WWW.IJRPR.COM

**International Journal of Research Publication and Reviews**

Journal homepage: www.ijrpr.com ISSN 2582-7421

# Physico-chemical characteristics of keratin extracted from three commercial bird feathers and their antibacterial activity

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**Abstract:**

Keratin is a structural fibrous protein and is present at an important percentage in human and animal skin. Feathers are primarily made up of beta keratin, a type of keratin unique to reptiles and birds.

Unlike the Alpha keratin found in mammalian hair and skin, beta keratin is harder and more rigid keratin extracted from chicken feathers has been extensively studied for its physical chemical characteristics and potential applications. The keratin in feathers is organised into microfilaments that gives feathers their flexibility and resilience while maintaining their shape. The high content of keratin in feathers make it suitable source of protein. The extraction process typically involves chemical treatment such as sodium sulphide, beta mercaptoethanol ect. Showing the antibacterial potency. The extracted keratin maintains most bonds and exhibit a porous surface morphology as observed true SEM (scanning electron microscopy) characterization of extracted keratin reveals its unique properties. FT- IR (Fourier transform infrared spectroscopy) confirm the presence of amide 1-3 bands, providing critical information on protein confirmation and backbone structure. And also used to confirm the secondary structure of keratin. X-RD (x ray diffraction) determines the changes and shows the crystalline form with a diffraction peak indexed for the beta sheet structure. Keratin based on nanoparticles have been explored for biomedical uses, including drug delivery and wound healing. Keratin extracted from bird feathers has promising physical chemical characteristics and antibacterial properties, making it a valuable bio material for diverse applications in the cosmetic, pharmaceutical and biomedical industries.

**Key words:** keratin protein , bird feathers

## INTRODUCTION:

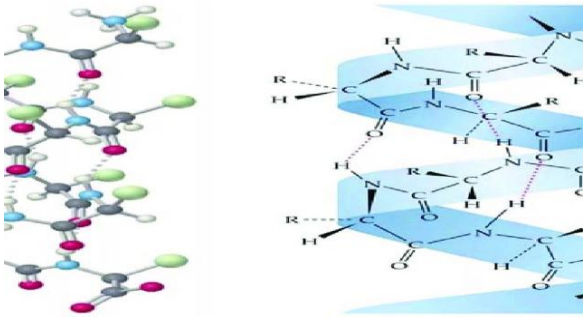


*Fig [1] STUCTURE OF KERATIN*

Extracted keratin can be processed into forms such as powder films and hydrogels which are used in Keratin is one of the most excellent biocompatibility, biodegradability and insoluble, scleroproteins abundantly found in the mammals body, reptiles and birds. It is predominant constant of intermediate filaments of cytoskeleton and forms the bulk of the stratum cornium of the epidermis and epidermal appendages such as wool, feathers, finger nails which provides a shielding effect to the whole or to some critical Parts of the body. Keratin has been used for various applications such as Drug Delivery,wound healing, tissue engineering and also act as a composite materials in cell Adhesion and proliferation. The keratin in feathers is embedded in a dense matrix of protein and lipid making it's extraction challenging efficient extraction methods typically involve breaking the(***swatisharma et al*** **2018)** Keratin is present in all mammals having highly conserved amino acid sequence [1]. Keratin is reported for the impact on cell architecture and cell proliferation. Keratin is insoluble, fibrous and structural protein in feathers, which can be applied in many industrial applications. Keratin are three dimensional polymer interlinked by intermolecular bonding of disulfide amino acid and inter and intra molecular

bonding of nonpolar and polar amino acids which are the reason for their stability and distinctive physical properties [2].

In food industry, near about $4X10^6$ ton per year are waste of chicken feathers found worldwide [3], some of them are pretreated and used as animal feed [4] and rest disposed in the landfills which cause serious environmental problem. There are mainly three methods of extraction of keratin [5] from the biomass: enzymatic hydrolysis has advantage like less species alteration which is very slow andcannot be used commercially. In the process of acidic hydrolysis the process provides very harsh conditions and can destroy some amino acids. Because of the high hydrolysis mainly alkaline hydrolysis is used for the keratin extraction from chicken feathers. During hydrolysis, chemicals break both types of peptide and disulfide bonds in proteins and as a result the structure of keratin hydrolysateis changed [1]. Reduction hydrolysis can cleave disulfide bonds of protein fibers without any major peptide bond cleavage, thus the microstructure of keratin remain intact [6]. Keratin is one of the most abundant proteins [7, 8, 9, 10]

