A

PROJECT REPORT ON

ISOLATION AND CHARACTERIZATION OF KERATIN PROTEIN FROM CHICKEN FEATHERS

SUBMITTED BY,

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SUBMITTED TO,

VIVEKANAND COLLEGE,KOLHAPUR (AUTONOMOUS)

DEPARTMENT OF BIOTECNOLOGY.

FOR PARTIAL FULFILMENT OF BACHELOR OF

SCIENCE IN BIOTECHNOLOGY

THE YEAR

2021-2022

UNDER THE GUIDANCE OF

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Assistant Professor, Department Biotechnology

"EDUCATION FOR KNOWLEDGE SCIENCE AND CULTURE"

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SHRI SWAMI VIVEKANAND SHIKSHAN SANSTHA'S

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Department of Biotechnology

Certificate

This is to certify that Shailesh Appa Budake

Exam Number 9307 has satisfactorily carried out his project report as per the syllabus prescribed by Bo'S Department of Biotechnology, Vivekanand College (Autonomous) for B.Sc- III Biotechnology (Entire).This project report represents his bonafied work during academic year 2021-2022.

Place: Kolhapur

Date: 21/5/22.

27/5/22

Project Guide

28/05/2022

Examiner

VIVEKANAND COLLEGE ESTD. JUNE 1964 KOLHAPUR-416 003

Head

Head

Department of Biotechnology (Entire)
Vivekanand College, Kolhapur (Autonomous)

DECLARATION

I hereby declare that the project work entitled "Isolation & Chararacterization of Keratin protein from chicken feathers" submitted to the Vivekanand College. Kolhapur for the award of the degree of "Bachelor of Science in Biotechnology is the result of bonafied work carried out by me under the guidance of Asst/Prof Mr. A.L. Upadhye .

I further declare that the results presented here have not been the basis for the reward of any other degree

Place:- Kolhapur

Date: 26/05/2022

Mr. Shailesh Appa Budake.

Acknowledgment

This project work is a successful outcome of the contribution and guidance of other person which express my deep gratitude.

I also express our thanks to Prof. Mr. S.G. Kulkarni Head of Department of Biotechnology, Vivekanand College, Kolhapur for availing me with the laboratory facilities to the Biotechnology Department to carry experiment work.

I also express our gratitude towards Prof. Mr.A.L.Upadhye my project guide for his guidance and who gave me encouragement and support throughout the course of study so that could complete my project work.

I also wish to express my gratitude to the laboratory staff for completing the project work. Lastly, express my gratitude to my parents, all our friends and classmates for their support and co operation. I am also grateful to all those who have directly or indirectly supported me in completion of this work.

Mr. Shailesh Appa Budake

INTRODUCTION :-

The present research work is regarding extracting natural keratin protein from chicken feathers by using different reducing agents. 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.

Feathers are bio-resource with high protein content more than 750 g per kg crude protein, Poultry slaughterhouses produce large amounts of feathers. Further, burning feathers in special installations is economically ineffective. Uncontrolled disposal of feathers is environmentally unacceptable. Five percent of the body weight of poultry is feathers; the slaughterhouse with a capacity of 50,000 birds, can easily produce 2-3 tones of dry feathers per day.

The keratin from feather shows an elevated content of the amino acids glycine, alanine, serine, cysteine and valine, but lower amounts of lysine, methionine and tryptophan, Reducing agents used in this research for reduction of disulfide bonds are thioglycolic acid. potassium cyanide, and sodium sulfide. The reductants act very quickly and without bringing about any chemical alteration or damage to the protein yield. Products prepared from the solutions behave as true proteins, and not as products of hydrolysis. Theirsolutions are precipitated by ordinary protein precipitants such as sulfosalicylic acid, and ammonium sulfate.

