



Alkali Enzymatic Extraction of Keratin Protein from Chicken Feather Waste in Bangladesh

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ABSTRACT

Keratin is a highly specialized fibrous protein, which is found in feathers, hair, wool and nails. Bioremediation of these waste materials is an issue requiring serious attention regarding environmental concern. In the present research work keratin protein is extracted from poultry chicken feather which is now treated as valueless product of poultry processing plants. Although it contains high keratin protein content, this byproduct is dumped continuously into the environment without further treatment. Millions of tons of chicken feathers are produced every year from poultry industry of Bangladesh which disposed off into environment without any industrial treatment. This protein rich valuable byproduct can be recycled and utilized effectively which has great economic and ecological importance. In this study, alkali-enzymatic hydrolysis was done for the extraction of keratin protein from feather. Desired result was found in reflux condensation system which yielded 76.2% protein hydrolysate compared to conventional hot plate hydrolysis which was yielded 52.63%. Detection and concentration of protein were determined by Biuret and Kjeldahl methods respectively.

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INTRODUCTION

The poultry industry in Bangladesh commercially growing rapidly early in 1990 due to its cheapest source of animal which is well accepted by all groups of people. This industry mainly produces chicken as well as other species like duck, pigeon, goose and small quantity of quail and guinea fowl. More than 60 lakh people are involved in the poultry industry in Bangladesh. The number of poultry firms were reduced to 55,000 in 2013 from 1,15,000 in 2007 due to the hit bird flu. In 1934 the government took initiatives to introduce improved breeds which brought the changes in the poultry industry and it continued with the crossbreeding between local and exotic poultry stocks until 1960 [1-2].

The poultry industry produces large amounts of waste that include solid waste such as waste feather, feed, abattoir waste and wastewater [3]. Feather waste which is a keratin protein resulting in huge quantities as byproduct from this industry and is regarded as important industrial waste [4-5]. An estimated over 65 million tons of feather waste are produced worldwide and feather waste contain about 90% keratin protein [6], 2-5 % Sulphur [7], 8% water and 1% lipid [8]. The structure of feather consists of a cuticle, cortex and medulla and the diameter varies from 40 to 150 μm [9]. Keratins are insoluble proteins [10] which belong to the scleroprotein groups extremely resistant to the action of

chemical, physical, and biological agents. Mechanical stability and high resistance to degradation of keratin are due to their hydrogen bonds, disulfide bonds, salt linkages and cross linkings [5]. Feathers constitute about 10% of total chicken weight [12]. At present, most waste feathers are disposed of as waste by landfilling in Bangladesh which leads to environmental pollution. As a protein rich byproduct this valuable waste can be recycled and utilized effectively which has great economic and ecological importance. Keratin protein of feather has potential industrial applications. It can be used in leather tanning in beam house operation, animal feed, organic fertilizer, adhesives, biomaterials and others [6-8]. Keratin is also an important raw material in the medical, cosmetic, pharmaceutical, and biotechnological industries.

Keratin hydrolysate (reduced, soluble keratin) from poultry feather can be prepared through alkaline [13-14], acid [15] and enzymatic treatment [16]. Goddar et al. [17] have studies the effect of acid, alkali and enzyme to convert the feather waste into natural protein. This was done by breaking of disulfide bonds of the keratin. The protein extraction process was slowing down as oxidizing agents such as bromine, permanganate and hydrogen oxide act very slowly during the breaking of disulfide bonds. In the presence of alkali, reducing agents act very quickly and dissolve the keratine but alkali alone does not dissolve it [17]. Combustion or land filling of feather waste does not contribute any benefit

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rather it brings negative impact on the environment having higher demand of energy and CO₂ production [18]. In order to ensure sustainable management of chicken feather waste, now importance are given to extract the keratin protein hydrolysate from poultry industries waste [19]. In this study, conventional hotplate as well as reflux hydrolysis using alkali and enzyme were carried out to extract the keratine hydrolysate from chicken feather waste. Poultry processing plant generates large amounts wastes and valuable by-products is shown in Figure 1. Both edible and non-edible wastes are produced where feathers are major contributors of non-edible by-products.

