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Research Article

Extraction and Characterization of Keratin from Waste Broiler Chicken Feathers

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Abstract: The current study was to extract the keratin protein from waste broiler chicken feathers. Due to their high keratin content, chicken feathers offer a valuable source of protein. The two steps involved are treating sodium sulfide (Na_2S) and then extracting the protein from the mixture. After the feathers have been broken down using reducing agents, dilute hydrochloric acid (HCl) is added to the solution to precipitate the protein. The precipitated protein was repeatedly rinsed with distilled water and dissolved in a sodium hydroxide (NaOH) solution to form a solution. The results indicate that a chicken feather has unique features. Keratin obtained from broiler chicken feathers had a yield of 81.1%, which is relatively high in cases of waste raw material conversion into value-added products. The prepared keratin was characterized by Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and thermogravimetric analysis (TGA) to investigate the chemical composition, thermal properties, crystallinity properties, and also physico-mechanical properties, including density. These findings suggest that chicken feathers can be used to extract protein.

***Keywords*:** protein, chicken feather, reducing agents, FTIR

1. Introduction

Proteins are necessary for human survival. They are one of the most important components of body tissue. Proteins are made up of amino acid components that are linked together by peptide bonds [1]. Peptide linkages are the amino connections that are produced by carboxylic acid and amino acid molecules [2]. A polypeptide bond connects the lengthy macromolecular chain of protein [3]. Some examples of proteins are keratin, collagen, elastin, and silk, which are fibrous compounds that have been widely investigated. Protein material can be synthesized in a similar way since their properties are similar [4].

Feather is commonly assumed to be a byproduct of chicken production. Some farmers raise poultry, particularly for their feathers. The number of feathers produced is significant. Each year, 2.3 billion pounds of feather are created for the 8 billion broilers produced. Each chicken has up to 125 mg of feather, and with over 400 million chickens processed each week around the world, the daily accumulation of feather waste surpasses five metric tons [5]. Feather fiber is comparable to the starch and cellulose found in paper and wood. Feather and wool are both made of keratin, but the surface area of a feather is significantly larger due to the smaller diameter of the fiber. As a result, in wool or cellulose fibers, the fiber can absorb more moisture. Feather fibers are naturally stable and long-lasting due to their

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crystal structure [6]. The quill (ranchis), barbs, and barbules are the primary components of a feather. The barb grows out of the quill, which is the primary shaft attached to the bird's body. Barbules are separated from barbs, and hooklets are connected by barbules. Despite the fact that the components are distinct, they all contain keratin. Because the quill section of the feather is brittle and fragile, it is not suitable for use as fiber. They can be utilized because they are sheet-like and fibrous [7-9]. Feather fiber can be employed in both hydrophilic and hydrophobic resins as reinforcements. This is due to the fact that 40% of 95 amino acids are found in the human body, 60% of keratin is hydrophobic, and the remaining 40% is hydrophilic [10].

The feather keratin contains a higher concentration of amino acids, i.e., glycine, alanine, serine, cysteine, and valine, but less lysine, methionine, and tryptophan. Sodium sulfide (Na_2S) is the reducing agent that is utilized in this study to reduce the disulfide bonds. Hydrochloric acid (HCl) is the most common protein precipitant used to precipitate the solution. Keratin is an essential element of tissues because it gives them a hard and fibrous matrix and allows them to bend in many directions, separating them [11]. Chicken feather keratin can be transformed into a natural protein that can be broken down by trypsin and pepsin and is soluble in alkalis and acids. By tearing the keratin disulfide bonds, this was made possible. Pleated sheets that have been twisted together in feather keratin are then stabilized and toughened by disulfide bonds. Therefore, the strength of keratin in chicken feathers can be decreased by dissolving these disulfide links, making the keratin soluble and capable of being transformed into a natural protein [12]. Novel discoveries could result from improvements in the extraction, purification, and characterization of keratin derivatives and uses for what is presently considered waste material that may lead to new opportunities for value addition.

