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Purification And Characterization Of Keratin From Chicken Feather

Kiruba M[1], Anandhi K[2] and Shanmugavadivu M[*]

PG Student, Department of Biotechnology, Dr.NGP Arts and Science College, Coimbatore, Tamilnadu, India.

[*]Associate Professor, Department of Biotechnology, Dr.NGP Arts and Science College, Coimbatore, Tamilnadu, India

Abstract: Keratins are proteins that form hard fibres, present in wool, horns, hoofs and feathers etc. In the poultry industry, the feathers constitute up to 10% of total chicken weight. Uncontrolled disposal of feathers from the poultry slaughterhouses is troublesome to environment. Primary purification phase is necessary for isolating the keratin from other materials. The purification was conducted by washing with distilled water, detergent and petroleum ether and lastly again with water. Reducing agent used were sodium sulphide. Keratin is precipitated and purified from the chicken feather using ammonium sulphate. The amount of Keratin protein in extract was calculated by Lowry's method. Presence of Keratin was identified with the help of SDS PAGE and it was used to determine the molecular weight. Keratin protein was characterized using FTIR. The analysis of FTIR confirmed the presence of chemical compositions such as carboxyl and amino groups in the protein samples. Keratin protein solution was used in cosmetic product and, especially for hair care products such as detergents, shampoos, conditioners etc.

Index Terms - **Keratin, Protein, Chicken Feather, Cosmetics, Keratin Shampoo**

I. INTRODUCTION

Keratin is a scleroprotein which is fibrous in nature. In vertebrates it presents in epidermally on body parts like scales, horns, fur, feather, hooves, hair etc. Nowadays, the increased consumption of poultry products has resulted in an increase in waste of about 8.5 billion tons of feather, which is created from the 24 billion chickens consumed annually. Due to the outdated and expensive disposal methods for feathers, there is a global environmental issue with feather waste. [Alashwal, *et al.*, 2019] On the other hand, feathers are a cheap, environmentally friendly and abundant source of protein (Keratin) that can be used in a variety of applications, including biomedical and cosmetic industries. Chicken feather keratin differs from other keratin in that it can stretch to approximately 6% of its length before breaking, whereas hair keratin can double in length [Ma B, *et al.*, 2016]. Recently discovered straightening creams can be used to treat frizzy, or wavy hairs. A variety of hair straightening compositions consisting of creams, shampoos, and conditioners are

currently available, but to produce a better and safer formulation, the correct composition must be selected [Suguna Jeganathan, 2015]. The keratin hydrolysates are used in a variety of cosmetic applications on the face and hair. The keratin peptides increase the hydration in the hair and give it luster and softness. They have a hydrating effect on the skin and are used in skin and hair moisturizer. [Barba C, *et al.*, 2008]. Keratin helps use of its products by improving their thermal and mechanical properties of high compatibility with hair and water. Keratin-based protein has biological evaluation and strengthening properties on relaxed hair. Shampoos containing keratin, strengthens human hair, repairing damaged hair and preventing further breakage [Atikah Bt Mad Nawi, 2013].

II. MATERIALS AND METHODS

2.1 Collection of Chicken Feather:

The country chicken feather sample was collected from a local farm in Coimbatore, Tamil Nadu.

2.2 Pretreatment of Chicken Feather:

The collected feather sample was then subjected to pre-treatment by first washing it with 70% ethanol. It was then soaked in petroleum ether for 24hrs, the sample was washed with distilled water and then air dried in a hot air oven at 40℃ for 3hours.The chicken feather was then weighted and powdered [Ramakrishnan N, *et al*.,2018]

2.3 Extraction of Keratin from Chicken Feather:

From the powdered chicken feather, 10g was weighed and 19.51g of sodium sulphide was added and made up to 500ml with distilled water. The mixture was kept for incubation in magnetic stirrer for 6hrs at 30℃. The solution was filtered and centrifuged at 10,000 rpm for 10mins at 30℃. The obtained solution was again filtered using a filter paper to collect the supernatant Arun Gupta, *et al.*, 2012.

2.4 Precipitation of protein using Ammonium sulfate:

70% of Ammonium Sulfate was dissolved in 500ml distilled water. The solution was stirred until all the ammonium sulfate particles were dissolved. The solution was then filtered to make it particle free [Schrooyen, P, *et al.*, 2001]. The chicken feather solution was then allowed to precipitate completely with 70% Ammonium Sulphate solution for 10mins at 10,000rpm. The pellet was collected and was allowed to dry in petri-dish at room temperature. The pellet was then grind using mortar and pestle to a fine powder. The powder was collected in Eppendorf tubes and stored.