The ability to flex in multiple directions without tearing is an important quality of keratin and it also provides a tough and fibrous matrix in tissues chicken feather keratins can be converted to natural protein soluble in alkali or acid and digestible by trypsin and pepsin. This was accomplished by breaking the disulfide bonds of the keratin. The B-keratins from feather have B-pleated sheets twisted together, and then stabilized and hardened by disulfide bonds. So by breaking these disulfide bonds in feathers, the strength of the keratin in the chicken feathers can be reduced thus become soluble and converted to natural protein.

As for oxidizing agents such as bromine, permanganate and hydrogen oxide act very slowly in breaking the disulfide bonds thus slowing down the protein extraction process. But in contrast, the reducing agents act very quickly and dissolve: keratin only at alkaline reaction (pH 10 to 13), but the action is not due to alkali alone. Products prepared from these reactions behave as true proteins and not as products of hydrolysis.

Few other previous researchers have worked on the extraction process of keratin. Researchers have studied the effect of the addition of various quantities of SDS on the rate of aggregation of the polypeptide chains and the rate of oxidation of cysteine residues during dialysis.

There are two types of keratin, alpha-keratin and beta-keratin. Alpha-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. Alpha-keratins amino acids sequences rich in cysteine and poor in hydroxyproline and proline. Beta-keratins are found in the hard tissues protein fibers such as bird feathers, nails, fish scales and others. The beta-keratins amino acids sequences are rich in small uncharged glycine and alanine and poor in cysteine, proline and hydroxyproline.

Keratin is insoluble in water and organic compound. The chemical properties of keratin are weak acids and bases. It is characterized by cystine content in the sequence of keratin amino acids and it can be

hydrolysed, reduced and oxidized. High strength ofkeratin is influenced by the two cysteine molecules bonded by disulphide honds. Keratin protein fraction is used in the formulation of anti-wrinkle treatment cream, sulfite hair straightener, conditioning shampoo and other personal care

▪AIM :- Isolation of Keratin protein from chicken feathers and its biochemical characterization.

▪OBJECTIVES :-

- To isolate Keratin protein
- Characterization with help of Thin Layer Chromatography
- FTIR analysis of extracted protein sample

MATERIALS AND METHODS

▪MATERIALS :-

Chicken feathers,Distilled water .

Glasswares:- silica gel plate ,glass rod,measuring cylinder ,flasks,test tubes.

Chemicals :- Amonium sulphate ,Diethyl ether,Na2SO4,Biuret reagent .

Equipments:- Test tube stand,Centrifuge,micropipette,filter paper.

METHODS:-

Step 1:- Pre-treatment of feathers

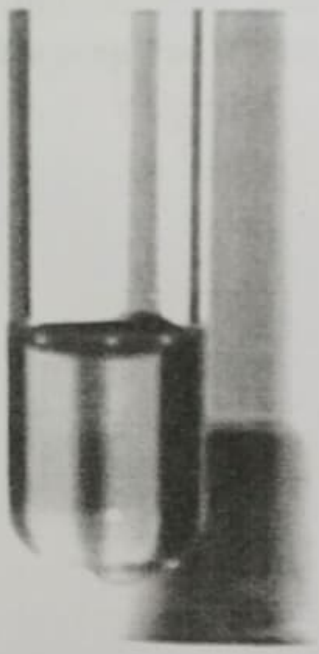
Chicken feathers are collected from chicken processing plants and soaked in ether for 24 hr. The main purpose is to clean the feathers from stains, oil and grease before processing it. The feathers are then washed with soap water and dried under sunlight. The dried feathers are then blended and kept carefully in scaled plastic bag.

Step 2:- Dissolving of Chicken Feathers

2 L of 0.5 M sodium sulfide solution is prepared in a 2 L conical flask. 50 g of the blended chicken feathers are weighed and added to the sodium sulfide solution. The solution is heated to the temperature of 30 °C, pH is maintained about 10-13 and the solution is continuously stirred for 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.

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. the reagent changed to purple colour in presence of peptide bonds, changes in the solution observed and recorded.



Analysis of the Sample:-

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.

The whole process repeated with 0.5 M thioglycolate solution and 0.5 M potassium cyanide solution replacing sodium sulfide solution. Both thioglycolate and potassium cyanide solutions are prepared with 0.1 N sodiumhydroxide.