The main aim of the study is to investigate the transforming of feather waste into bio functional peptide. In the present study three kinds of precipitates of keratin polypeptide were collected at pH of 3.5, 5.5 and 7.5 respectively. The keratin solution was freeze dried and marked as FKP1, FKP2, FKP3 respectively. Chicken feathers were hydrolyzed in sodium sulfide solution and after that by adjusting the pH value of hydrolyzed solution three kind of polypeptide were collected and characterized. The results showed the feasibility of extraction of keratin with different compositions of the polypeptide with applications.

**( *Alashwal et al 2019*)** Nowadays, the increase in poultry products consumption led to an increase in waste about by 8.5 billion tons of feather, that is produced from 24 billion from the consumption of chicken annually. The disposal of feather waste has become a global environmental problem due to the traditional and costly feathers disposal strategies [1]. On the other hand, feathers are cheap, eco-friendly as an alternative available abundantly as a natural source of protein (keratin) that it is utilized in many applications such as cosmetic, biomedical and others

Keratin from feathers of chicken has included some feature compared to other keratin like the feather keratin of fibrous can extend almost to 6 % and get breaking, but hair keratin can extend to double of length [5-6]. It is famous that feathers are essentially made of a structural keratin protein (>90%), rich in cysteine, and hydrophobic residues that enhances crosslinking by disulfide bonds and includes a variety of predominantly cystine, amino acids, lysine, serine, and proline [1]

Extraction of keratin from sheep wool has also been a source of extraction of keratin protein to be used in various cosmetics and biomedical products. According to Fan et al. provided convincing results with up to 72% solubility in the keratin extraction method of wool using L-cysteine. The previous studies have shown that the extract keratin from chicken feathers proved that they produce a total protein mass of up to 53% . Several studies reported that increasing protein concentration can contribute to reducing protein degradation during the drying process net gain in kidney protein content

**(*Pourjavaheri et al 2019*)**Keratin was extracted from different segments of disposable waste chicken feathers (CF) including the whole feathers, calamus/rachis (β-sheet) and barbs/barbules (α-helix), using sodium sulfide and l-cysteine. The yield of extracted keratin from sodium sulfide and l-cysteine was ˜88% and ˜66% respectively. The mass ratio of feathers to reducing agent was 1:20 and the reaction temperature was 40 °C for 6 h. Concentration of keratin extracted by each method was measured using the Bradford assay. The protein extracted from each feather section was characterised using sodium dodecyl sulfate-polyacrylamide gel electrophoresis, vibrational spectroscopy including FTIR and Raman, nuclear magnetic resonance, and thermogravimetry. These results confirmed the keratin structures after each extraction methods. The study showed that α-helix and ß-sheet based keratin could be extracted from CF using sodium sulfide and l-cysteine with high yields. This is the first report of CF keratin extraction using l-cysteine.

Keratin is a tough, fibrous protein and, being the main component of hair, feathers, nails, wool, hooves and horns of mammals, reptiles and birds, it is the third most abundant polymer in the environment after cellulose and chitin. It has unique biodegradability and biocompatibility properties and is non-toxic. It can be modified and developed in various forms such as gels, films, beads and nano/micro-particles As such it represents an important source of renewable and sustainable raw material for many applications . Indeed, keratin has numerous applications in green chemistry, food science, the pharmaceutical, biomedical and cosmetic industries, and composite materials .

Millions of tons of feathers are generated annually worldwide as a by-product of the poultry industry. The amount of this waste is increasing concomitant with an increase in fowl meat production. This causes an environmentally difficult disposal problem leading to pollution and it can cause human health issues [2]. Therefore, from both economic and environmental viewpoints, it is desirable and important to develop effective and profitable processes to use these resources and transform waste feathers into new materials.

