min using Tris–glycine–SDS running buffer. The gel was rinsed with distilled water, stained with Coomassie blue, destained with acetic acid, and dried [30].

2.6. Scanning electron microscopy (SEM)

SEM was used to examine the morphology and microstructure of keratin powder, including surface features, dimensions, particle distribution, and uniformity of the keratin powder. Analysis was conducted using a JSM-6510 SEM at magnifications of 2000x, 10,000x, and 20,000x. Prior to imaging, samples were sputter-coated with gold.

2.7. Differential thermal-thermogravimetric analysis (DT/TGA)

Thermal degradation of duck feather keratin was evaluated using a Perkin Elmer TGA Pyris 1 Thermogravimetric Analyzer (Melbourne, VIC, Australia). Samples were heated from 30 °C to 800 °C at a heating rate of 20 K/min under a nitrogen atmosphere to minimize thermal lag effects [31].

2.8. Particle size analysis (PSA)

PSA was performed to evaluate the particle size distribution and homogeneity of keratin. A sample concentration of 1 mg/mL was analyzed without further dilution using a Zeta Nano ZS (Malvern Instruments) at 25 °C. The results were expressed as the mean ± standard deviation [31].

2.9. X-ray diffraction (XRD) analysis

The crystal structure and particle characteristics of duck feather keratin were determined using XRD analysis. Following the method described by Duman and Kucuk [28], the samples were analyzed under Cu Kα radiation (λ = 0.154 nm) at 35 kV and 40 mA, within a scanning range of 10–50° 2θ and at a scanning rate of 5°/min. The crystallinity index (CI) of keratin was calculated using the Segal method, as expressed in Eq. (4) [32]:

$$C.I. = \frac{(I_9 - I_{14})}{I_9\ x\ 100} \times 100 \qquad (4)$$

where I_9 dan I_{14} represent the maximum crystalline diffraction intensities at 2θ = 9° dan 2θ = 14°. A higher CI value indicates a greater degree of crystallinity.

3. Results and Discussion

3.1. Yield

The highest yield was achieved with SE at pH 3 (92%), whereas the lowest was recorded for UAE at pH 5 (28%). The quantitative results (Table 1 and Fig. 4.) revealed significant differences in yield, demonstrating that extraction efficiency is governed primarily by the synergistic interaction between pH and extraction method.

Moderately acidic conditions (around pH 3) enhanced the protonation of acidic residues (Glu, Asp, terminal –COOH), thereby reducing electrostatic repulsion and facilitating coacervation and keratin precipitation. These results are consistent with pH-driven extraction optimization reported by Basukiwardojo et al. [17]. On the other hand, deprotonation of the carboxylate group increases electrostatic repulsion at pH values above 3 and reduces the effectiveness of coacervate production [26]. Another study emphasized the dual role of ultrasound in enhancing solubilization while influencing recovery efficiency [16].

Although UAE produced lower keratin yields compared with SE, it demonstrated superior preservation of molecular integrity. This was evidenced by the presence of sharp amide I and II peaks in the FTIR spectra, dominant SDS–PAGE bands at 40–60 kDa without excessive fragmentation, and more intact fibrillar morphology as observed by SEM. The application of UAE provides a sustainable pathway for processing duck feather waste from the poultry industry into high-value materials that align with circular bioeconomy principles.

Table 1. Comparison of the results of duck feather keratin extraction yield

pH	UAE	UAE (Mean ± SD)	SE	SE (Mean ± SD)
1	33		62	
	35	35.33 ± 2.52	60	61.67 ± 1.53
	38		63	
3	60		92	
	62	60.67 ± 1.15	90	90.33 ± 1.53
	60		89	
5	28		68	
	30	29.00 ± 1.00	67	66.67 ± 1.53
	29		65	

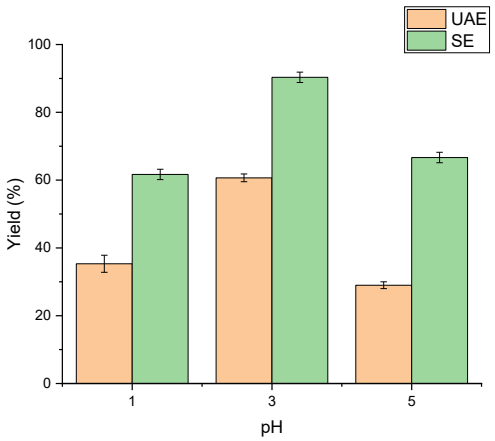

Fig. 4. Reproducibility test

3.2. Fourier transform infra-red (FT-IR) analysis

Fourier Transform Infrared (FTIR) spectroscopy was employed to characterize the functional groups of keratins extracted from duck feathers by ultrasonic-assisted extraction (UAE) and solvent extraction. Spectral measurements recorded in the wavenumber range of 4000–500 cm^{-1}. Fig. 5. shows characteristic absorption bands associated with the secondary structural components of keratin.