To the best of our knowledge, there are no reports on the extraction of keratin from waste broiler chicken feathers, despite the fact that there are some reports on the production of keratin. This research article to produce keratin from waste broiler chicken feathers emphasizes the use of biodegradable polymers from renewable resources.

2. Methodology

2.1 *Materials*

Raw broiler chicken feathers were collected from a chicken shop in Lucknow, India. Feathers were used for structure and property studies. Na_2S was 95% extra pure with yellow flakes, odorless, and had a molecular weight of 78.04 g/mol as procured from LOBA Chemie Pvt. Ltd., Mumbai, India. HCl was liquid with an assay (acidimetric) of 35 to 38% light yellow, non-volatile, non-combustible, and had a molecular weight of 36.46 g/mol and a density of 1.18 g/mL at 20 °C. It was generously given by Central Drug House Pvt. Ltd., New Delhi, India. Acetone was 99% extra pure, colorless, volatile, and had a molecular weight of 58.08 with a density of 0.79 g/cm^3. It was purchased from LOBA Chemie Pvt. Ltd., Mumbai, India. Distilled water, a form of purified water obtained by simple distillation free from impurities, was of laboratory grade.

2.2 *Extraction of keratin from waste chicken feathers*

The mechanism of extraction of chicken feather keratin via Na_2S is shown in Figure 1. A flow diagram of keratin extraction from waste chicken feathers is illustrated in Figure 2, which includes four steps: pretreatment, extraction, precipitation, and purification [13].

2.2.1 *Pre-treatment process*

In this step, firstly, chicken feathers were soaked in acetone for 24 hours to remove grease, oil, and strains, as well as microorganisms, then washed with soapy water to remove impurities. After that, the waste chicken feathers were cleaned and dried in the oven for 24 hours. This process effectively eliminated extraneous components and mitigated the foul odor [13].