2.5 Quantification of Crude Protein from Chicken Feather:

The amount of keratin protein in crude extract was estimated by Lowry's method. BSA was taken as the Standard. 0.1ml of the crude extract and 0.1ml of the keratin powder was taken as the test sample. BSA was taken in the concentration of 200µg, 400µg, 600µg, 800µg. [Vimalraj, *et al*., 2022]

2.6 Elution of Protein by Column Chromatography:

A cylinder-shaped glass column containing stationary phase (sephadex) was encountered slowly from the top with a liquid solvent (mobile phase). Keratin powder was dissolved in phosphate buffer (Mobile phase) that flew down the column with the help of gravity or external pressure applied. This technique was used for the purification of compounds from a mixture. Once the column was ready, the sample was loaded inside the top of the column. Keratin powder should be mixed with sephadex to make a fine powdered form for easy

distribution of sample in already packed sephadex gel column. Sample powdered mass should place on the top of the pre-packed sephadex column and sample should be covered with a layer of cotton. The mobile solvent was then allowed to flow down through the column. The compounds in mixture have different interaction ability with stationary phase (sephadex), and mobile phase, thereby would flow along the mobile phase at different time intervals or degrees. Each fraction was collected separately in a test tube and numbered consecutively for further analysis [Wolf Berthold and Joachim Walter, 2002].

2.7 Molecular Weight Determination of Keratin by Using SDS-PAGE:

SDS-PAGE technique was performed to confirm the keratin in crude extract and keratin precipitate powder. The percentage of SDS-PAGE gel is 10% [Xiao-chun Yin, *et al.*, 2013].

2.8 Fourier Transform Infrared (FTIR) Spectroscopy:

FTIR is useful for validating the identity of pure substances. The method is based on locating functional groups in molecules that vibrate when exposed to particular light wavelengths (either through stretching or bending in different ways). An FTIR spectrum is created by plotting the frequency of light (cm^{-1}) that the sample is exposed against the vibrations' intensity (% transmission) Daeid N.N 2019.

III. Formulation of Shampoo:

Shampoo was formulated using simple mixing process [Finn, R.K, 1959] of Keratin powder with other ingredients as given in the table:

Table 1: Composition of shampoo

S. No	Ingredients	Quantity	Uses
1.	Keratin powder	300mg	To prevent the hair fall
2.	Sodium Lauryl Sulphate	0.5 g	Surfactant
3.	Hydroxy Propyl Methyl Cellulose	1.5g	Thickening agent
4.	Glycerine	5ml	Viscosity Enhancer
5.	Methyl Paraben	0.18g	Preservative
6.	Almond oil	1ml	Fragrance, Antibacterial agent
7.	Distilled water	Up to 25 ml	Vehicle

IV. Pharmaceutical Evaluation of Shampoo:

The shampoo formulation was evaluated for different pharmaceutical parameters including physical appearance, determination of pH, consistency and foam stability.

4.1 Physical Appearance:

The formulated shampoo was observed for their visual appearance, transparency, colour, consistency [Vijayalakshmi *et al.,* 2018].

4.2 Determination of pH:

The pH of formulated shampoo was determined by using digital pH meter by dissolving 1gm shampoo in 100ml of water [Badi & Khan., 2014].

4.3 Consistency:

The consistency of formulated shampoo was determined by hand. A pinch of shampoo was taken and rubbed it with finger [Vijayalakshmi *et al.,* 2018].

4.4 Foam Stability Test:

The stability of foam was determined by using cylinder shake method. About 10ml of 1% formulated shampoo was taken in 50 ml measuring cylinder and shaken for 10 minutes. The total foam volume was measured after 1 minute and foam stability was determined by recording foam volume from 1 to 4 minutes [Patil *et al.,* 2015].

V. Result and Discussion:

5.1 Collection of Chicken Feather Sample:

The country chicken feather sample for the extraction of keratin was collected from a local poultry farm Coimbatore, Tamil Nadu

5.2 Extraction of Keratin from Chicken Feather:

The 10g of chicken feather was soaked in the 500ml sodium sulphide solution. Sodium sulphide reduced the chicken feather effectively. Quantitative identification of protein in the precipitate was confirmed by Lowry's Method. The solution turned purple after reagent was added and this was only possible if peptide bonds were present in it. In Sodium sulphide method, a yield of about 50%, based on the starting weight of the feather was also obtained when chicken feather was dissolved completely in sodium sulphide solution. Whereas the chicken feather was dissolved partially in potassium cyanide and thiglycolate. Around 45% of feathers in potassium cyanide solution and 70% feathers in thioglycolate solution could be filtered out after 6hrs of reaction. This concludes that sodium sulphide reduced the chicken feather efficiently than the other two reducing agents. It was observed that the dissolving ability of thioglycolic acid and potassium cyanide mainly depended on the pH of the solution [Gek Kee Chua, *et al.,* (2012).

