Malaysian Journal of Catalysis
http://mjcat.utm.my/
MJCat Research Article

Extraction and Characterization of Keratin Protein from Chicken Feathers using Alkaline Hydrolysis Method: Effects of Sodium Sulphide Concentration and Shelf-life Evaluation

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Article history:
Received 4 November 2023
Accepted 24 December 2023

ABSTRACT

This study investigated the extraction of keratin protein from chicken feathers through alkaline hydrolysis using sodium sulphide as a digesting agent. The protein was precipitated using hydrogen chloride and confirmed through biuret test, solubility test, sulphur test, and FT-IR analysis. The effect of varying sodium sulphide concentrations (0.5M, 0.75M, and 1M) on the extracted keratin was evaluated. Results showed that a higher concentration of sodium sulphide produced a higher yield of keratin, with 1M producing 65.8% yield. However, the shelf-life of wet keratin extracted using 1M concentration was four weeks, compared to six weeks for 0.5M and 0.75M concentrations. The dried keratin was unaffected after six weeks. The study suggests that a higher concentration of the reducing agent produced a higher yield of keratin protein but with a shorter shelf-life if drying was not carried out. The utilization of abundant waste generated by poultry industries is crucial in reducing pollution and creating opportunities for valuable product development. The extraction of keratin from chicken feathers provides an eco-friendly approach to waste management and creates opportunities for product development.

Keywords: *Keratin, Chicken feathers, Shelf-life, Yield, Drying, Hydrolysis*

| eISSN 0128-2581 |

1. INTRODUCTION

Keratin is a vital and abundant structural protein in humans and animals, which can be found in various industrial wastes such as slaughterhouse by-products, skin remains, animal hair, horns, hooves, and feathers [1-4]. These wastes are considered environmental pollutants due to their resistance to physical, chemical, and biological agents [5]. Keratin has a complex hierarchical structure that can be classified into primary, secondary, and tertiary structures. It also occurs abundantly in two forms which are α-keratins and β-keratins, with the former occurring in mammals and the latter in birds and reptiles [6], [7]. This work focuses on the extraction of keratin from chicken feathers, which have received significant attention in various applications, such as cosmetics, tissue engineering, and regenerative medicine, due to their biocompatibility, biological function, and biodegradability [8].

Chicken feathers are a rich source of keratin, with a large quantity being produced as a by-product of the poultry industry each year [9], [10]. Its primary structure consists of a repeating pattern of four amino acids: glycine, alanine, serine, and tyrosine. This pattern is repeated many times to form a long polypeptide chain. This unique sequence of amino acids gives chicken feather keratin its unique properties, including its strength, flexibility, and resilience. However, conventional methods of extracting keratin, such as acidic hydrolysis, can cause damage to some amino acids and consume a large quantity of reagents, which cannot be recycled [11]. Therefore, alternative methods, such as enzymatic hydrolysis and chemical-enzymatic treatment, have been proposed to produce hydrolyzed keratin protein [12]. Ionic liquids (ILs) have also been studied for their potential to dissolve biopolymers, including keratin, into useful materials for industrial applications [1].

The secondary structure of chicken feather keratin is characterized by alpha-helices, which are formed when the polypeptide chain twists around itself. The alpha-helices then associate with one another to form coiled-coil structures, which in turn associate to form protofilaments. The protofilaments then bundle together to form intermediate filaments, which are the structural components of keratin fibers. The tertiary structure of keratin involves the folding and packing of the intermediate filaments into a

three-dimensional structure. This three-dimensional structure gives keratin its unique properties and allows it to form strong and resilient fibers [13].

Alkaline extraction using sodium sulphide has been shown to be an efficient and economically favorable method that preserves the secondary structure of the protein, while the addition of an ionic surfactant such as sodium dodecyl sulphate can prevent the aggregation of keratin polypeptide chains [9]. Enzymatic catalysis hydrolysis is a promising alternative method, but its industrial application is hindered by the outer protective film and compact structure of the feather [12]. Therefore, developing effective and eco-friendly processes to extract keratin from poultry feathers is desirable from both environmental and economic perspectives [9].

2. MATERIALS AND METHODS

The use of chemical (alkaline) hydrolysis was employed in the extraction process with sodium sulphide as the reducing agent. The extraction method was carried out by different stages of experimental procedures as listed below and all the chemicals used were of analytical grade requiring no further purification.