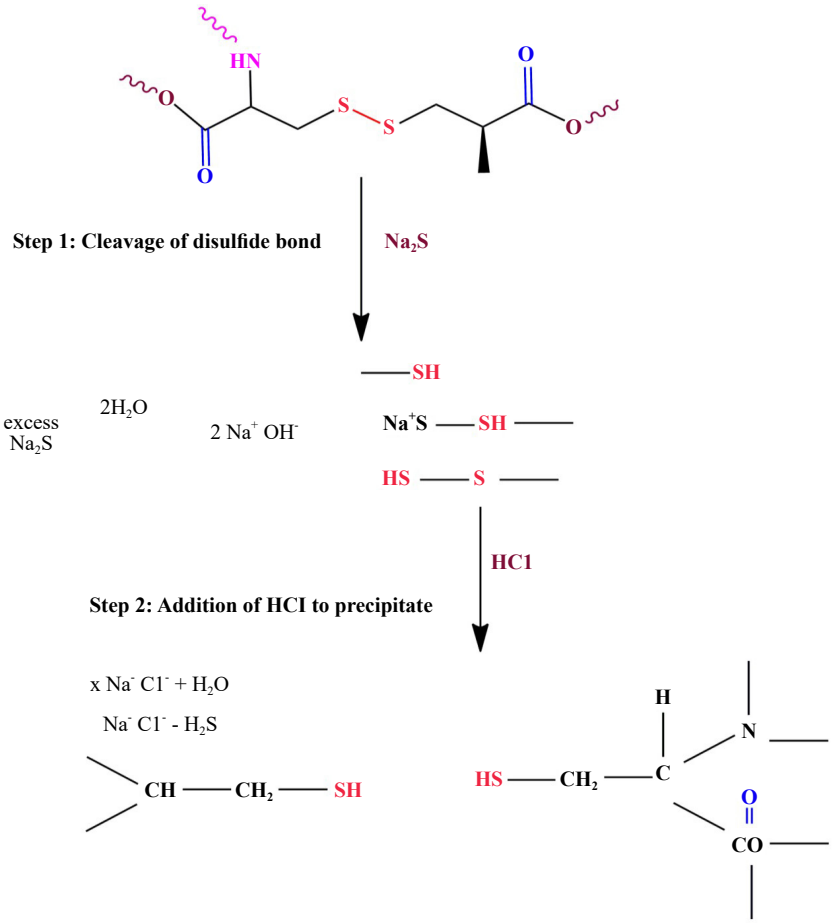

Figure 1. Mechanism of extraction of chicken feather keratin via Na_2S

2.2.2 *Extraction process*

In the extraction process, the dried waste chicken feather was first properly weighed, and then a solution of 0.5 M Na_2S was made in a 1 L flask. Then, the prepared solution of 25 g of weighed chicken feathers was added, and the solution was heated at 45 °C. The pH was kept between 10 and 13 in the basic medium, and the solution was properly stirred for 5 hours with the help of a mechanical stirrer. After that, the solution was centrifuged for 5 minutes at 10,000 rpm. The supernatant liquid was carefully collected, and then the remaining particles were filtered using filter paper.

2.2.3 *Precipitation process*

In the precipitation process, 100 mL of HCl solution was first prepared. Then, the filtrate solution obtained earlier was continuously stirred in a beaker, and the prepared HCl solution was added dropwise at a definite ratio. The solid particles were collected, and the supernatant liquid was collected separately. The whole process was carefully repeated.

2.2.4 *Purification process*

In this process, the solid particles were collected and then washed with deionized water, mainly 4 to 5 times, until the pH reached neutral. Then the solid particles were subjected to a drying process in petri dishes at a temperature of 45 °C for 3 to 5 hours to get a dried sample. The dried powder sample obtained was keratin.