MATERIALS AND METHODS

Raw material

Waste chicken feathers were supplied from local broiler house in Savar area, Dhaka. These were first washed with excess amount of tape water to remove stains, oil, dirt, blood and other impurities and dried in open air. Finally washed feathers were again dried in oven at 60-70°C for 24 hours. Then the dried waste feathers were cut into small pieces and kept carefully in sealed plastic bag.

Reagent, glassware, and apparatus

Sodium hydroxide, potassium hydroxide, and protease enzyme were used purchased from the local market. All the reagents used in this study were of analytical grade. Glassware's (Pipette, Beaker, Conical flask, measuring cylinder, Test tube, etc) used were of Borosil/Ranken. Magnetic hotplate, stirrer, reflux condenser, Kjeldahl apparatus, etc., were used.

Experimental procedure

Keratins are abundantly available as by-products mainly from poultry plants and slaughterhouses in the form of feathers,

hair, horns, hooves, and claws, etc., which have been discarded without proper utility (Figure 2). These by-products can be utilized by several chemical methods such as acid treatment, alkaline treatment, enzymatic treatment and combination of these chemicals to achieve cleavage of the disulfide bonds [11]. Alkaline and alkaline-enzymatic hydrolysis of chicken feathers are utilized in this research work. Flow chart of preparation of keratin hydrolysate from chicken feather is shown in Figure 3.

NaOH hydrolysis

10 g of cleaned and dried chicken feather was dissolved in an aqueous solution of sodium hydroxide. The hydrolysis was conducted with temperature not above 70-80°C and the hydrolysis duration was 4 hours using hotplate and stirrer. After the hydrolysis was completed, the hydrolysate was solidified in a magnetic hotplate to reduce the volume.

KOH hydrolysis

Cleaned and dried chicken feather of 10 g was taken and dissolved in an aqueous solution of potassium hydroxide. The hydrolysis was conducted with temperature not above 70-80°C and the hydrolysis duration was 4 hours using hotplate and stirrer. After the hydrolysis was completed, the hydrolysate was solidified using a magnetic hotplate to reduce volume.

Combined NaOH and KOH (N-K) hydrolysis

In this hydrolysis process, a combination of both NaOH and KOH was used. 10 g of cleaned and dried chicken feather was dissolved in an aqueous mixed solution of sodium hydroxide and potassium hydroxide. The hydrolysis was conducted with temperature, not above the 70-80°C and the hydrolysis duration was 4 hours using hotplate and stirrer. After the hydrolysis was completed, the hydrolysate was solidified using a magnetic hotplate to reduce volume.

