

Extraction of keratin from chicken feather and Spectral Characterization of protein by FTIR.

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Abstract:

The present study investigates the use of poultry and slaughterhouse waste product i.e. chicken feathers which are responsible for different environmental problems. Chicken feather is a good source of keratin which can be used in Cosmetics products as a source of protein. Collected samples soaked in petroleum ether for 24 hours to remove bloodstains and dust, dissolved chicken feather in sodium sulfide, protein precipitated by Ammonium Sulfate, and Protein purification done by dialysis bag. Qualitative analysis was done with the Biuret method and quantitative estimation of protein Folin (1951). The analysis lyophilized sample of FTIR confirm the characteristic peak was seen at 3417 cm^{-1} which indicates the presence of amide a group and suggesting the presence of Cysteine, Glutamic acid, Tryptophan, and Amide III respectively Thus it can be concluded from the FTIR analysis that the keratin protein of chicken feather is showing characteristics of secondary structure of proteins.

Keywords: Keratin, chicken feathers, FTIR, protein extraction, fibre.

Introduction:

Chicken feather is the by-product of poultry industry and slaughterhouses, which act as an environmental pollutant, degradation rate of chicken feathers is very slow causes many environmental issues. In world, five billion tons of chicken feather scrap is generated every year which disposed of by burning, landfill, and fodder supplements. (Vineis et.al. 2019). Chicken feathers have unique structures and properties not found in any natural or synthetic fibres and feather from other avian species. The low density, excellent compressibility and distinctive morphological structure of feather barbs make them unique fibers. Keratin is one of the important structural proteins (McKittrick et. al.2012). Keratin found in human and animal organs, presents disulfide bonds which are responsible for its chemical stability and high mechanical strength (Shavandi et. al.2017). Chicken feather is made of 90% keratin which may be a good source for cosmetics, shampoo, and hair treatment creams. (Alahyaribeik et. al.2020). Bird feathers are good source of keratin. According to a recent report, India's contribution alone is 350 million tons. The poultry feathers are dumped, used for landfilling, incinerated, or buried, which involves problems in storage, handling, emission control, and ash disposal. (Revathi et. al. 2015).

Materials and methodology

Chicken feathers were collected from Vishnupuri village, chicken feather dumping ground near to Swami Ramanand teerth Marthawada University, Nanded. Feathers soaked in petroleum ether 24 hours to remove dust and bloodstains. After 24 hours feathers were washed with soap water, dried in sunlight, blended, and kept in a sealed polythene bag. Extraction of keratin from chicken done by Gupta et. al. method i.e.-10 gm. of blended chicken feather added to 200ml of 0.5N sodium sulfide solution, solution stirred 6 hours up to compete for dissolve of blended feather with the help of magnetic stirrer, temperature maintained at 40o C and pH in between 10-13. This solution was filtered and Centrifuged at 10,000 rpm for 5 Minutes For protein precipitation 70% Ammonium sulfate and added dropwise, 50ml feather filtrate solution and 50 ml of 70% ammonium sulfate solution, centrifuged 10000 rpm for 5 min and supernatant was collected (Gupta et al. 2012).

For the process of protein purification, Dialysis bag was boiled in 2 % Sodium bicarbonate solution for 10 min. Then activated dialysis membrane was again boiled in distilled water for 10 min, and dipped into Ethanol solution. After addition of sample, the dialysis bag was sealed stirred on magnetic stirrer overnight in buffer (dialysate) at room temperature.

Qualitative analysis was done with the Biuret method and quantitative estimation of protein Folin (1951). The Fourier Transform Infrared Spectroscopy spectrum (FTIR) for detection of functional groups in sample was performed. FTIR spectra of keratin were recorded from 400 to 4000 cm^{-1} using a Shimadzu FT-IR spectrophotometer. For solid-state measurement, a pellet was prepared by mixing lyophilized keratin with Potassium Bromide.

Result and discussion

The extracted sample was analyzed for qualitative analysis by Biuret reagent and the results showed a positive test for purple color. Subsequently, the quantitative analysis of the keratin protein was used for the determination of protein content by Folin Lowry and the amount of protein was calculated from the standard graph. The amount of protein in the feather keratin was found to be 0.205 mg/ml. In the present study, the protein was extracted rapidly from chicken feathers. The amount of protein obtained from present study was then compared with the observations of bovine hoof reported by Kakkar et.al (2014).

The FTIR analysis of lyophilized sample powder revealed the presence of keratin in chicken feather The FTIR spectrum shows the characteristic peak at 3417 cm^{-1} Kakkar et al. (2014) analyzed the FTIR spectra and found characteristics peaks in between 1600-1700 cm^{-1} which exhibited the α and β confirmation of amide I. Observed characteristic peaks in the range of 1500-1560 cm^{-1} to confirm the presence of amide II conformation. These results conform with the present investigations. Similarly, the FTIR peaks at 1951 cm^{-1} , 1556-1587 cm^{-1} , 1311 cm^{-1} , and 1228-1251 cm^{-1} correspond to the previous studies of Andres (2000).