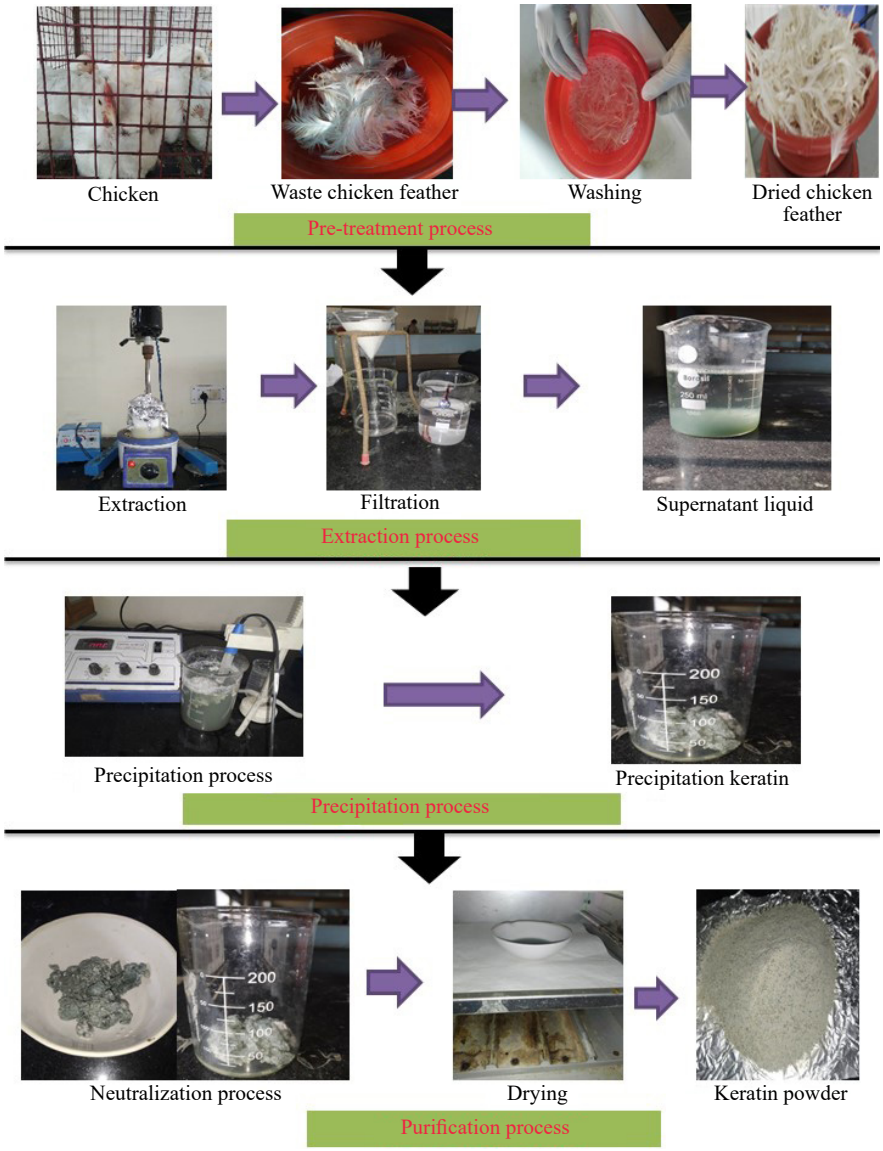

Figure 2. Flow diagram of keratin extraction from waste chicken feathers

2.3 *Yield calculation*

Yield percentage can be calculated by using Equation 1:

$$\text{Yield percentage (\%)} = \frac{\text{Total weight of obtained keratin}}{\text{Total weight of waste chicken feather}} \times 100 \quad (1)$$

2.4 *Physical property*

2.4.1 *Density analysis*

The density analysis of keratin powder was determined in accordance with American Society for Testing and Materials (ASTM) D-729 using a 25-mL specific gravity bottle made up of borosilicate glass (Nikam Scientific Company, Boisar, India). Equation 2 shows the calculation required.

$$\text{Density} = \frac{(c-a)}{(b-a)-(d-c)} \times D \tag{2}$$

where a = the weight of an empty pycnometer, b = the weight of a pycnometer with liquid, c = the weight of a pycnometer with a sample, d = the weight of a pycnometer with a sample and liquid, and D = the density of used liquid.

2.4.2 *Ash content determination*

The ash content of the sample was determined as per ASTM D-2584. Using a muffle furnace, the sample was put into the crucible, heated at 750 °C for 6 hours, and then cooled. The final weights were obtained as per ASTM standards. Equation 3 was used to compute the ash content (%) of keratin.

$$\text{Ash content (\%)} = \frac{\text{Residue weight (g)}}{\text{Sample weight (g)}} \times 100 \tag{3}$$

2.5 *Characterization*

2.5.1 *Fourier-transform infrared (FTIR) spectroscopy*

FTIR spectra were recorded at room temperature using a Thermo-Scientific Nicolet 6700. The FTIR spectrometer operates on a diamond disc in the range of 4,000 to 400 cm^{-1}.

2.5.2 *X-ray diffraction (XRD) analysis*

XRD measurements of keratin powder are carried out using an XRD unit (Model: D8 Advance Eco Bruker, Germany) with Cu kα radiation (40 KV, 30 mA) at a wavelength of 1.54 A°.

2.5.3 *Thermogravimetric analysis (TGA)*

The thermal analysis of keratin at room temperature up to 800 °C was evaluated using TGA instrument (Model: TGA-800 PerkinElmer, United States) at a heating rate of 10 °C/min under a nitrogen atmosphere. The samples were vacuum-dried at 40 °C.

2.5.4 *Scanning electron microscope (SEM)*

The morphological analysis of keratin powder was carried out using a SEM (Model: JEOL JSM-6490LV, Japan), and particle size analysis was measured using Image J software.