A broad absorption band centered at approximately 3280 cm^{-1} was attributed to O–H and N–H stretching vibrations, whereas the signal at 2964 cm^{-1} corresponded to C–H

stretching. The amide I band detected at 1630 cm^{-1} indicates C=O stretching vibrations correlated with α-helix and β-sheet conformations. Similarly, the amide II band (1530 cm^{-1}) was attributed to N–H bending coupled with C–N stretching, and the amide III band (1236 cm^{-1}) confirmed characteristic vibrational modes of the protein backbone. Additional absorption at 760 cm^{-1} corresponded to out-of-plane N–H bending, while the peak observed at 480 cm^{-1} was associated with disulfide (S–S) bond vibrations.

The close similarity of spectral profiles obtained from IL-UAE and SE derived keratin suggests that sodium sulfide-mediated reductive cleavage efficiently disrupts disulfide cross-links while preserving the integrity of the peptide backbone [33]. These observations confirm that the functional groups and secondary structure of keratin remain largely intact following both extraction strategies. Preservation of these structural motifs is particularly critical for downstream applications, as the stability of α-helix and β-sheet domains directly influences the mechanical performance of keratin, thermal resistance, and interaction with other biomolecules [34].

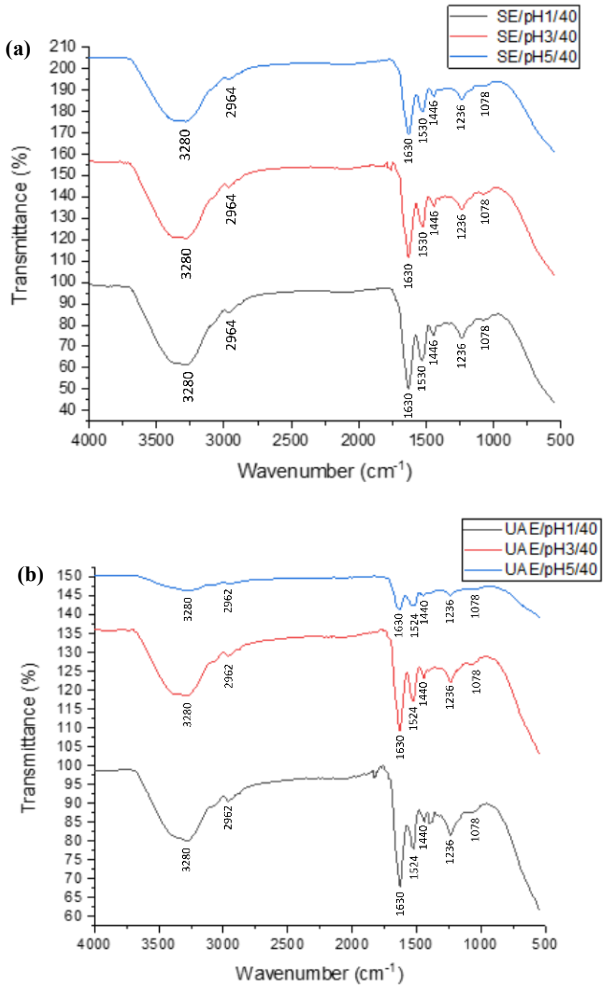

Fig. 5. Comparison of FTIR Analysis of keratin samples (a) UAE method (b) SE method

This observation is consistent with previous reports that also demonstrated structural preservation of keratin under sodium sulfide-based extraction [5,17,27]. The persistence of stable amide bands reinforces the structural robustness of keratin under diverse extraction conditions, highlighting its potential for advanced applications, including biomedical scaffolds, controlled-release matrices, composite films, and sustainable biopolymers, while simultaneously providing a value-added strategy to mitigate duck feather waste and support circular bioeconomy practices.

3.3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-Page) analysis.

The molecular weight distribution of soluble proteins in keratin was determined through SDS-PAGE gel electrophoresis analysis (Fig. 6.). Samples U3 (UAE/pH3/40) and S3 (SE/pH3/40) displayed highly intense and dark bands in the molecular weight range of 15–25 kDa, which corresponds to the typical molecular weight range of β-keratin in poultry feathers [1,5]. The band intensity in SE/pH3/40 was noticeably higher than in UAE/pH3/40, indicating that the SE method extracted a greater amount of protein under optimal pH conditions. These results further confirm the role of pH in enhancing extraction efficiency, as pH 3 promotes the formation of more stable protein coacervates, allowing keratin to be more easily separated from the feather matrix [26]. No additional bands outside this range were observed, suggesting that the extracted protein was relatively pure. This finding is consistent with the reports by Lawrence et al. and Basukiwardojo et al. [17,24] which demonstrate that Na_2S as a reducing agent can yield high-purity keratin without degrading its primary structure.