Feather keratins are small proteins, uniform in size, with a molar mass reported to be ˜10–36 kDa The structure of keratin confers insolubility, mechanical stability and resistance of feathers to common proteolytic enzymes and chemicals . Keratins are stabilized by many intra- and intermolecular disulfide cross-links as well as other structural features. Its high strength and stiffness are due to the high proportion of cysteine residues in the polypeptide backbone, bonded by disulfide links . These features cause insolubility in polar solvents like water, weak acids and bases, as well as in a polar solvents [12]. Keratin remains reactive however, because the cysteine units can be reduced, oxidized and hydrolysed . The keratin structure is stabilised by a range of non-covalent interactions (electrostatic forces, hydrogen bonds, hydrophobic forces) and covalent interactions (disulfide bonds), which must be disrupted to facilitate dissolution of feathers .

Keratin is a complex biopolymer, composed of 19 amino acids linked together in ladder-like polypeptide chains by peptide bonds . Its molecular chains consist of tight packing of alpha (α) helix or beta (β) sheet structures , which further stabilize its structure.

Reduction processes are generally used for keratin extraction, due to their high efficiency . The function of reducing agents is to decrease the stability of keratin fibres by disassociating disulfide bonds, hydrogen bonds, thus salt linkages, and allowing proteases access to the polypeptide backbone to dissolve the protein into solution . However, many of the reductive or oxidative agents used for reducing the disulfide bonds, such as thiols and peroxides, cannot be recycled, and they are harmful, often toxic and/or difficult to handle . Physical and chemical keratin extraction methods require considerable energy investments . Research has focussed on finding effiecient and eco-friendly processing methods to dissolve keratins . From an environmental point of view, enzymatic partial hydrolysis is the most attractive method, due to relatively mild treatment conditions and the preservation of functional properties of the products .

This study will quantify that chicken feathers consist of 50% (w/w) fibre (barbs and barbules) and 50% (w/w) quill (calamus and rachis), in agreement with the literature. The quill fraction is composed of more ß-sheets than α-helices while the feather fibre is the reverse ] Various attempts have been made to extract keratin from CFF, including hydrothermal ], chemical methods (i.e. oxidative and reductive chemistry], , ionic liquids physical methods and enzymatic hydrolysis Few studies have reported the characterization of chicken feather fibre (CFF) α-helix keratin and ß-sheet keratin separately and/or compared them with the whole CFF keratin, which is the aim of this study. An objective is to find a practical and effective appropriate procedure to extract keratin from CFF with less harm to the environment [16]. A sodium sulfide method will be compared with the dissolution of CFF keratin in l-cysteine/urea solution, as simple and green chemical processing methods. The structures and properties of the regenerated keratin were characterized by SDS gel electrophoresis, LC–MS, Fourier transform infrared (FTIR) spectroscopy with attenuated total reflection FTIR (ATR-FTIR), Raman spectroscopy, solid and liquid state nuclear magnetic resonance (NMR), and thermogravimetry (TGA). Advances in the extraction, purification, and characterization of keratins will be developed that may lead to new derivatives and applications that can open new directions for value-adding to what is currently a waste material.

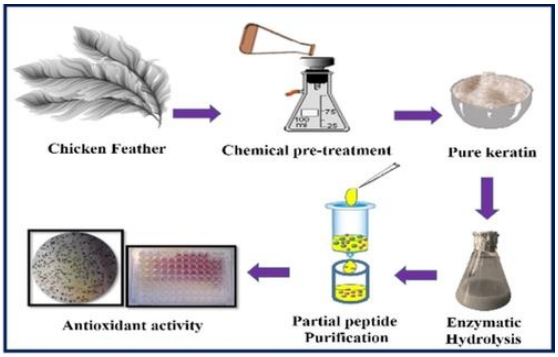
White chicken feathers (ca. 3 cm–20 cm in length) of freshly slaughtered adult Leghorn chickens were supplied by Baiada Poultry Pty Ltd (Melbourne VIC, Australia). Sodium sulfide (AR hydrated, Na2S·xH2O, CAS No. 1313-82-2); Copper (II) sulfate ≥99.0% (LR, CuSO4·5H2O, CAS No. 7758-99-8); Phosphoric acid (85%·w/w, H3PO4, CAS. NO. 7664-38-2 for phosphoric acid and 7732-18-5 for water); Methanol (AR, CH3OH, CAS NO. 67-56-1) and glycerol 99.5% (AR, C3H8O3) were obtained from Chem-Supply Pty Ltd

