Extraction and characterization of keratin from chicken feather waste biomass: a study

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***Abstract*—**Keratins are proteins that form hard fibers, present in wool, horns and hoofs, feathers etc. The feathers consist of up to 10% of total chicken weight. Uncontrolled disposal of feathers from the poultry slaughterhouses industry is troublesome to environment. Primary purification phase is necessary for isolating the keratin from other materials. The present work was conducted to isolate the chicken feathers and to extract the keratin from processed biomass. The purification was conducted by washing with ddH_2O, detergents and ether and lastly again with boiled water at (100°C). Reducing agents used were sodium sulfide and sodium sulfite. The precipitates obtained in the form of pellet were washed 2-3 times with ddH_2O in order to remove foul smell and residues of sodium sulfide and HCl can be washed properly. To prepare the soluble form sodium hydroxide was added. Among the reducing agents tested Sodium sulfide showed good dissolving power as compared to others. Sodium sulfide with 0.3M and 0.5M concentrations dissolved the chicken feather efficiently. Keratin protein was dialyzed by ddH_2O using cellulose membrane (12 MWCO) which retained the protein size ~12 kDa. The concentration of keratin was studied by Bradford method (1.6mg/ml) and physiochemical characterization was done by FTIR and TGA analysis. The presence of alkane, carboxylic acid, amine, thiocarbonyl groups and disulfide bond was confirmed by FTIR analysis. The purified keratin is a promising substrate for the synthesis of cosmetic and pharmaceutical products.

Keywords— Feather, keratin, cosmetic products, environment, industry

1. INTRODUCTION

The large amount of feathers are discharged from the poultry industry every year particularly with fast food and very less amount of that is useful as a packing material, embellishment and forage. The feathers are biodegradable and to dump them large dumping area is required but the release of the landfill leachate produces heavy metals, organic and inorganic chemicals as well as pathogens; which can pollute the groundwater. Thus, no disposal on time creates environmental problem [1-2]. Hydrolyzing keratin from feather to produce amino acids is a kind of solution, but it is far from industrial applications. Therefore, it becomes a necessity to recycle feathers to make the discharge ecofriendly. Across the world, chicken feathers are among the unique, easily available and inexpensive byproducts. Chicken feathers are rich in keratin. Keratin is a valuable biopolymer [3]. Out of all other major structural proteins, such as collagen, elastin and myofibrillar proteins present in vertebrates, keratins have large amount of disulfide bonds. The presence of hydrophobic amino acids and disulfide crosslinking, make the keratins as insoluble in polar as well as non-polar solvents. The mechanical strength of feather and wool keratin is similar and abundantly found in nature as a hard keratin [4-5]. Number of techniques are available to dissolve hard keratin such as by using reduction and oxidation reactions [6-7], enzymatic hydrolysis [5] and ionic liquids [8-9].

The main aim of this study is to know the best concentration of the reducing agent and further the characterization of the extracted keratin. In the present study keratin protein were collected by using the process of isoelectric point precipitation. Reduced feathers were hydrolyzed by using different precipitants and best one was selected for the further studies. Precipitates of keratin protein at specific pH value were collected and characterized. The collected keratin was dissolve in alkali and also the keratin sample was freeze dried. Characterization includes the keratin yield with different concentrations of extraction solution and precipitants, dry mass, molecular weight and IR spectroscopy.

2. MATERIALS AND METHODS

A. Materials

Chicken feathers were collected from chicken processing plant at Jaya Gading, Kuantan, Malaysia. All chemicals were of analytical grade and were purchased from Sigma Aldrich (Selangore, Malaysia). Cellulose dialysis membranes (12MWCO) having average flat width of 33mm were purchased from Sigma Aldrich.

B. Pretreatment of feather and precipitation

First, the feathers were washed to remove all the blood and dirt. Then, the feathers were soaked in detergent for 2 h. After washing with ddH_2O feathers were soaked in diethyl ether and left overnight. The feathers were then washed with hot water (100°C) and distilled water and dried under sunlight approximately 48 hours. The dried feathers were then blended or cut into small pieces and kept carefully in sealed plastic bag. Keratin was extracted by alkaline hydrolysis from chicken feathers. 50g of feathers were dissolved in 2 L of different concentration of sodium sulfide (0.1, 0.3, 0.5M). The solution was heated to 60°C, stirred by mechanical stirrer having rpm 300 for 1-6 h for each concentration of sodium sulfide. The hydrolysate was separated by sieve to remove any

undigested material and then centrifuged (5810R, Eppendorf North America) at 10,000 rpm for 10 min. The supernatant was taken and precipitated with different precipitants (HCl, C_2H_5OH, acetone) and selected the best precipitant.