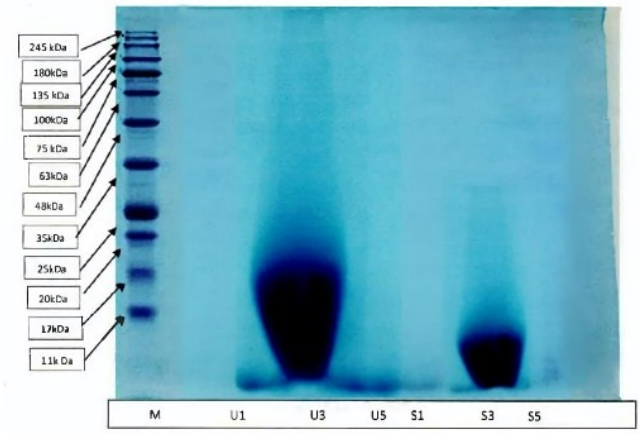

Fig. 6. Results of SDS-Page analysis of keratin from both methods (UAE and SE) and pH variations (1, 2, 3)

3.4. Scanning electron microscopy (SEM)

SEM micrographs of keratin extracted from duck feathers using the UAE and SE methods at different pH values (Figure 3) show different morphological characteristics (at magnification of 1000x and 20,000x).

At pH 1 (Fig. 7(a) and 7(b)), keratin exhibited dense and heterogeneous particles as a result of incomplete dissolution, whereas at pH 5 (Fig. 7(e) and 7(f)), larger and denser aggregates were observed due to reduced electrostatic repulsion. At pH 3 (Fig. 7(c) and 7(d)), spherical particles were formed and further assembled into a porous network, indicating partial unfolding of keratin chains that promoted self-assembly

into nanostructured aggregates, consistent with the observations of Chen et al. reported that intermediate pH conditions facilitate the formation of nanostructured keratin assemblies [6]. Compared with SE, the UAE method produced finer and more homogeneous particles, which can be attributed to ultrasonic cavitation that disrupts agglomeration and enhances dispersion. This morphological transition is also in agreement with Sun et al. who demonstrated that under mildly alkaline conditions keratin tends to precipitate as dense aggregates with rougher surfaces, driven by stronger inter-chain hydrogen bonding and reduced solubility [26]. This observation is consistent with reported literature [1]; pH has a significant effect on keratin morphology. The rough and microporous surface topology contributes to enhanced interfacial bonding characteristics when keratin is used as a biocomposite reinforcement agent, corroborating the structure-function paradigm as established in recent studies on keratin-based composite systems [34].

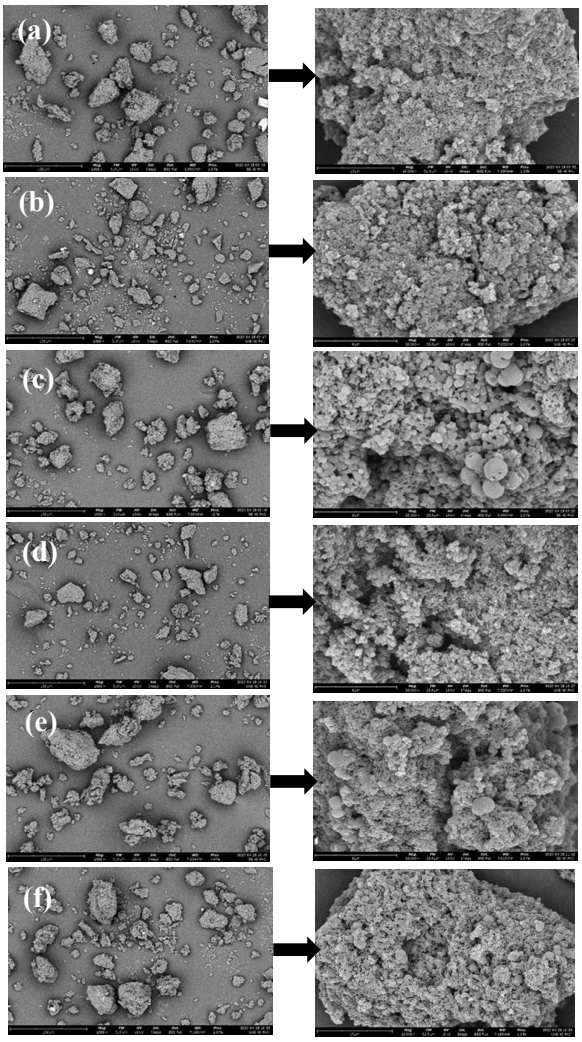

Fig. 7. Analisis SEM keratin dari bulu bebek, (a) SE/40/pH1, (b) UAE/40/pH1, (c) SE/40/pH3, (d) UAE/40/pH3, (e) SE/40/pH5, (f) UAE/40/pH5