3. Results and discussion

3.1 *Yield calculation*

Keratin is extracted with an 81.1% yield from waste chicken feather, which is relatively high in terms of converting waste raw material into a value-added product [14]. These were previously reported as 84.5% at 80.9 ℃ in 9.5 hours using Design-Expert concomitantly [10]. Keratin yield percentage depends on different Na_2S concentrations, temperature, and time.

3.2 *Determination of density using the pycnometer method*

A pycnometer was used to calculate the density of a homogeneous solid material that is insoluble in a working liquid, i.e., water. Next, a known-density liquid (diesel) is put into the pycnometer; this liquid is fully insoluble in powder. Such properties are important in the valorization of feathers in several applications, such as yarn and

composites. The density of chicken feathers was calculated and obtained as 0.891 g/cc. The reason for the light weight and low density of the chicken feathers might be due to their honeycomb structure. The extracted keratin density of 44.8% was higher than that of the chicken feather [15]. Considering that the density of keratin powder increases as the amount of disulfide concentration decreases, this may be due to the free volume decreasing.

3.3 *Ash content determination*

Ash content signifies the presence of an inorganic component in keratin. Ash content can be used as one of the specific factors for keratin quality indication because, with an increase in ash content, the keratin purity level will increase. 2 g of keratin, which was previously weighed, was placed in a crucible to determine the ash content. The keratin sample was heated for 6 hours at 800 °C in a muffle furnace. After 6 hours, the crucible was pulled out of the furnace and cooled in the desiccator for 30 minutes. The extracted keratin has an ash content of 2%.

3.4 *FTIR analysis*

The analysis of FTIR confirmed the existence of chemical compositions in pure feathers, and keratin extracted from broiler chicken feathers was determined. The pure feather spectra can be seen in Figure 3. The characteristic peaks at 3,296.9 cm^{-1} signify O-H stretching, whereas the peak at 2,925.4 cm^{-1} represents C-H stretching [16]. The peaks at 1,660 cm^{-1}, 1,533.7 cm^{-1}, and 1,216.8 cm^{-1} justify the existence of the C=O stretch of amide I, the C-H stretch and N-H stretching of amide II, and the C≡N stretch of amide III band, respectively; the 418 cm^{-1} C-S stretch is associated with monosulfide and disulfide bonds [17]. The FTIR spectra of pure keratin display the characteristic peak at 3,407.7 cm^{-1}, which signifies O-H stretching and N-H stretching, whereas the peak at 2,922.19 cm^{-1} represents C-H stretching. The peaks at 1,636.5 cm^{-1} and 1,384.24 cm^{-1} justify the existence of the C=O stretch of the amide I band and the C≡N stretch of the amide III band in the extracted product, respectively. Meanwhile, the peak at 767.24 cm^{-1} is associated with NH out of plane bending. The presence of disulfide bonds in keratin is found at 484.6 cm^{-1} [18]. These FTIR analyses confirmed that the resultant product was derived from waste chicken feather building block amino acids forming peptide groups of keratin protein.