C. Protein Purification

The precipitant collected were added into 100ml of distilled water and washed by stirring. The solution was centrifuged at 10,000 rpm for 5 mins and solids were collected. The collected precipitate were then dissolved in 25ml of 0.2M sodium hydroxide and then centrifuged again for 5 min at 10,000 rpm. The supernatant was collected carefully and pellet form was discarded. The washing step was repeated two times. The supernatant collected was dialyzed against 1L of distilled water using cellulose tube for two days. The outer water was changed thrice a day. The dialyzed keratin protein was subjected to freeze dry (EQPCL 066, LABCONCO, USA) for further analysis and characterization.

D. Characterization and analysis of keratin sample

The protein concentration of keratin sample was measured by the Bradford protein assay method at different hour of dissolution, using Bovine serum albumin as a standard. The amount of dry mass of keratin sample was measured by pouring 10ml of keratin solution into petri plate and then dried under oven for 24 h. Before pouring the petriplates were weighted and labeled and after 24 h they again weighed and the difference was calculated. The microstructure of keratin was examined by Fourier Transform Infrared Spectroscopy (FTIR Thermo Nicolet Avatar 370, USA) with the wavelength number ranging from 4000 to 400cm^{-1} in the transmission mode. Thermo gravimetric analysis (TGA Q500V6.7 Build 203) was carried for the keratin by using platinum pan at a heating rate 10°C/min under nitrogen rich environment over a temperature range of 20°C to 600°C.

3. RESULTS AND DISCUSSION

A. Characterization of the extracted keratin

Three different concentrations (0.1, 0.3M and 0.5M) of the sodium sulfide were used to see the difference on the dissolution of chicken feather. The reading of every hour noticed by separating 20 ml from1-6 h. The precipitation was done for each sample and protein was purified and dissolved in NaOH. The concentration of protein present was 1.6 mg/ml estimated by using Bradford test. The sample protein showed blue color in the presence of Bradford dye. Again the results showed that with the increase in the dissolving time the concentration of protein also increased (figure 1). The results are shown in the graph given below for each concentration of sodium sulfide. But the effect of both (0.3M and 0.5M) concentrations was more or less similar so 0.3 M was selected for further experiments (figure 2,3). Calculation of dry weight showed that the amount in initial hours was lesser with moderate solubility which increased concomitantly with time (figure 4).

Three different type of precipitant (HCl, Ethanol, Acetone) were used to precipitate the proteins. The best result was shown by HCl. So, HCl will be selected for the further studies.

The color of keratin protein solution turned to pale yellow after three days from dark yellow. From the dialysis, sulfate and some of alkane group have been diffused out from the membrane tubing; clearly showed that the molar mass of those sulfate and alkane smaller than membrane tubing molecular weight cut off which is 12 kDa due to 1 $gmol^{-1}$ is equivalent to 1 Da. The sample was filtered by whatmann filter paper and the small particles then can able to separate easily.

FTIR

The FTIR spectra in the region 4000-400cm^{-1} for extracted keratin are given in figure 5. The absorption bands appeared for the sample keratin before and after dialysis are mainly assigned to the peptide bonds (-CONH-). The bands originate in the vibration of peptide bonds are amide I-III [10-14]. The peaks at 1672, 1598 and 1276cm^{-1} are showing vibrations known as amide I, II and III which is the result of β-sheet [15] which shows that the extracted keratin have similar secondary structure as feather fibers.