5.3 Ammonium Sulfate Purification:

70% Ammonium sulfate in 350ml of crude extract was centrifuged at 10,000rpm for 10 mins. The pellet was collected and ground to fine powder using mortar and pestle. The total amount of precipitated keratin powder is 3g. Hence the total yield is 30%. Ammonium sulfate as expected, precipitated the protein and at the same time used to purify the protein. It was also confirmed that the 700g of Ammonium sulfate was

dissolved in 1litre of distilled water. This solution was added in 1:1 ratio to feather filtrate solution, which yielded 53% keratin powder [Arai, K. M, *et al.,* 1983].

5.4 Identification and Quantification of Protein in Chicken Feather:

To identify and quantify the keratin protein, Lowry's Method was used. The amount of keratin protein in the sample was estimated using a standard curve of a selected standard protein solution such as BSA. The reading was taken at 660nm. It was found that the precipitated crude extract contained 0.51mg/ml of protein and Keratin powder contained 0.31mg/ml of protein. It was also noted confirmed that the presence of protein was confirmed by biuret test. In that method the total amount of protein was estimated of about 26.5g [Arun Gupta, *et al.,* 2012].

5.5 Elution of Keratin Using Column Chromatography:

Sephadex was mixed with buffer then column chromatographic elution was done using buffer. The composition of the fractions was collected during column chromatographic separation. Totally 6 fractions were collected and named as F1, F2, F3, F4, F5 and F6.

5.6 Confirmation of Protein in Fraction:

In the Fractions, the presence of protein was confirmed qualitatively

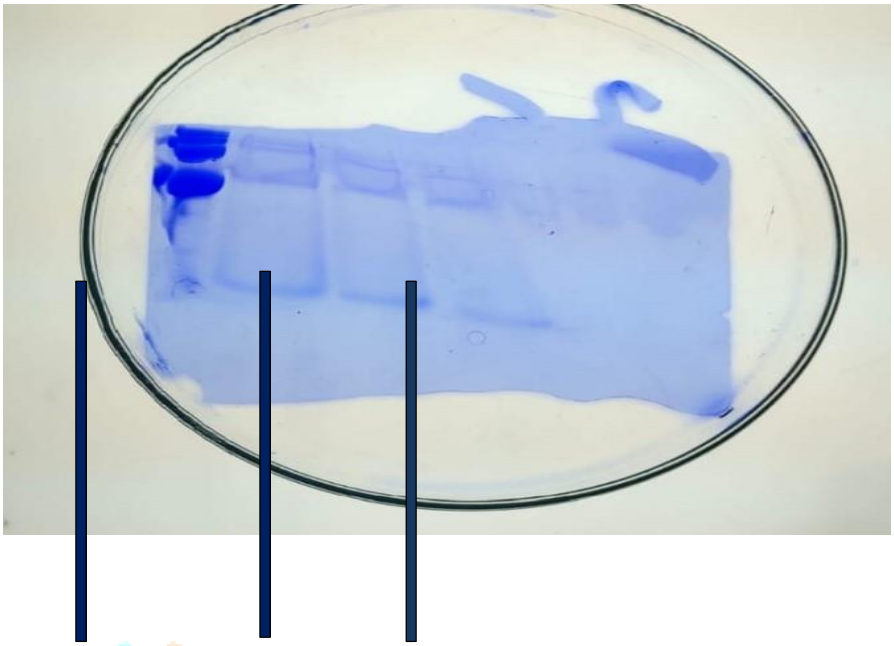
Keratin powder	F1	F2	F3	F4	F5	F6
Protein [ninhydrin]	-	+	+	+	-	-
Million's reagent	-	+	+	+	-	-

Table 2: Confirmation of keratin protein in Column Fraction

5.7Molecular Weight Determination of Keratin by Using SDS-PAGE:

The molecular weight of the chicken feather crude extract and keratin powder sample was determined by SDS-PAGE. The keratin can be determined by the bands obtained in the lane. The molecular weight of chicken feather keratin range approximately 20KDa. The protein marker is BSA (Bovine Serum Albumin). It was confirmed that the microfibril keratin range is 20-36 kDa. The result showed the molecular weight of extracted keratin is agreed with MALDI –TOF MS [Xiao-chun Yin, *et al.,* 2013].

Fig 1: SDS-PAGE



BSA Crude Extract Keratin Powder

5.8 FTIR characterization of keratin powder from chicken feather:

The samples were carried out in Shimadzu spectrometer with Agilent Micro lab Suite. Frequencies of absorbed radiation are detected by an infrared spectrometer, and a plot of absorbed energy against frequency, called 'infrared spectrum'. It was confirmed that the C-N bond, N-H bond, and C=O bond confirmed the presence of carboxyl group and amino groups, the two groups that will only present in amino acids [Bellisola and Sorio, 2012].