2.1 Collection and pretreatment of waste chicken feathers

Chicken feathers were collected from Samaru market, latitude 11.1617 ˚N and longitude 7.6479 ˚E. The feathers were washed with distilled water to remove all the accompanied blood and dirt. It was then soaked in detergent for 2 hours, and washed with distilled water, after which it was oven dried at 60 ˚C for 12 hours. The dried feathers were soaked in diethyl ether for 12 hours to cleanse it from stain, oil and grease, then washed with hot water (100 ˚C) followed by distilled water and then oven dried at 60 ˚C for 48 hours to remove its moisture content. The dried chicken feathers were ground using a laboratory milling machine at mesh size 0.25 mm and stored in a sealed plastic bag at room temperature.

2.2 Keratin protein extraction

1 L of 0.5 M sodium sulphide solution was prepared in a 2000 mL beaker. 25 g of the ground chicken feathers was weighed and added to the sodium sulphide solution. The solution was heated to the temperature of 60 ˚C at a pH of 10 and stirred continuously for 6 hours using a magnetic stirrer. The solution was centrifuged at 10,000 rpm for 10 minutes. The supernatant liquid was carefully collected then filtered using the burchner funnel with filter paper of 110 mm to make it particle free.

2.3 Protein precipitation

After the extraction process, the pH of the keratin solution was adjusted to 3.5 by addition of HCl to precipitate the protein. The solution was then centrifuged at 10,000 rpm for 6 minutes and the solid particles carefully collected.

2.4 Protein purification

400 mL distilled water was added to the keratin protein collected and stirred (washing). The solution was centrifuged at 10,000 rpm for 6 minutes and the solid particles collected carefully for further analysis and characterization.

The above steps were repeated for sulphide concentration 0.75 M and 1 M.

2.5 Analysis and characterization of the keratin protein

2.5.1 General test (Biuret test)

5% sodium hydroxide solution and 1% copper sulphate solution were prepared. 2 mL of each keratin solution (0.5 M, 0.75 M, 1M) was mixed with the sodium hydroxide solution in the ratio 1:1. Three drops of the copper sulphate solution were added to the mixture solutions. Changes in the solution were observed and recorded.

2.5.2 Differentiating test (Solubility test)

10 mL of distilled water was added to each keratin protein in a test tube. The test tubes were then shaken vigorously for a minute and observations recorded.

2.5.3 Confirmatory test (Sulphur test)

40% sodium hydroxide solution and 2% lead acetate solution were prepared. 2 mL of each keratin protein was mixed with the sodium hydroxide solution in the ratio 1:1. The solution in the test tubes were held on flame and boiled for 1 minute, then cooled under tap. 5 drops of the lead acetate solution were added to the mixture solution and changes observed and recorded.

2.5.4 Fourier Transform Infrared Spectroscopy

Functional groups of the extracted protein were investigated by mixing samples into KBr pellets using Fourier transform infrared (FT-IR) absorption spectrum Agilent spectrometer (30 scans with 8 cm^{-1} resolution) in the wavenumber range of 650–4,000 cm^{-1}.

2.5.5 Shelf-Life

A sample of the wet keratin extracted was segregated and kept in a sealed container, while some part was shadow dried for 72 hours at room temperature, crushed into fine powder and kept in another sealed container. Both samples were then observed for 6 weeks.

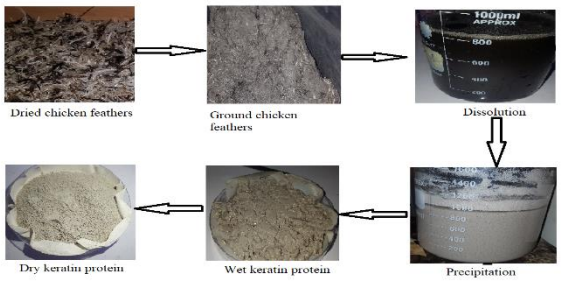

Figure 1. Schematic diagram of the keratin extraction process

3. RESULTS AND DISCUSSION

3.1 Extraction of Keratin from Chicken Feathers

Alkaline hydrolysis was carried out to extract keratin from chicken feathers using 0.5M, 0.75M and 1M concentration of the reducing agent, sodium sulphide (Na2S). Table 1 shows the physical observation, moisture content, yield and shelf-life after extraction.