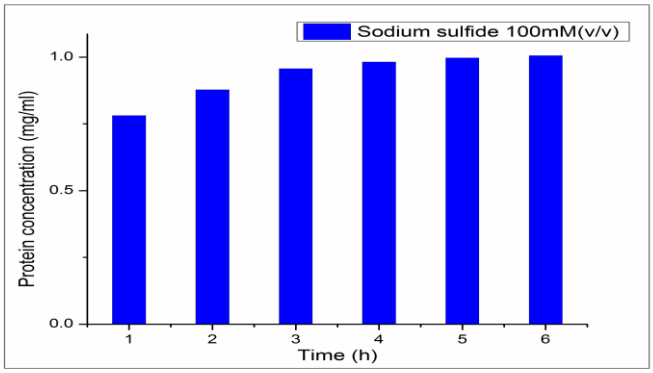

Figure 1: Effect of incubation time on the protein concentration dissolved in 100 mM of sodium sulfide.

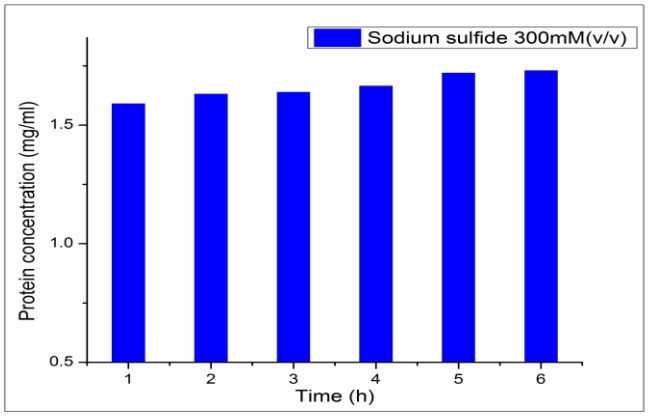

Figure 2: Effect of incubation time on the protein concentration dissolved in 300 mM of sodium sulfide.

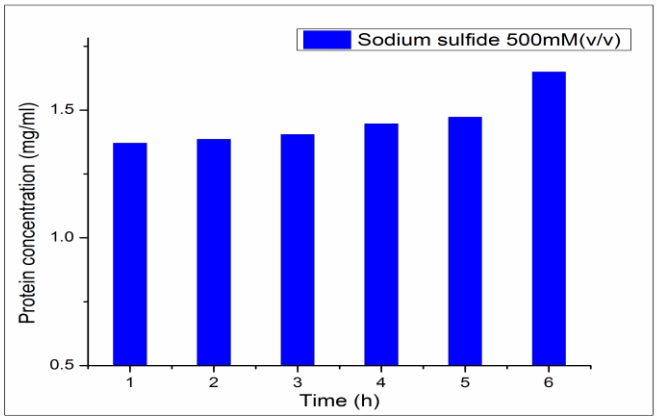

Figure 4: Effect of incubation time on the protein concentration dissolved in 500 mM of sodium sulfide.

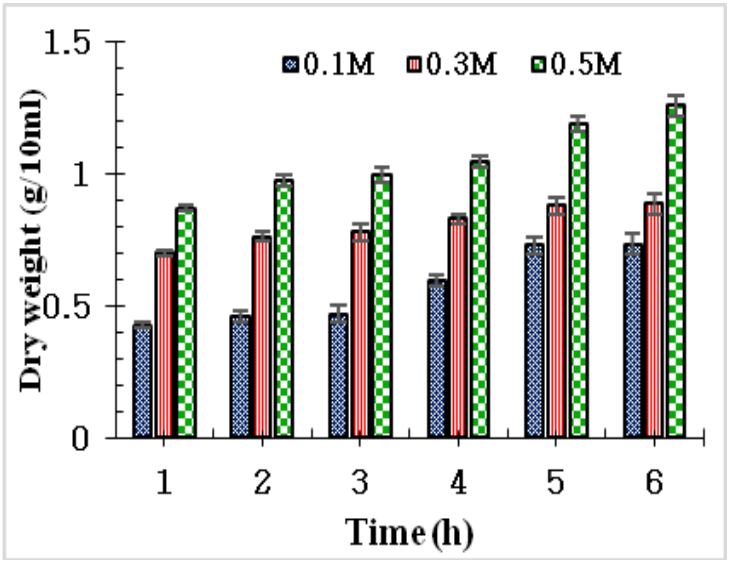

Figure 6: A comparison of dry weight of the protein obtained after each 1 h in different concentrations of sodium sulfide 0.1M, 0.3M, 0.5M (v/v).