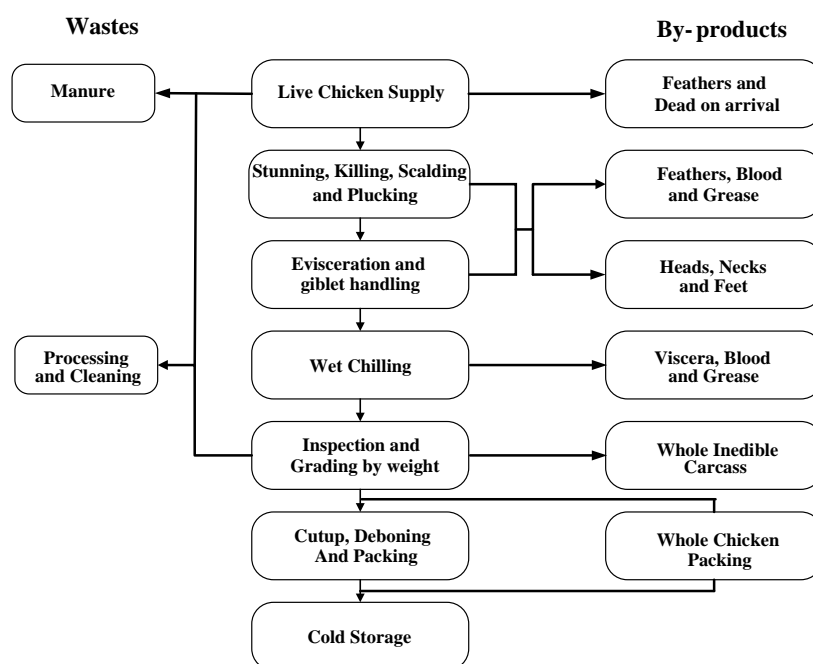


Figure 1. Wastes generated from a poultry processing plant [19]



Figure 2. Waste chicken feather; a) Feather waste in slaughterhouse and b) Cleaned and dried feather waste

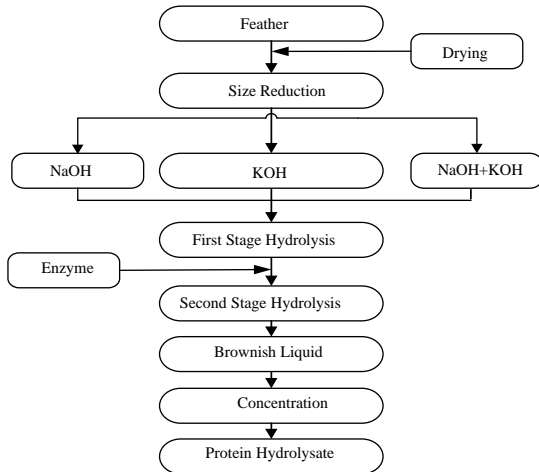


Figure 3. Flow chart of preparation of keratin hydrolysate from chicken feather

Alkaline-enzymatic hydrolysis

The alkali concentrations which gave the maximum protein content in hotplate hydrolysis were used in the alkali-enzymatic process using a reflux condenser. After the hydrolysis was completed, the hydrolysate was solidified using a magnetic hotplate to reduce volume.

Analysis

Protein of the extracted hydrolysate was identified by the biuret test and protein concentration was determined by the Kjeldahl method in a digester Gerhardt (Germany). Functional groups of protein hydrolysate were determined by FTIR.

a) Biuret test

The Biuret test is based on the ability of Cu(II) ions to form a violet-colored chelate complex with peptide bonds (-CONH-groups) in alkaline conditions. This test confirms the presence of proteins in the sample. In this test, 2 ml of extracted hydrolysate solution was taken in a dry test tube. Added 3 drops of 10% NaOH and 3-6 drops of 0.5% CuSO₄ to the sample test tube [20].

b) Kjeldahl method

In this work, 0.5g of hydrolysate sample was taken in a Kjeldahl flask and digested with 15 ml concentrated sulfuric in the presence of a mixture of Na₂SO₄ and CuSO₄ in ratio of 5:1. The digested solution was neutralized with 40% NaOH

and distilled into a 4% boric acid solution. The borate anions formed was titrated with 0.5M H₂SO₄, which was converted to nitrogen in the sample [20].

Yield

The percentage of yield can be derived from the following equation [21]:

$$\text{Percent yield} = (\text{Actual yield} / \text{Theoretical yield}) \times 100 \quad (1)$$

where actual yield is the amount of product obtained from hydrolysis, and theoretical yield is 90% [6].

RESULTS AND DISCUSSION

FTIR

The FTIR spectrum of keratin protein from chicken feather waste in the region 500-4000 cm⁻¹ is shown in Figure 4. The absorption bands that appeared for the hydrolysate are mainly assigned to the peptide (-CONH-). The bands that originate in the vibration of peptide bonds are amide I-III. The peaks at 1689.64, 1543.05 and 1107.14 cm⁻¹ are indicating the vibrations known as amide I, II and III [13].

Biuret and Kjeldahl methods

Figure 5 shows the change of color of the extracted hydrolysate from grey (a) to purple color (b) after the addition of biuret reagent. This change of color represents the presence of proteins in the extracted hydrolysate.

