

## Separation of Natural Keratin Protein from The Chicken Feather Waste



### Biotechnology

**KEYWORDS :** Chicken feather, sodium sulfide, UV-Vis spectroscopy, FT-IR and keratin

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### ABSTRACT

*Protein is an important nutrient needed by our body to maintain body structures and is an important ingredient for cosmetic products. Chicken feathers have high level of keratin protein content and can become a suitable protein source. So, the present research has conducted to extract keratin protein from chicken feathers. The chicken feathers were reduced using sodium sulfide solution and later on separating the protein from chemicals. The percentage of keratin protein is evaluated by use of biuret test and FTIR analysis. The analysis by FTIR confirmed the presence of carboxyl acid and amino groups in the protein solution. The biuret test helps in determining the concentration of protein obtained from different methods. Thus these two tests confirm the presence of protein in the solution. From this research, it is concluded that keratin protein can be extracted from chicken feathers.*

### 1. Introduction

Enormous quantities of chickens are being consumed every day in the society that produces a large amount of feathers waste in poultry industries. So far, feathers are known to have been chemically and physically prepared to be used as feather meal as well as digestible nutritional protein for animal feed. Over 90% of the dry weight of hair are proteins called keratins, which have high disulfide content, from the amino acid cysteine. Feather keratin shows an elevated content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan (Toshihiro and Daguang, 2008).

Keratin-consisting materials have always been plentiful in the nature but restricted in practical usages, mainly because of their insolubility and non-degradability by the ordinary proteolytic enzymes. New developments of keratinase production have attracted many attentions to apply keratinase in poultry industry. Feathers waste in poultry industries present a high-quality supply of keratins. This valuable source of keratin could be used either as a source of fertilizer glues and films, or many selected amino acids, and proteins which are applied in animal feed industry (Yamauchi and Yamauchi, 2002).

Poultry industry is continuously producing increasing amount of poultry meat and noticeable quantities of organic residues such as feather, bone meal, blood, offal and so on. Chicken feathers, making up about 5% of the body weight of poultry, are a considerable waste product of the poultry industry being produced in world-wide (Martelli et al., 2006). Disposal of waste feathers is a major concern for poultry industry and accumulation of this huge volume of the waste feathers results in environmental pollution and protein wastage. Currently a minor quantity of waste feathers is used in other industrial applications such as clothing, insulation and bedding, producing biodegradable polymers and enzymes and also as a medium for culturing microbes. A higher quantity of pretreated feather is utilized to produce a digestible dietary protein feedstuff for poultry and livestock (Schrooyen et al., 2001).

The reducing agents help in decreasing the stability of keratin fibers in the solid form found in feathers. These reagents will break down disulphide bonds, hydrogen bonds and salt linkages of the keratin fibers in order to dissolve it into protein solution. Currently there is an increasing interest in the development of materials that are environment friendly, obtained from renewable resources. The main renewable materials are obtained from polysaccharides, lipid and proteins. Proteins are polymers formed by various amino acids capable of promoting intra- and inter-molecular bonds, allowing the resultant materials to have a large variation in their functional properties (Arun

et al., 2012).

Keratin was classified into  $\alpha$ - and  $\beta$ -keratins. Feather is mainly made from  $\beta$ -keratin with molecular mass of 10 kDa. On the other hand,  $\alpha$ -keratin consists of two subfamilies, which are acidic keratins with 40–50 kDa (type I) and neutral/basic ones with 55–65 kDa (type II). The cysteine contents of chicken feather and human hair were approximately 7–8% and 17–18%, respectively (Martelli et al., 2006).

Hence, the present research has carried out to be extracting natural keratin protein from chicken feathers by using reducing agent.

### 2. Materials and methods

#### 2.1. Collection of the feather and pre treatment

Chicken feathers were collected from the chicken processing plants at Madippakkam, Chennai. The same were soaked in ether for 24 hr to remove the stains, oil and grease. The feathers are then washed with soap water and dried under sunlight. The dried feathers are then blended and kept carefully in sealed plastic bag.