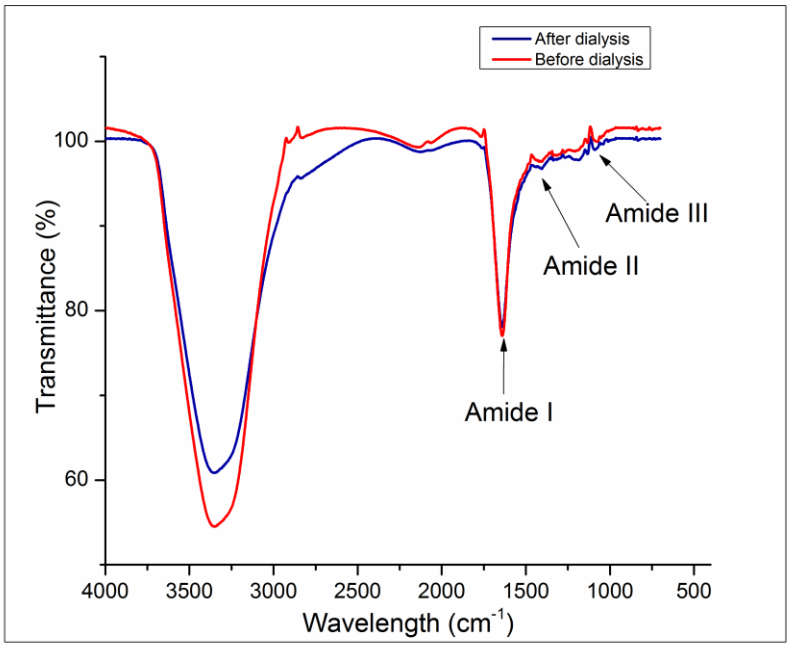

Figure 5: FTIR analysis of the keratin sample before and after dialysis

TGA

The thermal stability and degradation of the extracted keratin was studied by using TGA. As shown in figure 6. The extracted keratin was showing two stages of decomposition started to degrade between 61°C and 267°C and produced a significant amount of residue. There was occurrence of sharp weight loss from 200°C to 400°C, which is associated with helix structure denaturation and skeletal degradation and destruction of chain linkage, peptide bridges [16]. There are several chemical reactions occur which decompose keratin into lighter products and volatile compounds such as CO_2, H_2S, HCN and H_2O [17]. Thermal degradation started at 61°C and become faster above 158°C.

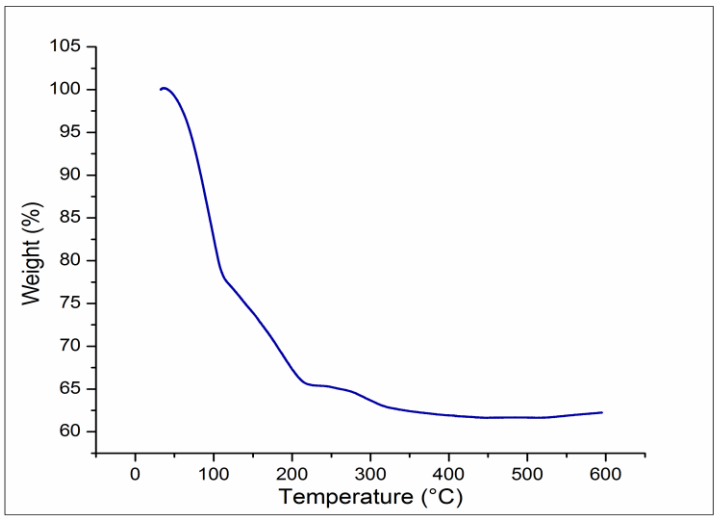

Figure 6. Thermogravimetric analysis of extracted keratin protein

4. CONCLUSION

This study was conducted for the extraction of keratin protein using sodium sulfide which was most appropriate reducing agent and also to optimized the different factors affecting the extraction process then, finally the properties of extracted keratin were studied. The whole process and research plan appears to be economic, scientifically sound and technically strong. The production of keratin and its application for the synthesis of products of commercial value will bridge a gap between academia and industry. We are very hopeful that it will definitely benefit the society, institution as well as play important role to boost up the economic growth of country.

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