Protein concentrations (crude protein) of the extracted hydrolysates of various methods were determined by the Kjeldahl method, that are shown in Tables 1-3.

Moisture content

About 3g of waste feather was taken and then cut into small pieces for analysis of moisture content. Moisture content was found 11.05% using moisture meter (Shimadzu MOC63u).

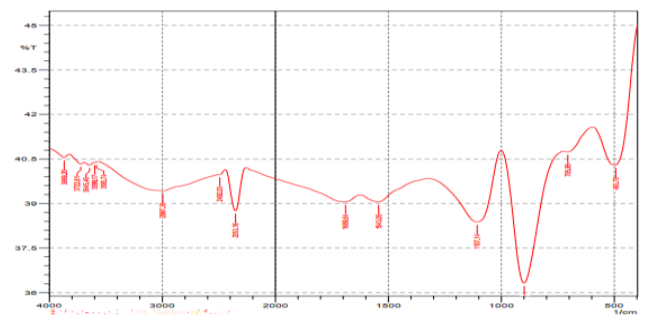


Figure 4. FTIR spectrum of chicken feather hydrolysate

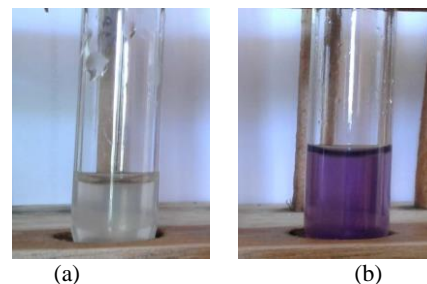


Figure 5. Biuret test; a) before the addition of biuret reagent, b) after addition of biuret reagent

TABLE 1. Crude protein analysis by Kjeldahl method (single, hot plate); Sample weight 10g

Chemicals (g)		Temperature (°C)	Time (h)	Protein (%)
NaOH	KOH			
0.6	-	75-80	8	24.32
0.8	-	75-80	8	14.27
1.0	-	75-80	8	27.83
1.2	-	75-80	8	33.51
-	0.6	75-80	8	27.83
-	0.8	75-80	8	32.43
-	1.0	75-80	8	23.61
-	1.2	75-80	8	23.61

Alkaline hydrolysis (Hot plate system)**a) NaOH hydrolysis**

Figure 6 shows the effect of NaOH concentration on feather hydrolysate. The NaOH concentrations were varied from 6 to 12% based on featherweight. Kjeldahl method of protein

analysis shows that the maximum crude protein was achieved at 12% concentration of NaOH. About 33.51% protein was extracted at 12% concentration. The lowest protein extraction was obtained at 8 % concentration of NaOH which was only 14.27%.

b) KOH hydrolysis

Figure 7 shows the effect of KOH concentration on feather protein hydrolysate. The KOH concentrations were varied from 6 to 12% based on featherweight. After an 8% concentration of KOH, the percentage of protein extraction was decreasing determined by the Kjeldahl method. The highest protein extraction has occurred at 8% concentration which was 32.43%.

Combined NaOH-KOH (N-K) hydrolysis

Figure 8 shows the effect of combined NaOH-KOH (N-K) concentration on feather protein hydrolysate determined by the Kjeldahl method. Here combined concentration ratios were used as 1:1, 1:2 and 2:1 of 6, 8, 10 and 12% concentration of N-K respectively. The highest protein extract 44.91 was found from 10% (1:1 = 5% NaOH + 5% KOH)

TABLE 2. Crude protein analysis by Kjeldahl method (combined NaOH: KOH hydrolysis, hot plate); Sample weight 10g