Obtained Peak	Peak Position	Group	Class	Peak Details
3286.70	3200 – 3550	O-H Stretching	Alcohol	Strong, Broad
	2500 – 3300	O-H Stretching	Carboxylic acid	Strong, Broad
	3267 – 3333	C-H Stretching	Alkyne	Strong, Sharp
1635.64	1680	C=O Stretching	Secondary amide	Strong
	1680	C=O Stretching	Tertiary amide	Strong
	1650	C=O Stretching	8 - Lactam	Strong
	1626 – 1662	C=C Stretching	Alkene	Medium
	1600 – 1650	C=C Stretching	Conjugated alkene	Medium
	1580 – 1650	N–H bending	Amine	Medium
	1566 – 1650	C=C Stretching	Cyclic alkene	Medium

1072.42	1000 – 1400	C–F Stretching	Fluoro compound	Strong
	1020 – 1250	C–N Stretching	Amine	Medium
	1050 – 1085	C-O Stretching	Primary alcohol	Strong
678.94	550 – 850	C-Cl Stretching	Halo compound	Strong
	665 – 730	C=C Bending	Alkene	Strong
	515 – 690	C-Br Stretching	Halo compound	Strong
610.79	550 – 850	C-Cl Stretching	Halo compound	Strong
	515 – 690	C-Br Stretching	Halo compound	Strong
555.50	550 – 850	C-Cl Stretching	Halo compound	Strong
	515 – 690	C-Br Stretching	Halo compound	Strong
	500 – 600	C-l Stretching	Halo compound	Strong

Table 3: Functional group analysis of the F2 and F3 fraction of the extract of the keratin power from FTIR Spectroscopy

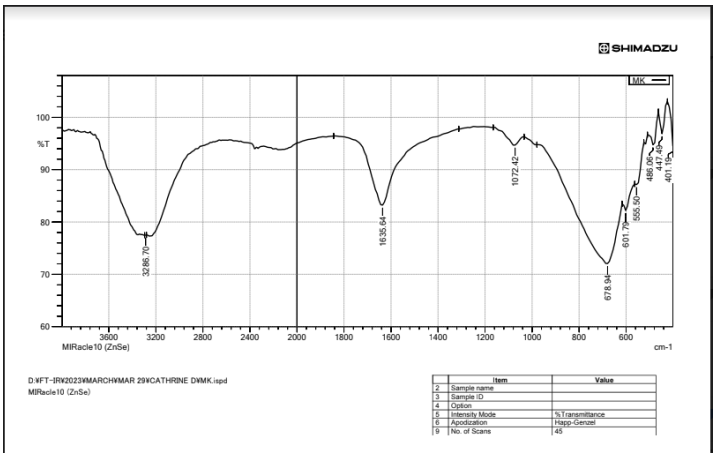


Fig 3: FTIR Result

5.9 Formulation of Shampoo

Shampoo was formulated using a simple mixing process. Shampoo was formulated by adding the required amounts of other ingredients

5.10 Evaluation of Formulated Shampoo

Physical appearance:

The prepared shampoo showed good characteristics in terms of appearance on the visual inspection of the formulation. The appearance was viscous by nature. Shampoo is typically in the form of a viscous liquid with some exception of waterless solid form such as a bar.

Colour:

Without the use of any colouring agents, the shampoo came out to be a natural White colour.

Transparency:

The formulated shampoo was transparent and had good odour. The hair got benefits from transparent shampoo in a number of ways, including enhanced softness, better manageability, and less frizz. It was the perfect product for people with dry or damaged hair because it aids in detangling the hair and reducing breakage.

Determination of pH:

The pH of the shampoo resulted at 6.86, which is important for improving and enhancing the quality of hair. Minimizing irritation to the eyes, and stabilizing the ecological balance of the scalp.

Foam Test:

Few drops of shampoo were taken in my hand and rubbed with water. 1ml of shampoo was dissolved in 9ml of distilled water and shaken vigorously. The obtained foam remained for 2-3minutes.

Consistency:

The consistency of the formulated shampoo was smooth. The choice of material to be utilised in shampoo formulation is heavily influenced by the needed consistency of the finished product.

VI. Conclusion:

Chicken feather was the by-product of the poultry industry and slaughterhouse. Environmental issues are caused by chicken feathers. Extraction of keratin from waste materials is a beneficial process and also environmentally friendly. Purified keratin after quantification could be used in the formulation of anti-wrinkle treatment cream, sulfite hair straightener, conditioning shampoo and other personal care products. Keratin shampoo was made from Chicken Feather. The presence of protein was confirmed by Lowry's method. Molecular weight determination of keratin was done SDS-PAGE and FTIR spectra produced ideal results.

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