Table 1. Physical appearance of extracted keratin

Concentration (M)	0.5	0.75	1
% Moisture Content (MC)	70	74.5	78.1
% Protein Yield	55.7	61.6	65.8
Shelf-life (weeks)	4	6	6
Physical Appearance	Most compacted	Moderately compacted	Least compacted with a smoother appearance

The wet keratin (keratin before drying) extracted for the 1 M concentration appeared to be smoother and loose indicating high amount of water present. While the 0.5 M concentration was the least loose, showing low amount of water. The solubility of the feathers is dependent on the concentration of Na^2S, temperature and process time [14].

As seen in Table 1, the concentration of the reducing agent affects the appearance, associated water as well as the yield of the extracted protein. The amount of keratin yield in the final product depends on the degree of hydrolysis of the feather. Hence, the 1 M concentration shows the highest degree of hydrolysis. This contradicts the work of Gindaba et al. [15] which says that at 0.5 M the yield decreases on increasing concentration of the solvent. The increasing pattern of keratin yield is caused by peptide and disulphide bonds cleavage. The effect of concentration of sodium sulphide at constant temperature and pH is shown in Figure 2.

The samples of wet and dried keratin proteins were observed for 6 weeks under atmospheric conditions. At week 4, the 1 M wet keratin was observed to have some microbial growth while the 0.5 M, 0.75 M and all the dried keratins appeared the same. At week 6, there was some microbial growth on the 0.5 M and 0.75 M wet keratin and none for the dry keratins of all concentration. The onset of the contamination for the 1 M keratin was due to the high amount of moisture present providing the environment for the growth.

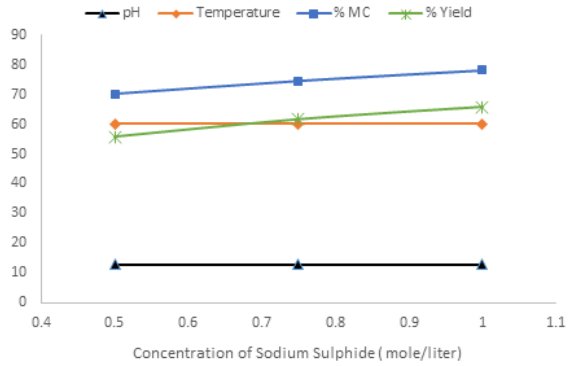

Figure 2. Effect of concentration of sodium sulphide at constant temperature and pH

3.2 Characterization of the Extracted Protein

3.2.1 Biuret test

The protein solution remained its usual colour (ash) on the addition of the NaOH solution. It turned purple however on the addition of the copper sulphate solution indicating the presence of peptide linkage as shown in Figure 3. This is a general test for all types of protein where the Cu^{2+} ions react with the nitrogen of the peptide bond to form a purple colour complex.

Figure 3. Biuret test for extracted protein from chicken feather

3.1 Extraction of Keratin from Chicken Feathers

Alkaline hydrolysis was carried out to extract keratin from chicken feathers using 0.5 M, 0.75 M and 1 M.

3.2.2 Solubility test

On addition of water, the keratin was observed to be insoluble as shown in Figure 4. This is due to the presence of the disulphide bonds present making it insoluble. It differentiates it from the rest of the proteins that are highly soluble owing to the hydrophilic amino acids present in them.

Figure 4. Solubility test for extracted protein from chicken feather

3.2.3 Sulphur test

The protein solution turned black on the addition of the lead acetate shown in Figure 5, confirming the presence of cysteine which is a major component of keratin. Upon boiling of the keratin solution with sodium hydroxide, the sulphur present in its amino acids i.e., cysteine is converted to inorganic sodium sulphide. It then reacts with lead to form lead sulphide that forms black coloured pigments inside the solution.

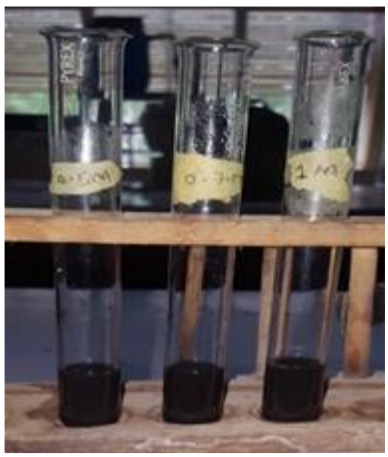

Figure 5. Confirmatory test for extracted protein from chicken feather

3.3 Fourier Transform Infrared (FT-IR)

Keratin is a natural polymer with a complicated structure, hence the need for Fourier Transform of Infrared (FTIR) to understand all details of structure of keratin. The characteristic bands and signals from these spectra were analysed in order to confirm the products as keratin. Figure 6-9 shows the characteristic absorption bands for the keratin extracted.