Concentration (%)	Chemicals	Ratio		Temperature (°C)	Time (h)	Protein (%)
6	NaOH: KOH	0.3	0.3	75-80	8	31.07
	NaOH: KOH	0.2	0.4	75-80	8	26.73
	NaOH: KOH	0.4	0.2	75-80	8	32.83
8	NaOH: KOH	0.4	0.4	75-80	8	44.91
	NaOH: KOH	2.66	5.34	75-80	8	23.39
	NaOH: KOH	5.34	2.66	75-80	8	33.12
10	NaOH: KOH	0.5	0.5	75-80	8	23.96
	NaOH: KOH	3.33	5.34	75-80	8	31.05
	NaOH: KOH	5.34	2.66	75-80	8	32.80
12	NaOH: KOH	0.6	0.6	75-80	8	23.48
	NaOH: KOH	0.4	0.8	75-80	8	23.10
	NaOH: KOH	0.8	0.4	75-80	8	21.99

TABLE 3. Crude protein analysis by Kjeldahl method (alkali-enzymatic, reflux hydrolysis); Sample weight 10g

Enzyme (Proteolytic)		% of Chemicals (Based on Feather wt.)	Temperature (Alkaline hydrolysis) (°C)	Time (Chemical hydrolysis) (h)	Enzyme Ratio (%)	Temperature (enzymatic hydrolysis) (°C)	Time (enzymatic hydrolysis) (h)	Protein (%)
Chemical	Ratio							
NaOH: KOH	1:1	10	75-80	8	1	50-60	5	55.71
NaOH: KOH	1:1	10	75-80	8	2	50-60	5	76.2
NaOH: KOH	1:1	10	75-80	8	3	50-60	5	58.60
NaOH: KOH	1:1	10	75-80	8	4	50-60	5	34.38
NaOH: KOH	1:1	10	75-80	8	5	50-60	5	41.17
NaOH: KOH	2:1	12	75-80	8	1	50-60	5	60
NaOH: KOH	2:1	12	75-80	8	2	50-60	5	40
NaOH: KOH	2:1	12	75-80	8	3	50-60	5	43
NaOH: KOH	2:1	12	75-80	8	4	50-60	5	40
NaOH:KOH	2:1	12	75-80	8	5	50-60	5	42

combined N-K concentration and the lowest protein extract 21.99 was found from 6% (2:1= 4% NaOH+ 2% KOH) combined N-K concentration.

Alkaline hydrolysis (Reflux hydrolysis)

The NaOH-KOH concentration of combined hydrolysis that yielded the maximum protein hydrolysate was used in the reflux system. Figure 9 shows the increase of protein extract in the reflux system than the conventional combined hot plate system. Here N and K represent the NaOH and KOH respectively. The highest percentage of protein determined by the Kjeldahl method was 52.63 found at 10% of combined alkaline hydrolysis which ratio was 1:1N and K respectively. The lowest percentage of protein 33.34 was obtained at 6% combined N-K hydrolysis and the ratio was 1:1N and K respectively.

Alkaline-enzymatic Hydrolysis (Reflux system)

Alkaline-enzymatic reflux hydrolysis yields a better percentage of crude protein among other hydrolysis methods. Figure 10 shows the Alkaline-enzymatic Hydrolysis using the reflux system and % of crude protein was determined by the Kjeldahl method. In the first stage of alkaline hydrolysis, a 10% combined 1:1 (N-K) concentrated alkaline solution was added which was the highest crude protein yield in alkaline hydrolysis. In the second stage, 1 to 5% concentration of proteolytic enzyme was used for enzymatic hydrolysis. Among them, 2% concentration gave the highest protein percentage which was 76.20%. After that crude protein percentage was gradually decreasing. Also, according to Equation (1), percent yield is equal to 84.67%.

Hot Plate hydrolysis, reflux hydrolysis, and final product of chicken feather hydrolysate are shown in Figure 11.

