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EXTRACTION OF KERATIN PROTEIN FROM WHITE CHICKEN FEATHERS AND THEIR NUTRITIONAL APPLICATION IN SOCIAL WELFARE

Biochemistry

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ABSTRACT

Keratins are insoluble structural proteins with highly crossed-linking, which constitutes the major components of tough protective tissues, such as hair, wool, feather, horn, and nail, and so on. Protein is an important nutrient needed by human body to maintain the body structures and it is a chief constituents for cosmetic products. Chicken feathers having huge amount of keratin protein content and become a suitable protein source. There are two processes that involved in dissolving chicken feathers using different reducing agents and second one separating the protein from chemicals. Reducing agents used are thioglycolic acid and sodium sulphide. Once the feathers are dissolved in reducing agents, ammonium sulfate solution used for the solution of precipitation of protein. The precipitated protein is washed with distilled water for several times and sodium hydroxide solution is used to obtain protein solution form After the methods of precipitation, washing and dissolving the protein solution, the percentage of keratin protein is evaluated by means of biuret test. From this processes, it can be concluded that protein can be extracted from chicken feathers. The keratin protein solution can be used for several purposes such as anti-aging cream, shampoo, and conditioner and for medical purposes such as bone replacement and bone graft.

KEYWORDS

Chicken feather, Reducing agents, Protein precipitation, FTIR, Keratine.

INTRODUCTION

Feathers are produced in huge amount as a by-product in the poultry farm globally.Chicken feathers are bio-resource which constitutes almost 90% protein and produced huge amount as feather waste from poultry processing plant worldwide. Uncontrolled disposal of feathers may cause serious environmental problems, since during incineration of feathers produce huge amount of carbon dioxide (CO_2) as well as landfillings which results the production of toxic substances like hydrogen sulfide (H_2S), ammonia (NH_3)etc. environmentally unacceptable[1].Almost 5% percent of the body weight of chicken constitutes feathers. Approx 2-3 tones of dry feathers produce per day from a slaughterhouse by capacity of 50000 birds. Keratins are insoluble structural proteins with highly crossed-linking, which constitutes the major components of tough protective tissues, such as hair, wool, feather, horn, and nail, and so on[2]. Natural keratin protein extracted from chicken feathers by using various reducing agents. The reducing agents is a biochemical agents which reduces the stability of keratin fibers in the solid form found in feathers. These reagents break down the disulphide bonds, hydrogen bonds and salt linkages of the keratin fibers to dissolve it into protein solution. Proteins are polymers of various amino acids which involved in the formation of intra-molecular and inter-molecular bonds, it provide their functional properties. Feather keratin consists high amount of the amino acids like glycine, alanine, serine, cysteine and valine, while lysine, methionine and tryptophan are present as low constituents. There are various types of reducing agents used in the reduction of disulfide bonds are thioglycolic acid, potassium cyanide, and sodium sulfide. The reductants involved in the production of the proteins without alteration of chemical composition or damage to the protein. Produced solutions are precipitated by the protein precipitants such as sulfosalicylic acid and ammonium sulfate. Keratins produced by chicken feather can be converted to natural protein[3]. These are soluble in alkali or acid and digested by enzyme trypsin and pepsin, since it also breaking the disulfide bonds of the keratin. The β-keratins from feather have β-pleated sheets secondary structure twisted to each other and then it stabilized and hardened by disulfide bonds. So the breaking of disulfide bonds in feathers reduces the strength of the keratin in the white chicken feathers becomes soluble and it can be converted to natural protein. An oxidizing agents such as bromine, permanganate and hydrogen peroxide act very slowly in breaking of disulfide bonds which slow down the process of extraction of protein[4].While the reducing agents reduced quickly and dissolve keratin only at alkaline medium at pH 10-13. Detergent like SDS (Sodium Dodecyl Sulfate) are also involved in aggregation of the polypeptide chains and the rate of oxidation of cysteine residues during dialysis[5]. It has been shown that the extraction of keratins from wool with an aqueous solution of urea, 2-mercaptoethanol and sodium dodecyl sulfate (SDS)[6]. It is found that the sodium dodecyl sulfate (SDS), accelerated the extraction of keratin protein and increased the extraction yield of protein. It also stabilized the aqueous solutions of protein after removal of urea by dialysis against water containing (0.08 wt %) 2-mercaptoethanol. The surfactant forms a complex with the keratin and is removed by dialysis much slower than other low molecular mass compounds. Determination of amino acid sequence of a single polypeptide chain, β-4, from fowl feather barbs[7]. The β-4 chain found to consist of 96 amino acid residues and their molecular weight 10kDa in the form of S-carboxymethylation. It has been studied that the effect of different organic acids on the structure of wool protein[8]. There are two types of keratin, α-keratin and β-keratin. α-keratins are found in the soft tissues protein fibers of sheep wool, hair and skin. It is twisted together and formed like a rope strand. α-keratins are rich in cysteine amino acids while poor in hydroxyproline and proline amino acid sequences. While β-keratins are found in the hard tissues protein fibers such as bird feathers, nails, fish scales etc. The beta-keratins amino acid sequencesis rich in small uncharged glycine and alanine while poor in cysteine, proline and hydroxyproline. The resulting products of hydrolysis of keratin have also proven that, it is useful for the production of fertilizer, cosmetics, biomedicals (decontamination), textiles (fibers modification), Bioactive hydrolysates[9].