3.5. Differential thermal-thermogravimetric analysis (DT/TGA)

The thermal analysis of keratin extracted at pH 3 using UAE and SE (Fig. 8) revealed two distinct thermal degradation stages. The initial weight loss below 100 °C was attributed to moisture evaporation. At this stage, SE derived keratin exhibited a higher mass loss, indicating a larger fraction of loosely bound water. This behavior is associated with the more porous and hygroscopic protein matrix produced by SE, which retains more surface water. In contrast, the combined mechanical and thermal effects of ultrasonic cavitation during UAE increase solubilization and result in a more homogeneous keratin structure with lower porosity, thereby reducing the amount of loosely bound water remaining in the sample [5].

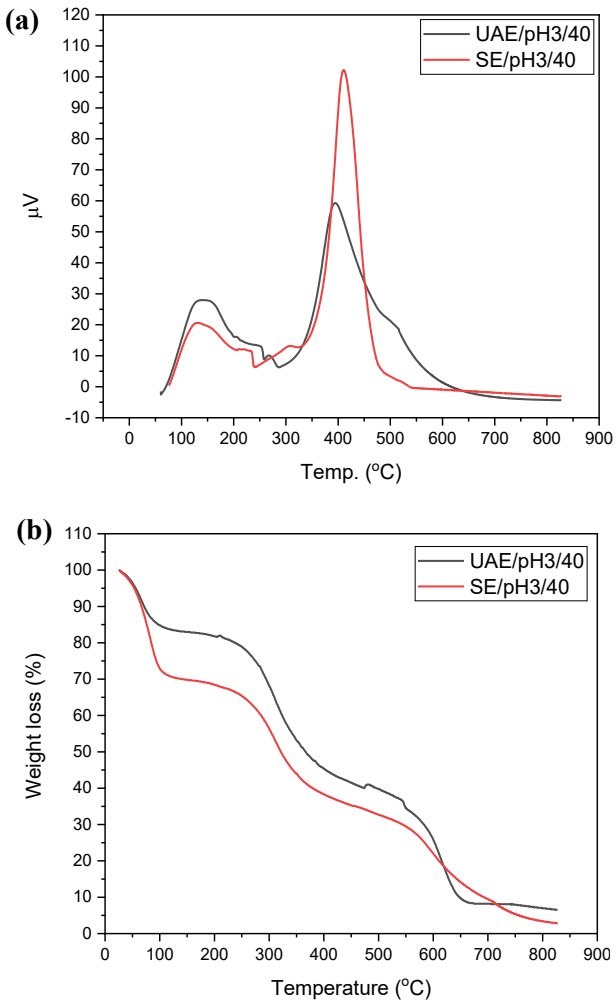

Fig. 8. DTA (a) and TGA (b) graphs of extract keratin from duck feather

The main degradation stage, occurring between 250 and 500 °C, corresponded to the cleavage of peptide bonds, disulfide bridges, and secondary structural elements, consistent with the typical thermal degradation pathway of keratin. Keratin obtained via SE displayed a sharper DTG peak with higher intensity (100 µV) and higher residual mass (8.65%), indicating localized thermal degradation associated with stronger cross-linking. In contrast, UAE derived keratin exhibited a broader peak with lower intensity (60 µV) with lower residual mass (5.46%), indicating slower decomposition kinetics and improved thermal dispersion.

In the temperature range of 600 and 700 °C, a distinct difference between the two extraction methods was observed. UAE derived residues demonstrated a steeper mass decline,

indicating that the remaining components were more reactive and more easily decomposed, while SE samples underwent gradual decomposition. The absence of distinct endothermic or exothermic peaks in DT/TGA at this region suggests that the process involves progressive oxidation or secondary pyrolysis with small enthalpy changes, where heat absorption and release occur simultaneously and effectively compensate for each other.

These results are consistent with the finding [16] as ultrasound-assisted extraction modifies protein aggregation and produces keratin with higher content of volatile components and altered thermal stability. Furthermore, the thermal resistance of keratin is largely governed by the density of disulfide cross-links and interchain hydrogen bonding, both of which may be partially disrupted under UAE conditions [36], thereby accounting for the reduced residual mass and broader decomposition profile.

3.6. Particle size analysis (PSA)

Particle size distribution analysis (Fig. 9) revealed that keratin obtained by UAE exhibited a slightly larger average size compared to SE, while exhibiting a lower polydispersity index (PI < 0.3), indicating a more homogeneous particle size distribution. Such a narrower size distribution is critical as it directly correlates with the stability and consistency of keratin's functional performance.