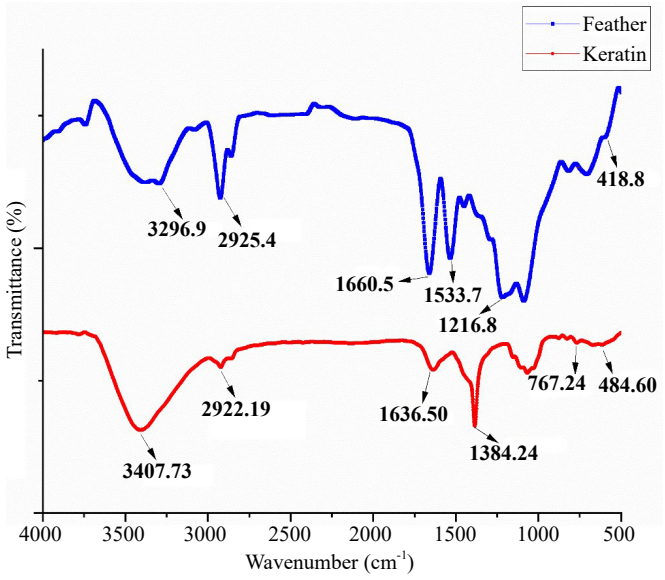

Figure 3. FTIR spectra of chicken feathers and extracted keratin

3.5 *XRD analysis*

The analysis done was by wide-angle X-ray diffraction (WAXD) to determine the crystal phase of the samples. Figure 4 represents the XRD pattern of the resultant product; the pattern shows that the obtained product indicates a semicrystalline nature. The equatorial reflection 2θ is between 16° and 31° for the α-helix structure and 9° and 10° for the β-helix structure, respectively. Both diffraction peaks show an amorphous nature [19]. The diffraction peaks of 10.61 and 19.55 indicated the α and β helix patterns. The intensity of the peaks indicates that there was more β-sheet conformation in the extracted keratin. The found data showed the effect of the solvent and reducing agent on the crystallization of keratin and confirmed the tendency of keratin to form β-sheet structures and Na_2S-cast forms.

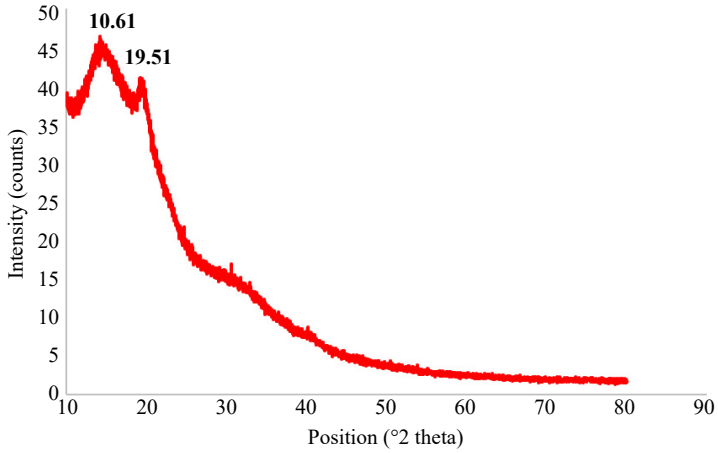

Figure 4. XRD analysis of extracted powder keratin

3.6 *TGA*

The TGA of the resultant product is performed at a 10 °C/min heating rate in a nitrogen atmosphere at room temperature up to 800 °C. The thermal decomposition of the extracted keratin is shown in Figure 5. There is a weight loss of keratin in two stages, which occur in the temperature range of 95 °C to 800 °C. The first weight loss in keratin weight happened between 45 °C and 100 °C and was attributed to the loss of bound water. The weight loss in the temperature range of 245 °C to 450 °C is mainly due to the decomposition of other products and volatile compounds, and it was degraded by 85% at about 800 °C [20].