Table 1: The Amino Acid Composition of Chicken Feathers

Amino Acid	µM/mg Protein*1	% Amino Acid in Feather Keratin
Aspartic Acid	0.351	4.36
Threonine	0.335	4.05
Serine	1.282	13.47
Proline	0.775	1.01
Glutamic Acid	0.624	9.9
Glycine	1.003	7.57
Alanine	0.412	3.66
Valine	0.617	7.23
Cystine	0.078	2.11
Methionine	0.017	0.025
Isoleucine	0.326	4.73
leucine	0.50	7.25
Tyrosine	0.102	1.85
Phenylalnine	0.267	4.11
Lysine	0.039	0.57
Histidine	0.001	0.016
Arginine	0.377	6.57

*Based on sample as 100% protein (1Micro mole per milligram of protein) Keratin is insoluble in water and organic compound. The

chemical properties of keratin are weak acids and bases. It is characterised by cystine content in the sequence of keratin amino acids and it can be hydrolysed, reduced and oxidized. High strength of keratin is influenced by the two cysteine molecules bonded by disulphide bonds digit. Keratin protein is useful in the formation of anti-wrinkle treatment cream, sulfite hair straightener, conditioning shampoo and other personal care[10].

MATERIAL AND METHODS

Collection of feathers

White Chicken feathers were collected from chicken processing plants and kept in ethanol for 24 hr. During this process feathers were cleaned from stains, oil and grease etc. Then washed with the soap water of feathers simultaneously dried under sunlight. After that the dried feathers are then blended and kept carefully in sealed plastic bag.

Dissolving of chicken feathers

500mL of 0.5M sodium sulfide solution was prepared in a 1000mL conical flask. 12.5g of the grinded chicken feathers was added to the sodium sulfide solution. The solution was incubated at temperature 35°C and pH between10-13 and the solution was continuously stirred for 6 hours on rotary shaker. Then the solution was filtered and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected carefully and filtered by using whatman filter paper no.1 to make it particle free.

Preparation of ammonium sulfate solution-70% $(NH_4)_2.SO_4$

700g of ammonium sulfate was dissolved in 1000ml deionized water. The solution was stirred until all the ammonium sulfate particles were dissolved. The solution is then filtered to make it particle free.

Protein precipitation

The filtrate feather solution was collected in beaker and stirred it. Add ammonium sulfate solution gradually 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 10 minutes and the solids particles were carefully collected separately and repeat this steps 2-3 times.

Protein purification

The solid particles collected and added into 100ml distilled water and mixed it properly with magnetic stirrer (washing). The solution is then centrifuged at 10,000 rpm for 10 minutes and the precipitates was collected carefully. The collected solid materials were then dissolved in 100ml of 2M sodium hydroxide solution. Then solution was centrifuged again at 10,000 rpm for 10 minutes and all the supernatant were collected carefully and stored while the solid precipitates are discarded. These steps repeatedly 2-3 times.

Biuret test

The determination of protein concentration is an essential technique in all aspects of protein studies and proteomics.To prepared 1% copper sulfate solution and 1% potassium hydroxide solution. The 5ml of the collected solution was mixed with potassium hydroxide solution with 1:1 ratio. Then 3-4 drops of copper sulfate solution was added to the mixture solution. Physical changes in the solution was observed and recorded. The solution was analyzed under uv-vis to obtain its absorbance at 540nm. The whole process repeated with 0.5M thioglycolate solution and solution replacing the sodium sulfide solution. Thioglycolate prepared with 0.1N sodium hydroxide.

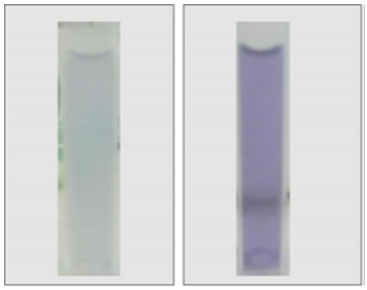
RESULTS AND DISCUSSION

It has been shown that white chicken feathers was dissolved completely in sodium sulfide solution, where as partially dissolved in thioglycolate solution. Almost 80% feathers in thioglycolate solution can be filtered out after 6 hours of reaction. It can be concludes that sodium sulfide reduces chicken feather efficiently than the other reducing agents.The presence of protein is confirmed by biuret test. The solution turned purple after reagent is added and this is only possible if peptide bonds are present in it. The more peptide bonds in it, the higher the intensity of the purple color as seen in figure 1. Also the difference between the purple colors of the different solutions is visible seen in figure 1. This is in accordance with absorbance of biuret solution and amount of protein obtained in the end of the research. The higher the amount of chicken feather dissolved the higher the protein obtained table 2.