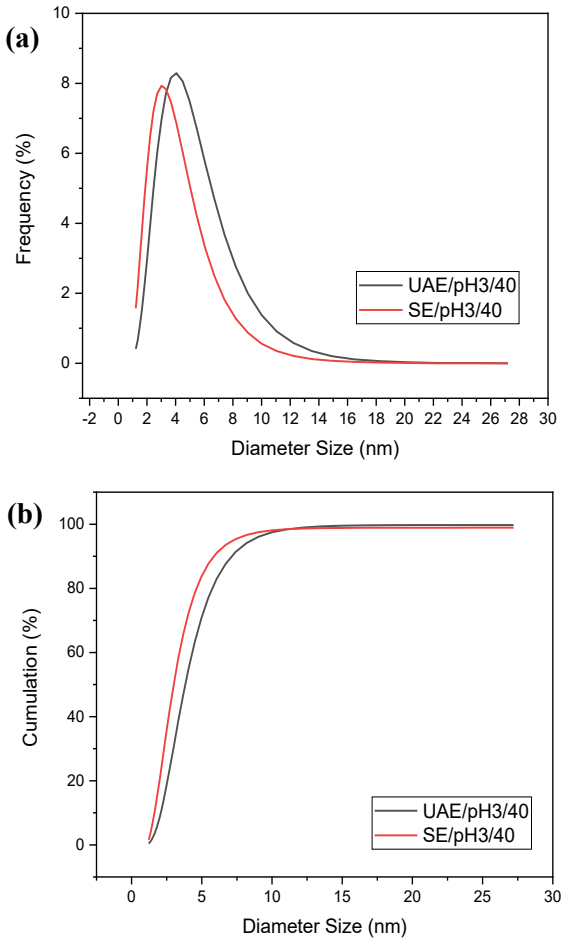

Fig. 9. Results analysis (a) particle size distribution (b) Cumulative particle size distribution of UAE/pH3/40 and SE/pH3/40 keratin

Keratin extracted via the SE method exhibited an average diameter (Dav) of 4.56 nm (D10 = 1.58 nm, D50 = 2.96 nm, D90 = 5.69 nm) with a PI of 0.3005, as the solvent-based system relies primarily on diffusion and slow chemical reactions, rendering the particles more prone to aggregation and yielding a broader size distribution. In contrast, UAE-derived keratin had a larger average diameter of 5.68 nm (D10 = 2.06 nm, D50 = 3.82 nm, D90 = 7.1 nm) with a lower PI of 0.2627; this behavior is attributed to ultrasonic cavitation, which generates shear forces and microjets capable of disrupting particle agglomerates and increasing surface area, thereby producing more uniform and well-dispersed particles [37]. These findings are consistent with the results reported [38], demonstrating that high-energy physical methods such as ultrasonication are more effective in achieving narrower particle size distributions than conventional chemical techniques. The tendency toward a more homogeneous particle size distribution in UAE was further supported by SEM observations, which revealed smoother morphology and evenly dispersed particles. Such controlled size distribution and uniform morphology are of paramount importance for enhancing stability, biocompatibility, and the predictable behavior of keratin in biomaterial and pharmaceutical applications [38].

3.7. X-ray diffraction (XRD) analysis

XRD analysis (Fig. 10) confirms the semi-crystalline nature of keratin extracted from duck feather, characterized by two dominant diffraction peaks at 2θ = 9° and 2θ = 22–24°. The first peak corresponds to the α-helix structure, while the second peak reflects overlapping contributions from random coil and β-sheet domains. The SE/pH3/40 sample displayed slightly higher peak intensities at both positions compared with UAE/pH3/40, indicating a higher degree of crystalline ordering. In contrast, the diffraction pattern of UAE/pH3/40 showed broader diffraction peaks, indicative of reduced crystallite size and increased amorphous content. This structural alteration can be attributed to ultrasonic cavitation, whereby the collapse of microbubbles generates shear forces and localized high pressures that disrupt hydrogen bonding among polypeptide chains, thereby reducing structural order and increasing the amorphous fraction [16,40].