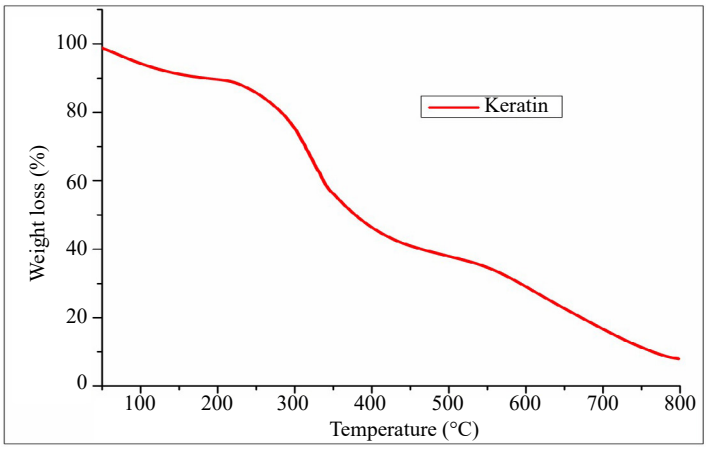

Figure 5. TGA of extracted keratin

3.7 *SEM analysis*

The morphology of keratin was investigated by SEM analysis at different magnifications of 300X, 400X, and 500X, as shown in Figure 6. The powder keratin was found to have a smooth surface with a non-homogeneous nature and texture. This nature of morphology may be due to the broader polydispersity of the keratin protein. The sample surface is found to be rough, which means it can attach to other materials without any difficulty, and as a result, it can be used as a biodegradable filler. The sample size is noticed on the microscale in the range of 39 µm to 85 µm. There is the formation of agglomeration in the particles, along with a large number of noticeable pores on the sample surface [21].

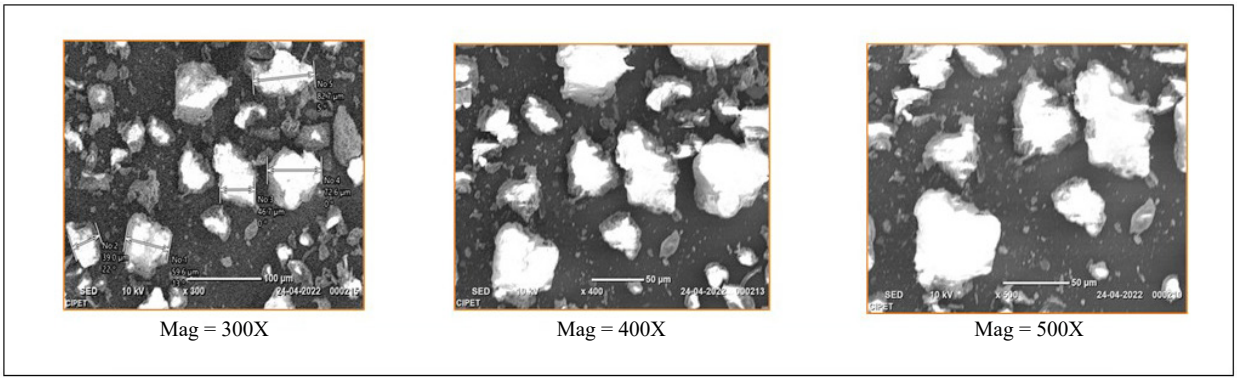

Figure 6. SEM analysis of keratin at different magnifications of 300X, 400X, and 500X

4. Conclusion

It is a perfect resource to obtain keratin protein because broiler chicken feathers contain 90% crude protein and cause environmental problems owing to their excessively long decomposition. It can be concluded that the extracted keratin from broiler chicken feathers using Na_2S followed the following order of chemical processes, which involved an alkaline hydrolysis method. Keratin obtained from waste chicken feathers had a yield of 81.1%, which is relatively high in the case of waste raw material conversion into a value-added product. The resultant product is characterized by using FTIR and XRD techniques, which display the relevant peaks that confirm the product quality. The analysis by FTIR confirmed the existence of chemical compositions such as carboxyl acids and amino groups in the protein sample. The TGA curves for extracted keratin from Na_2S demonstrated nearly comparable breakdown behavior, demonstrating the purity of keratin, which shows its onset degradation temperature at 280 °C. The value-added product, i.e., keratin, had a density of 1.29 g/cc. The surface roughness of the particles was readily scanned in the SEM images, along with the presence of spongy microporous particles noticed in the microscale range of 39 µm to 85 µm. The resulting keratin protein has the potential to be employed for a variety of applications, including anti-aging creams, shampoos, and conditioners, as well as for medical and biomedical uses such as bone replacement, bone grafts, and tissue engineering.

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Conflict of interest

There is no conflict of interest in this study.

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