#### 2.2. Sodium sulfide treatment of chicken feathers

50 g of the blended chicken feathers were weighed and added in to 2 L of 0.5 M sodium sulfide solution, which was prepared in the conical flask. The solution is heated to the temperature of 30°C, pH is maintained in between 10-13 and the solution is continuously stirred upto 6 h. The solution is then filtered and centrifuged at 10,000 rpm for 5 min. The supernatant liquid carefully collected then filtered using filter paper to make it particle free.

#### 2.3. Preparation of ammonium sulfate solution

700 g of ammonium sulfate is dissolved in 1 L deionized water. The solution is stirred until all the ammonium sulfate particles are dissolved. The solution is then filtered to make it particle free.

#### 2.4. Protein precipitation

The feather filtrate solution collected earlier placed in a beaker and stirred. Ammonium sulfate solution is added slowly drop wise. The ratio of feather filtrate solution and ammonium sulfate solution added is 1:1. The solution is then centrifuged at 10,000 rpm for 5 min and the solids particles are carefully collected. The supernatant liquids are collected separately and step 2 and 3 are repeated with it.

#### 2.5. Protein purification

The solid particles collected are added into 100 mL deionized

water and stirred (washing). The solution is then centrifuged at 10,000 rpm for 5 min and the solids are gathered carefully. The collected solid particles are then dissolved in 100 mL of 2 M sodium hydroxide solution. The solution is then centrifuged again at 10,000 rpm for 5 min and all the liquids are collected carefully and stored while the solids are discarded. The precipitating, washing and dissolving steps are repeated 3 times.

### 2.6. Biuret test

1% copper sulphate solution and 1% potassium hydroxide solution are prepared. The 5 mL of the solution collected is mixed with potassium hydroxide solution with 1:1 ratio. Three drops of copper sulphate solution is added to the mixture solution. Changes in the solution observed and recorded.

### 2.7. Protein measurement using UV-Vis and nanodrop

The solution is analyzed under UV-vis to obtain its absorbance and the absorption maxima ( $\lambda_{max}$ ). Further, 2  $\mu$ l of protein solution was loaded on the sample loading region of Nanodrop instrument to measure the concentration of the protein.

### 2.7. Fourier Transform Infrared (FT-IR) spectrometer analysis

The solution collected after purification is analyzed in FTIR and its wavelength graph obtained and compared with the standard graph. The solution fully precipitated using ammonium sulfate, the solids are separated weighed and its weight recorded. FT-IR spectra were recorded on a Fourier Transform spectrometer Perkin-Elmer 2000 with a 4  $\text{cm}^{-1}$  resolution and 16 scans. All spectra were recorded at room temperature. The molar concentration of 1 varied from  $5 \times 10^{-5}$  to  $5 \times 10^{-3}$  M (in saturated solutions) and of models from  $5 \times 10^{-4}$  to  $5 \times 10^{-1}$  M. Depending on concentration of the compound and the IR region, different cells were applied: quartz cells from 1 to 50 mm and KBr cells from 0.066 to 2.66 mm. The  $\nu$  (C=O) frequency region was decomposed with the help of the PEGRAMS program using the mixture of Gaussian and Lorentzian functions.

## 3. Result and discussion

In India, chickens have been consumed several hundred million per year and most of their feather was abandoned as garbage. Therefore, recycling of the feather is desirable as a valuable bio-material resource. The reducing agents help in decreasing the stability of keratin fibers in the solid form found in feathers. These reagents will break down disulphide bonds, hydrogen bonds and salt linkages of the keratin fibers in order to dissolve it into protein solution. Currently there is an increasing interest in the development of materials that are environment friendly, obtained from renewable resources.

The pre treated chicken feather was dissolved completely in the sodium sulfide (0.5 M) solution. This concluded that 0.5 M sodium sulfide could has reducing capability of chicken feather. The presence of protein is confirmed by biuret test. The solution turned purple after reagent is added and this is only possible, if peptides are present in it. The technique was used already by the earlier researchers (Arun et al., 2012) and they confirmed the presence of protein by biuret test. The more peptide bonds are in it, the higher the intensity of the purple colour.