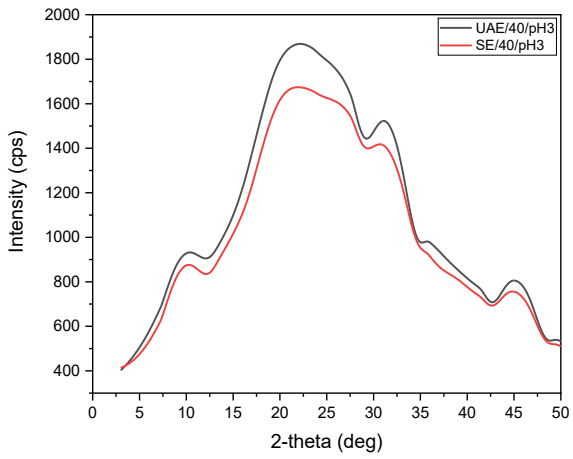

Fig. 10. XRD analysis of duck feather keratin UAE/pH3/40 dan SE/pH3/40

These findings are consistent with recent studies reporting that ultrasonic treatment reduces keratin crystallinity, as evidenced by peak broadening in XRD patterns and decreased crystallinity percentages, highlighting the disruptive effect of high-energy sonication on ordered keratin domains [16]. Similarly, Basukiwardojo et al. [17] demonstrated that combining ionic liquids with ultrasonication yielded keratin with more dispersed morphology and altered secondary structures compared to conventional solvent-based extraction. These results support the interpretation that UAE tends to enhance amorphous characteristics, whereas SE preserves a greater crystalline fraction.

Recent reports further emphasize that keratin obtained via ultrasonic or ionic liquid-assisted extraction generally exhibits higher amorphous content and smaller crystallite sizes than keratin from conventional methods. Such structural features enhance surface area and biocompatibility, making the material more suitable for applications requiring solubility and biological interactions. Conversely, keratin with higher crystallinity, typically obtained through conventional extraction, provides superior mechanical stability, rendering it advantageous for scaffolds or composite materials [17,18].

4. Conclusion

This study demonstrated the significant influence of Ultrasonic-Assisted Extraction (UAE) on the quality of keratin extracted from duck feathers using an ionic liquid system. Although UAE at pH 3 resulted in a lower yield (60%) compared to Solvent Extraction (SE) at the same pH (92%), the structural analyses revealed distinct advantages. FTIR and SDS-PAGE confirmed the preservation of functional groups and β-keratin bands, SEM and PSA showed finer morphology with more homogeneous particle size distribution, and TGA/DTA combined with XRD indicated broader degradation profiles and increased amorphous content. These results suggest that ultrasonic cavitation plays a key role in enhancing surface area, solubility, and biocompatibility, making UAE-derived keratin more suitable for biomedical and functional applications, whereas SE remains favorable for producing higher yield and crystalline stability. Thus, both methods exhibit complementary strengths, underscoring the importance of tailoring extraction strategies to specific application requirements while promoting the sustainable valorization of duck feather waste.

Acknowledgments

The authors gratefully acknowledge the financial support from the Indonesian Ministry of Education, Culture, Research, and Technology through the National Competitive Research Grant Scheme, which enabled the successful completion of this research.