Absorbance of Biuret Test Solutions

Table 2: Absorbance of biuret test solutions and amount of protein obtained

PROTEIN SAMPLES	ABSORBANCE@ 540nm	AMOUNT OF PROTEIN
1.Thioglycolate Solution	0.322	4.2
2.Sodium Sulfide Solution	1.321	6.3



Sample 1 Sample 2

Figure 1 Biuret solutions of all two samples.

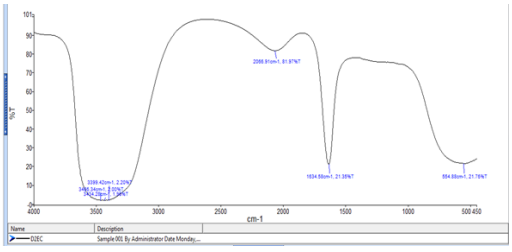


Figure 2: FTIR RESULT OF CRUDE KERATINE

The FTIR (fourier transform infrared spectroscopy) also confirmed the presence of amino and a carboxyl group in the sample, the two groups confirms the presence of amino acids. The secondary structure of keratin was also analyzed using FTIR spectroscopy. Thus the product obtained at the end of the research confirmed true keratin protein without the presence of any foreign materials. Absorbance is proportional to the concentration of a solution. So increasing the absorbance also shows higher protein concentration. It was observed 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 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 NH_2^+ group of the diamino acids and the COO- group of the dicarboxylic acids can be broken. Due to some reasons these 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 not like thioglycolate solution or potassium cyanide solution 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 sodium hydroxide plays an important role in dissolving feathers. So in order to maximize the dissolving ability of thioglycolate solution the exact amount of sodium hydroxide need to be calculated.

The total mass of the protein obtained by using different reducing agents are as follows sodium sulfide (53%), potassium cyanide (29.6 %) and thioglycolic acid (8.8%).This is in accordance with the result of the UV-Vis analysis. The protein sample also analyzed using Fourier transform infrared spectroscopy. The graph obtained matched the standard graph of protein solution. The wavelength obtained from the infrared spectroscopy confirmed C-N bond, N-H bond, and C=O bond are in the sample thus the presence of carboxyl group and amino groups the two groups that will only present in amino acids are undeniable. composition of amino acid in table1 Therefore the sample confirmed true protein.

FACTORS AFFECTING THE SOLUBILIZATION OF KERATIN

It has been shown that Na_2S (sodium sulfide) under highly alkaline

conditions (pH 13) was used for the solubilization during extraction. The blended feathers were dissolved in Na_2S at diferent concentrations 150, 300 and 550 mM. The feathers fractions showed maximum solubility with 550 mM Na_2S after 6 h and It has been shown that there were no changes after 6 h. At lower concentration 150 and 300 mM, the feathers biomass was partially dissolved with rachis and most of the undissolved feathers portion remains in the beaker. The solubility of feathers was attributed to breakage of disulfde bonds hydroxyl ions in basic medium digit. The dissolved samples of feathers were precipitated using NaOH. The effect of diferent amount of NaOH used on the precipitation of keratin is shown in Table 2

Table 2 Quantitative effect of NaOH on the dissolution of the extracted Keratin

SL.NO.	Keratin powder (mg)	2 N NaOH added (ml)	Consistency
1.	500	2	Very thick
2.	500	5	Mild thick
3.	500	10	Thick
4.	500	15	Diluted
5.	500	20	More diluted

DISCUSSION

Different concentrations of glycerol (2–10%) was mixed with extracted keratin solution to produce plastic films and then investigated for its characterization. It is an important to improve the strength of the film; thus, it will open a new gateway for the research on bioplastics using waste biomass. During the process of extraction it was observed that the extracted keratin solution when kept at room temperature had a tendency to aggregate. It is found that the sodium dodecyl sulfate (SDS), accelerated the extraction of keratin protein and increased the extraction yield of protein. It also stabilized the aqueous solutions of protein after removal of urea by dialysis against water containing (0.08 wt %) 2-mercaptoethanol. The surfactant forms a complex with the keratin and is removed by dialysis much slower than other low molecular mass compounds. This study have been reported that the extraction of keratin from biomass of chicken feather waste using alkaline hydrolysis and optimize the process of extraction. The extracted keratin has been characterized to study its physical and chemical properties. Finally, the keratin particles were used to develop a bioplastic film using microcrystalline cellulose as a nanoadditive. The properties of the regenerated film were evaluated to validate the industrial potential of keratin powder.

CONCLUSION

During this research it can concluded that the keratin protein are alternative alternative source of nutrition as well as industrial applications. Since chicken feathers consist of 90% crude protein and it is major environmental problem due to its time consuming decomposition, it is an ideal material to obtain keratin protein. The chicken feathers were first dissolved using reducing agents and protein precipitated out from the solution. Chicken feathers can also to be use for the production of fuel. Finally we provided an efficient method to utilize the waste biomass of poultry industry.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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