Absorbance is proportional to the concentration of a solution. So increasing the absorbance also shows higher protein concentration. Earlier researchers have observed and reported that the highest absorbance is in sodium sulfide reacted solution and lowest is in the thiglycolic acid reacted solution. The dissolving rate of feathers in thioglycolate solution and potassium cyanide solution is low because the reaction will be highest only if the solution is highly alkaline with pH of the solution is in the range from 10 to 13. This is because in the alkaline state the proton will be removed from the amino group and the ionic bond formed by electrostatic attraction of the  $\text{NH}_3^+$  group of the di-amino acids and the  $\text{COO}^-$  group of the dicarboxylic acids can be broken (Arun et al., 2012).

Due to some reasons ionic bond must be broken first to reduce the disulfide bonds of the keratin and dissolve the feathers. The

sodium sulfide solution is readily alkaline in which sodium hydroxide has to be added to make it alkaline with the pH between 10 to 13. This explains why even though both solutions are reducing agents they cannot reduce the disulfide bonds. Because without alkaline state proton cannot be removed thus cannot break the ionic bond. So, the present research is confirmed that sodium hydroxide plays an important role in dissolving feathers.

Totally 34.8 g of protein was obtained from 50g of chicken feather waste, after the reduction by sodium sulfide (0.5 M). The optimum absorption was detected in between 260-280 nm using UV-Vis spectrophotometer (Fig-1). The protein sample was analyzed using Fourier transform infrared (FT-IR) spectroscopy. The spectrum was matched with the standard graph of the protein solution. The sample graph obtained from the FT-IR is confirmed the presence of C-N bond, N-H bond and C=O (Fig.2). Accordingly, the two groups confirm the presence of amino acids. Hence, the product obtained at the end of the research confirmed true keratin protein without the presence of any foreign materials.

## 4. Conclusion

The present research has carried out to find an alternative source of keratin protein. As, the chicken feathers consist of 90% crude protein and pose an environmental problem due to its time consuming decomposition. In this research, sodium sulfide was used to dissolve the chicken feathers, it mainly depends on the pH of the solution, which is controlled by the amount of sodium hydroxide added to the solution. Ammonium sulfate as expected precipitated protein and at the same time used to purify the protein. The presence of the protein was confirmed by the biuret test and FTIR (fourier transform infrared spectroscopy) analysis. So, the present research will be helpful to recover huge amount of keratin protein from the chicken feather waste. The derived keratin protein can be used for several purposes such as, animal feed, anti-aging cream, shampoo, and conditioner & for medical purposes also.

## Acknowledgment

The authors are thankful to the University Grants Commission (UGC), New Delhi for the financial support of this research through DSK-PDF scheme. Special thanks are extended to the Centre for Biotechnology, Anna University for providing the laboratory and research support.

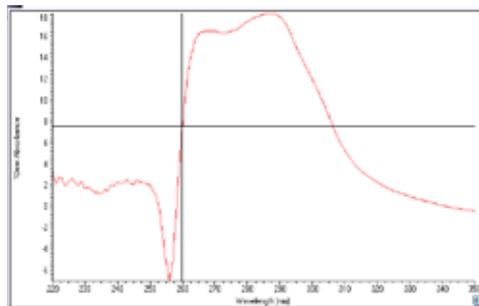


Fig. 1. Measurement of the concentration of keratin using UV-Vis and nano drop

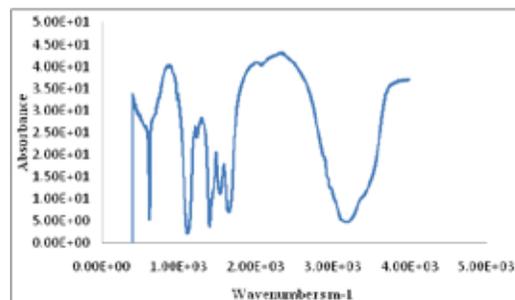


Fig. 2. FT-IR spectrum obtained from the final product (Keratin).

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