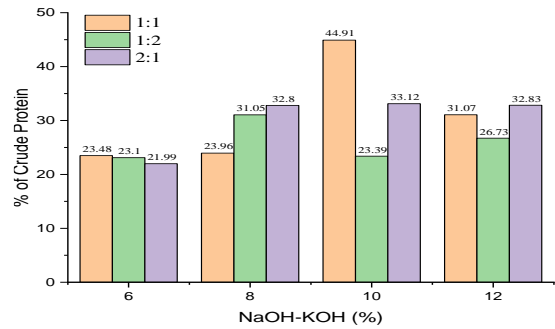


Figure 8. Effect of combined NaOH-KOH (N-K) concentration on % of Crude Protein

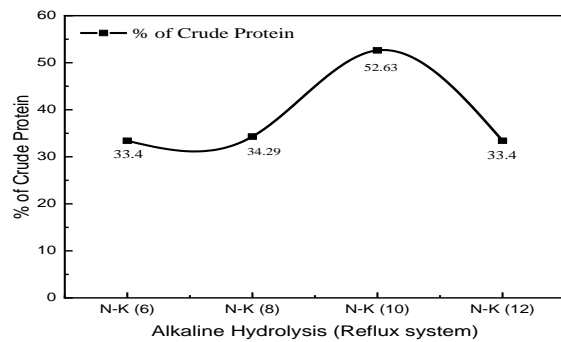


Figure 9. Effect of Alkaline Hydrolysis (Reflux Hydrolysis) on % of Crude Protein

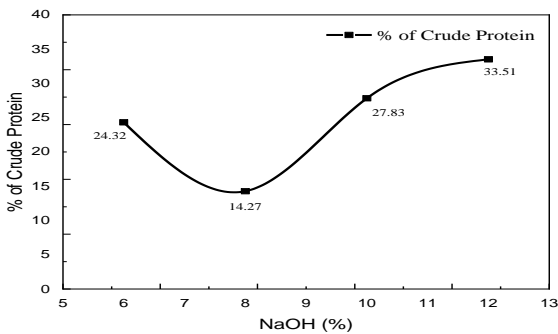


Figure 6. Effect of various NaOH concentration on % of Crude Protein

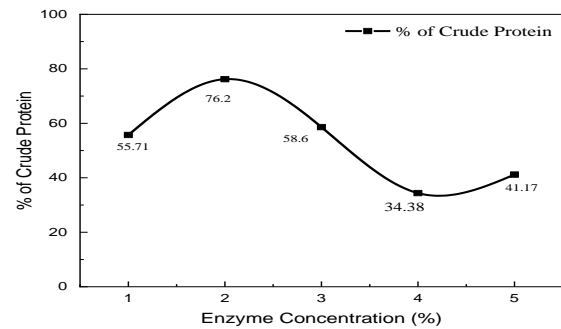


Figure 10. Effect of Alkaline-enzymatic Hydrolysis (Reflux system) on % of Crude Protein

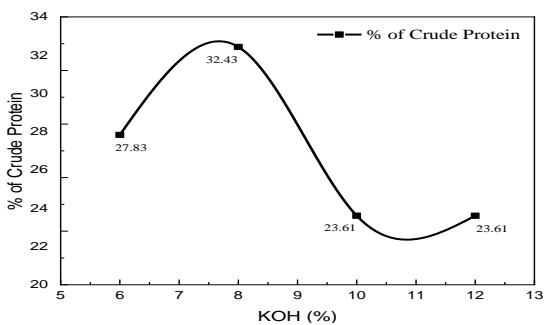


Figure 7. Effect of KOH concentration on % of Crude Protein

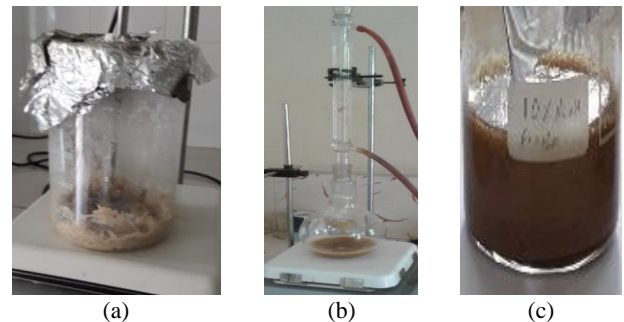


Figure 11. a) Chicken feather hydrolysis hot plate, b) Reflux Hydrolysis process of chicken feather, and c) Final product

APPLICATIONS OF CHICKEN FEATHER HYDROLYSATE

Hydrolyzed keratinous material contains 20 amino acids with irregular structure, it can be used as slow-releasing nitrogen fertilizer livestock feed, biodegradable films, coating, glues, and others. By interacting with cosmetics keratin helps in retaining moisture in the skin as a result keratin hydrolyzate has a huge application in cosmetic industries [14-20]. Keratin protein solution can be used for medical purposes like bone replacement and bone graft [23].