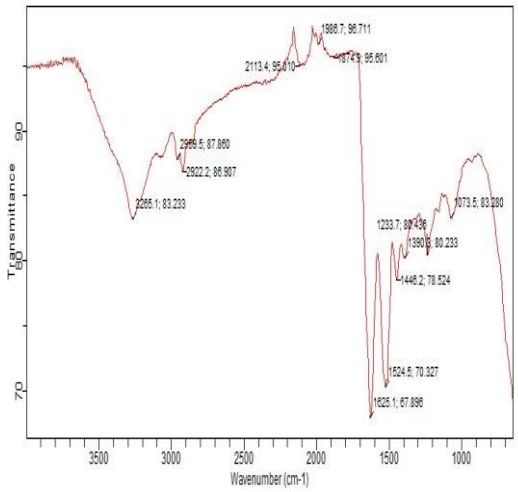

Figure 6. FT-IR of chicken feathers

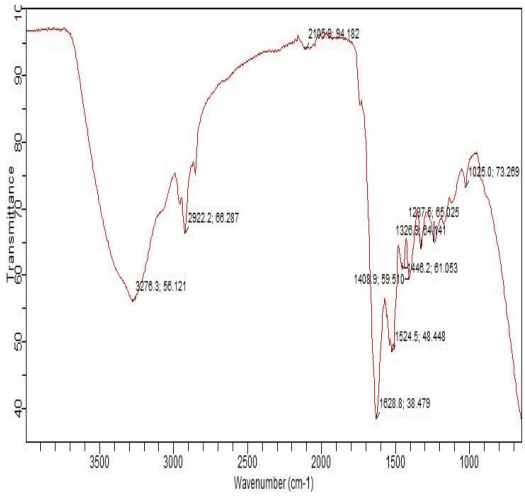

Figure 7. FT-IR of Extracted Protein of 0.5 M

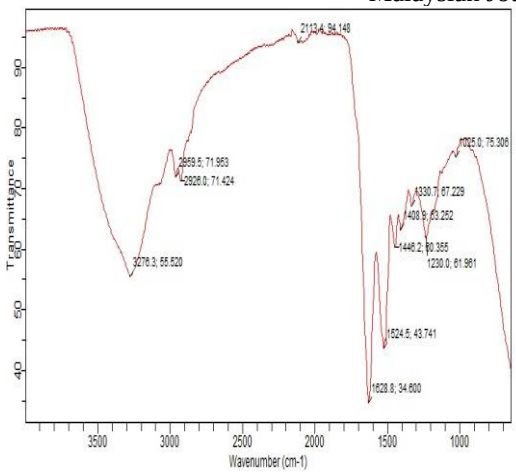

Figure 8. FT-IR of Extracted Protein of 0.75 M

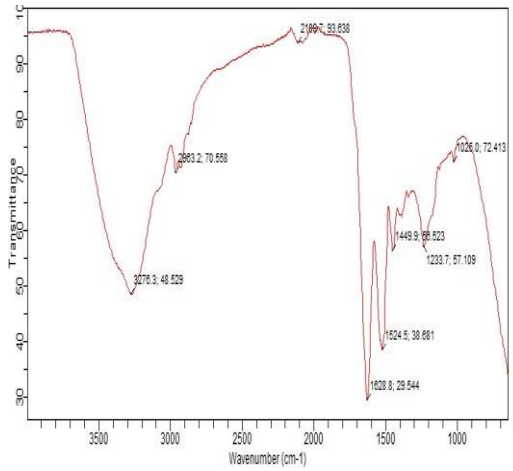

Figure 9. FT-IR of extracted protein of 1M

4. CONCLUSION

Sodium sulphide proved to be a highly effective dissolution agent, while hydrogen chloride demonstrated excellent precipitating properties. Notably, at a concentration of 1M alkalis, the extracted keratin exhibited a loose appearance, with a moisture content of 78.2%, a yield of 65.8%, and a shelf-life of only 4 weeks. These findings suggest that higher concentrations of the dissolution agent led to greater keratin extraction. Furthermore, this extraction process is suitable for scaling up from the laboratory to the industrial level.

ACKNOWLEDGEMENTS

The research team will like to acknowledge Ahmadu Bello University Zaria and Petroleum Technology Development Funds for providing research laboratories.

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