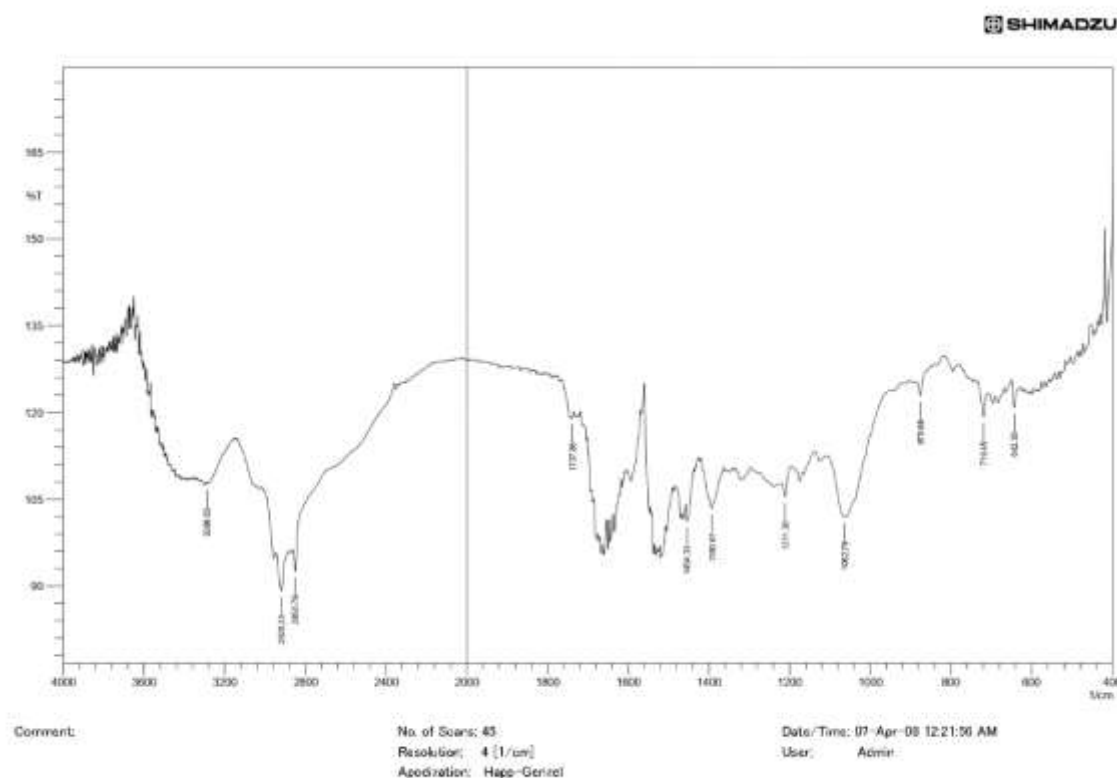


Fig. FTIR result of lyophilised final product.

The results were obtained after subjecting the lyophilized sample to FTIR analysis. It is evident from the figure that the characteristic peak was seen at 3417 cm^{-1} which indicates the presence of amide a group. The figure also illustrates the presence of α and β conformation of the extracted protein and the Peaks were seen 1600-1700 cm^{-1} . Some of the peaks were are also observed at 1951 cm^{-1} , 1311 cm^{-1} , 1556-1587 cm^{-1} , and 1228-1251 cm^{-1} suggesting the presence of Cysteine, Glutamic acid, Tryptophan, and Amide III respectively. Thus it can be concluded from the FTIR analysis that the keratin protein of chicken feathers is showing characteristics of secondary structure of proteins.

Conclusions

The present work primarily deals with keratin derived from chicken feathers. Since chicken feathers consist of 90% crude protein, the feather waste can be utilized for rapid extraction of keratin with different solvents of which Sodium Sulphide was the optimum choice due to its reliability to dissolve the protein. Hence Sodium Sulphide was used to obtain the desired protein present from chicken feathers.

The crude sample was then subjected to Ammonium Sulphate precipitation and dialysis and the results demonstrated that the amount of protein although was quantitatively estimated, protein content was low due to certain substances that interfered that led to a decrease in the amount of protein.

The FTIR analysis of the lyophilized sample confirms that keratin being a fibrous protein exhibits secondary structure. Thus the extracted and partially purified keratin powder has few important properties which can be further explored for amino acid analysis and hence seems to be a promising source for various applications such as its use in textiles industries, biomedical, and tissue engineering.



Fig. Chicken Feather Dumping Ground at Vishnupuri, Nanded



Fig. Sampling location on Google map



Fig. Pre-treatment of chicken feather with petroleum ether for 24 hours.



Fig. Dissolution of Chicken Feather in sodium Sulphide Solution.



Fig. keratin obtained by using freeze drying.

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