Keratin based materials can be served as raw material in the production of wound healing agents, regenerated films, building materials, compostable packaging materials, wood adhesives, porous foam, mats, biomaterials, gels and feather meal [7, 10, 24].

Keratin hydrolysate (2-3%) is used in tanning with chromium sulfate during leather processing, which increases the exhaustion of chromium in tanning bath more than 90%. In chrome tanning, water-soluble keratin peptides reactions completed in two steps. Firstly, the low molecular weight peptides react with chromium and finally, collagen in leather reacts both with free chromium and chromium-keratin complex. At very high temperatures and pressure, chicken feathers can be converted into water-soluble peptides in an autoclave by the reaction with lime and sodium hydroxide. As a result, a retanning- cum- filling agent has been employed [25]. Due to the presence of fictional groups on the backbone and side chains, keratin proteins are useful as adsorbent for the removal of toxic pollutants[26]. Feather could be a valuable raw material for making lightweight composite used automobile and aviation industry [27].

After all, the collected chicken feathers are converted into biodegradable plastics by polymerization reaction. Herein, the waste feathers are formed into fine powder and then amalgamated simultaneously to form long-chain keratin molecules. Finally, this mold is heated at 170°C to give various shapes. To prepare Technical nonwoven textile and to improve metal ion removal capacity, chicken feather fiber was treated chemically [28].

CONCLUSION

Millions of tons of chicken feathers are generated every year from the Bangladesh poultry industry. Improper disposal of this waste creates a solid waste problem in the environmental. This waste can be hydrolyzed to keratin protein under alkaline-enzymatic treatment. The optimum conditions were found 10% combined NaOH-KOH (1:1, 5% NaOH, 5% KOH) solution by reflux condensation which yielded 76.20% crude protein. If this value-added by-product of the poultry industry can be utilized in a proper way then it will bring benefits to our economy as well as the environment.

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Persian Abstract

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چکیده

کراتین یک پروتئین فیبری بسیار خاصی است که در پرها، مو، پشم و ناخن‌ها یافت می‌شود. تجمع زیستی این مواد زائد موضوعی است که نیازمند توجه جدی به دغدغه‌های زیست محیطی است. در پژوهش حاضر، پروتئین کراتین از پر مرغ طیور استخراج می‌شود که اکنون به عنوان محصول بی‌ارزش واحدهای فرآوری طیور مورد استفاده قرار می‌گیرد. اگرچه حاوی پروتئین کراتین بالایی است، اما این فرآورده به طور مداوم و بدون هیچ تصفیه بیشتر در محیط دفع می‌شود. میلیون‌ها تن پر مرغ هر ساله از صنعت طیور بنگلادش تولید می‌شود که بدون هیچگونه تصفیه صنعتی در محیط زیست دفع می‌شود. این محصول با ارزش پروتئین غنی از پروتئین قابل بازیافت و استفاده موثر است که از اهمیت اقتصادی و اکولوژیکی زیادی برخوردار است. در این مطالعه، هیدرولیز قلیایی آنزیمی برای استخراج پروتئین کراتین از پر انجام شد. نتیجه مطلوب در سیستم تراکم رفلاکس مشاهده شد که ۷۶/۲ درصد پروتئین هیدرولیز پروتئین را در مقایسه با هیدرولیز معمولی صفحه داغ که به میزان ۵۲/۶۳ بود، به دست آورد. شناسایی و تعیین غلظت پروتئین به ترتیب به روش Kjeldahl و Biuret تعیین گردید.