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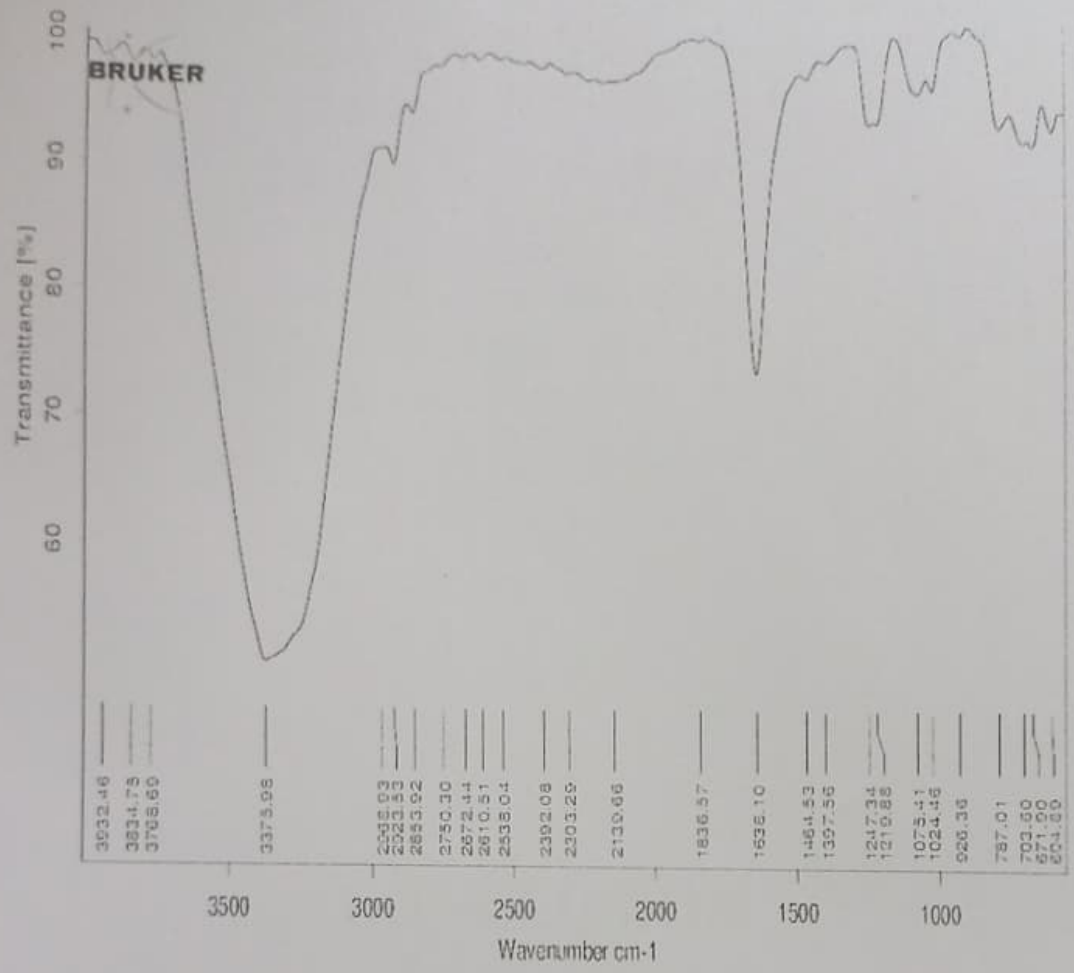

D:\FTIR DATA\VCK\Alin Pathan\KERATIN Extract.0	KERATIN Extract	Instrument type and / or accessory	5/25/2022

CONCLUSION:-

1]Thus we conclude that,the isolation of keratin protein from chicken feathers and its biochemical characterization was performed with the help of Biuret reagent ,where the reagent changed to purple colour in presence of peptide bonds.

2]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. Thus the product obtained at the end of the research confirmed true keratin protein without the presence of any foreign materials.