References

1. N. Raydan, *Development of new adhesives based on keratin extracted from duck feathers for the production of composite materials*, Chemical Science. Université de Pau et des Pays de l'Adour, 2024
2. E. Commission, D.-G. for Agriculture, and R. *Development, EU agricultural outlook for markets, income and environment 2022-2032*. Publications Office of the European Union, 2022.
3. Park, G., Lee, K., Lee, Y., Kim, Y., Jeon C., Lee, O., Kim, Y., Son, H. *Biodegradation and valorization of feather waste using the keratinase-producing bacteria and their application in environmentally hazardous industrial processes*, J. Environ. Manage. 346 (2023) 118986.
4. Ismoyowati., Suswoyo, I., Rosidi., Mugiyono, S., and Hidayat, N., *The Effect of Environmental Factor, Population and Age of Duck on Egg Production*, Anim. Prod., vol. 22 (2020) 118–125.
5. Alvarez, S., Raydan, N., Svahn, I., Gontier, E., Rischka, K., Charrier, B., Robles, E., *Assessment and Characterization of Duck Feathers as Potential Source of Biopolymers from an Upcycling Perspective*, Sustain., 15 (2023).
6. Chen, H., Gao, S., Li, Y., Xu, Hui-Juan., Li, W., Wang, J., and Zhang, Y., *Valorization of Livestock Keratin Waste: Application in Agricultural Fields*, Int. J. Environ. Res. Public Health, 19 (2022).
7. Dubey, D. K., Agarwal, S., Yadav, M.P., Goswami, A., Ravat, A., *THE STUDY OF THE EFFECTS OF IMPROPER HAZARDOUS WASTE DISPOSAL ON ECOSYSTEMS*, Indian J. Sci. Res., 4 (2024) 130–136.
8. Ouakarrouch, M., El Azhary K., Laaroussi, N., Garoum, M., and Kifani-Sahban, F., *Thermal performances and environmental analysis of a new composite building material based on gypsum plaster and chicken feathers waste*, Therm. Sci. Eng. Prog., 19 (2020) 100642.
9. Chilakamarry, C. R., Mahmood, S., Saffe, S. N. B. M., Arifin, M. A. B., Gupta, A., Sikkandar, M. Y., Begum, S., Narasaiah, B., *Extraction and application of keratin from natural resources: a review*, 3 Biotech, 11 (2021) 1–12.
10. Tran, C. D., and Mututuvari, T. M., *Cellulose, Chitosan and Keratin Composite Materials: Facile and Recyclable Synthesis, Conformation and Properties*, ACS Sustain. Chem. Eng., 4 (2016) 1850–1861.
11. Wu, Y., Gao, H., Liu, X., Qin, C., Wang, B., Xu, J., Zhai, C., & Nie, Y., *Efficient and regulable extraction of keratin from yak hair using imidazolium-based ionic liquids*, J. Mol. Liq., 388 (2023) 122725.
12. Wang, J., Zhao, Z., Yuan, H., Gao, H., and Nie, Y., *Effective Extraction of Keratin from Human Hair under Mild Conditions Using DES Based on Choline Chloride and Ethanolamine*, ACS Sustain. Chem. Eng., 12 (2024) 534–542.
13. Nuutinen, E. M., Virtanen, T., Lantto, R., Vähä-Nissi, M., and Jääskeläinen, A. S., *Ductile keratin films from deep eutectic solvent-fractionated feathers*, RSC Adv., 11 (2021) 27512–27522.
14. Eslahi, N., Dadashian, F., and Nejad, N. H., *An investigation on keratin extraction from wool and feather waste by enzymatic hydrolysis*, Prep. Biochem. Biotechnol., 43 (2013) 624–648.
15. Moktip, T., Salaipeth, L., Cope, A. E., Taherzadeh, M. J., Watanabe, T., and Phitsuwan, P., *Current Understanding of Feather Keratin and Keratinase and Their Applications in Biotechnology*, Biochem. Res. Int., 1 (2025).
16. Qin, X., Yang, C., Guo, Y., Liu, J., Bitter, J. H., Scott, E. L., and Zhang, C., *Effect of ultrasound on keratin valorization from chicken feather waste: Process optimization and keratin characterization*, Ultrason. Sonochem., 93 (2023) 106297.
17. Basukiwardojo, M. M. S., Kusumawati, N., Asri, M. T., Dzulkarnain, S. A., Al hafidl, A. N., Kahfi, A., Nabila, M. A., Setiawan, F., Isyrak, L., and Rahmawati, K., *Ionic liquid ultrasound-assisted extraction (IL-UAE) for duck feather keratin and in silico evaluation as a potential procollagen n-endopeptidase inhibitor*, Commun. Sci. Technol., 10 (2025) 75–86.
18. Feroz, S., Muhammad, N., Dias, G., and Alsaiari, M. A., *Extraction of keratin from sheep wool fibres using aqueous ionic liquids assisted probe sonication technology*, J. Mol. Liq., 350 (2022) 118595.

19. Serna-Vázquez, J., Ahmad, M. Z., Boczkaj, G., and Castro-Muñoz, R., *Latest insights on novel deep eutectic solvents (DES) for sustainable extraction of phenolic compounds from natural sources*, Molecules, 26 2021).
20. Jahan, K., Ashfaq, A., Younis, K., Yousuf, O., and Islam, R. U., *A review of the effects of ultrasound-assisted extraction factors on plant protein yield and functional properties*, J. Food Meas. Charact., 16 (2022) 2875–2883.
21. Córdova, A., Catal´an, S., Carrasco, V., Farias, F. O., Trentin, J., L´opez, J., Salazar, F., and Mussagy, C. U., *Sustainable assessment of ultrasound-assisted extraction of anthocyanins with bio-based solvents for upgrading grape pomace Cabernet Sauvignon derived from a winemaking process*, Ultrason. Sonochem., 112 (2025).
22. Bouizgma, K., Rabbah, N., Abbas, Z., and Abourriche, A., *Unlocking sustainable extraction of natural antioxidants: Green solvents, smart technologies, scalability and future directions*, Sep. Sci. Technol., 60 (2025) 657–683.
23. Zhang, W., Boateng, I. D., and Xu, J*., How does ultrasound-assisted ionic liquid treatment affect protein? A comprehensive review of their potential mechanisms, safety evaluation, and physicochemical and functional properties*, Compr. Rev. Food Sci. Food Saf., 23 (2024) p. e13261, 2024.
24. Lawrance, A., Mary, D. L. R., Felicita, A. E. S., and Arockianathan, P. M., *Physico-chemical Characteristics of Keratin Extracted from Three Commercial Bird Feathers and their Antibacterial Activity*, Asian J. Chem., 36 (2024) 1803–1811.
25. Lyu, l., Su, S., Lu, J., Zhou, X., and Gao, Y., *Structural characteristics and sound absorption properties of different kinds of waste feather fibers*, Text. Res. J., 93 (2023) 2138–2148.
26. Sun, J., Santiago, G. M., Yan, F., Zhou, W., Rudolf, P., Portale, G., and Kamperman, M., *Bioinspired Processing of Keratin into Upcycled Fibers through pH-Induced Coacervation*, ACS Sustain. Chem. Eng., 11 (2023) 1985–1994.
27. Pourjavaheri, F., Pour, S. O., Jones, O. A. H., Smooker, P. M., Brkljača, R., Sherkat, F., Blanch, E. W., Gupta, A., Shanks, R. A., *Extraction of keratin from waste chicken feathers using sodium sulfide and L-cysteine*, Process Biochem., 82, (2019) 205–214, 2019.
28. Meko, O. A., Eraga, S. O., and Arhewoh, M. I., *Effect of Extraction Parameters on Some Properties of Keratin obtained From Waste Chicken Feathers*, Trop. J. Nat. Prod. Res., 8 (2024) 7423–7430.
29. Rosarina, D., Narawangsa, D. R., Chandra, N. S. R., Sari, E., and Hermansyah, H., *Optimization of Ultrasonic—Assisted Extraction (UAE) Method Using Natural Deep Eutectic Solvent (NADES) to Increase Curcuminoid Yield from Curcuma longa L., Curcuma xanthorrhiza, and Curcuma mangga Val*, Molecules, 27 (2022).
30. Škerget, M., Colnik, M., Zemljič, L. F., Gradišnik, L., Semren, T. Z., Lovakovi´c, B. T., and Maver, U., *Efficient and Green Isolation of Keratin from Poultry Feathers by Subcritical Water*, Polymers (Basel)., 15 (2023) 1–15.
31. Tinoco, A., Gonçalves, F., Costa, A. F., Freitas, D. S., Cavaco-Paulo, A., and Ribeiro, A., *Keratin:Zein particles as vehicles for fragrance release on hair*, Ind. Crops Prod., 159 (2021) 113067.
32. Duman, Ş., and Küçük, M., *Production and characterization of keratin microparticles obtained from wool fibers by cryogenic milling method*, Part. Sci. Technol., 40 (2020) 933–941.
33. Mattiello, S., Guzzini, A., Giudice, A. D., Santulli, C., Antonini, M., Lupidi, G., and Gunnella, R., *Physico-Chemical Characterization of Keratin from Wool and Chicken Feathers Extracted Using Refined Chemical Methods*, Polymers (Basel)., 15 (2023) 1–15.
34. Xu, X., Wang, Z., Li, M., Su, Y., Zhang, Q., Zhang, S., Hu, J., *Reconstructed Hierarchically Structured Keratin Fibers with Shape-Memory Features Based on Reversible Secondary-Structure Transformation*, Adv. Mater., 35 (2023).
35. Jain, A., Behera, M., Mahapatra, C., Sundaresan, N. R., and Chatterjee, K., *Nanostructured polymer scaffold decorated with cerium oxide nanoparticles toward engineering an antioxidant and anti-hypertrophic cardiac patch*, Mater. Sci. Eng. C, 118 (2021).
36. Banasaz, S., and Ferraro, V., *Keratin from Animal By-Products: Structure, Characterization, Extraction and Application—A Review*, Polymers (Basel)., 16 (2024).
37. Senthilkumar, N., Chowdhury, S., and Sanpui, P., *Extraction of keratin from keratinous wastes: current status and future directions*, J. Mater. Cycles Waste Manag., 25 (2023) 1–16.
38. Shubha, A., Sharmita, G., and Manaswi, R., *Recent advances in preparation and biomedical applications of keratin based biomaterials*, Biotechnol. Sustain. Mater., 1 (2024) 1–27.
39. Giannelli, M., Guerrini, A., Ballestri, M., Aluigi, A., Zamboni, R., Sotgiu, G., and Posati, T., *Bioactive Keratin and Fibroin Nanoparticles: An Overview of Their Preparation Strategies*, Nanomaterials, 12 (2022).
40. Wang, Y., Liu, J., Zhang, Z., Meng, X., Yang, T., Shi, W., He, R., and Ma, H., *Insights into Ultrasonication Treatment on the Characteristics of Cereal Proteins: Functionality, Conformational and Physicochemical Characteristics*, Foods, 12 (2023).
41. Amin, S., Abbas, M., Ashgar, Z., Ghani, N., Shaheen, S., Hassan, F., Akram, R., and Yousaf, H. S., *Extraction of Keratin from Chicken Feathers and its Application in the Treatment of Contaminated Water: an Eco-Friendly Approach*, Brazilian Arch. Biol. Technol., 67 (2024).