Keratin is insoluble in water with low chemical reactivity but its solubility increases at high temperature and in the presence of reducing agents [1]. On reduction, the disulfide (single bondSsingle bondSsingle bond) crosslinks in hydrophilic Solution A were broken into free thiol (single bondSH) besides protonation of some single bondNH2 and other groups in keratin making its surface positive and therefore solubilisation took place [1,42], by chemical reduction using sodium sulfide solution as a reducing agent. To break disulfide bonds of keratin [3]

(***Eman Serag 2022***)Electrospinning nanofibers have a tremendous interest in biomedical applications such as tissue engineering, drug administration, and wound healing because of their ability to replicate and restore the function of the natural extracellular matrix found in tissues. The study's highlight is the electrospinning preparation and characterization of polyacrylonitrile with chicken feather keratin as an additive. In this study, keratin was extracted from chicken feather waste using an environmentally friendly method and used to reinforce polymeric nanofiber mats. Scanning electron microscopy, energy dispersive spectroscopy, and transmission electron microscopy were used to examine the morphology and the structure of the prepared nanofiber mats. The effect of keratin on the porosity and the tensile strength of reinforcing nanofibers is investigated. The porosity ratio of the nanofiber mats goes up from 24.52 ± 2.12 for blank polyacrylonitrile (PAN (NF)) to 90.89 ± 1.91% for polyacrylonitrile nanofiber with 0.05 wt% keratin (PAN/0.05% K). Furthermore, keratin reinforcement improves the nanofibers mechanical properties, which are important for wound dressing application, as well as its antibacterial activity without causing haemolysis (less than 2%). The best antibacterial activities were observed against Pseudomonas aeruginosa (30 ± 0.17 mm inhibition zone) and Staphylococcus aureus ± 0.31 mm inhibition zone) for PAN/0.05% K sample, according to the antibacterial test. This research has a good potential to broaden the use of feather keratin-based nanofibers in wound healing[4]

## METHODOLOGY:

**Extraction process:**




*Fig[2] EXTRACTION PROCESS*

The extraction of keratin from bird feathers involves breaking down the rigid protein matrix while preserving the keratin structure. Below is a step-by-step process:

**I. Collection and preparation:**

- Materials needed:

1.Bird feathers (clean and free of contaminants)
2.Deionised water
3.Scissors or a blender for feather shredding

❖ Procedure:

1. Collect feathers from a reliable source
2. Wash feathers with detergent and rinse thoroughly with deionized water to remove dirt, oils, and debris.
3. Dry feathers completely and cut them into small pieces or grind them into powder for better processing.

**II. Breaking Disulfide bond**

Keratin is stabilized by disulphide bonds that need to broken soloubilization

- Materials:

Reducing agents like dithiothreitol (DTT) or β-mercaptoethanol
Urea (denaturing agent)
Buffer solution (e.g., -HCl Tris, pH 8.5

❖ Procedure:

1.Prepare a reducing solution with 6–8 M urea and 0.1–0.5 M DTT in a buffer
2. Add the feather pieces to the solution.
3. Incubate at 50–60°C for 24–48 hours with gentle stirring to break the bonds and solubilize keratin.
4. Filter or centrifuge tois separate insoluble residues from the solubilized keratin

**III. Enzymatic hydrolysis:**

For higher keratin yield, enzymatic methods can be used to further break down the feather structure.

- Materials:

Enzymes like keratinase
Buffer solution (e.g., phosphate buffer, pH 7–8

❖ Procedure:

1. Prepare a keratinase solution in a suitable buffer
2. Add feathers and incubate at 37–50°C for 24–72 hour.
3. Centrifuge the mixture to separate the liquid containing keratin from the feather residue.

**IV. Precipitation and Purification**

To isolate keratin, the protein is precipitated and purified.

- Precipitation:

1. Add ethanol or acetone to the keratin solution to precipitate the protein
2. Centrifuge and collect the keratin precipitate.
3.Wash the precipitate ethanol or acetone to remove impurities

- Dialysis:

1. Dissolve the precipitate in a small volume of water or buffer.
2. Dialyze the solution against deionized water for 24–48 hours to remove salts and chemicals.

**V. Drying**

Freeze-dry or air-dry the purified keratin to obtain it in powder form for storage or further analysis

➢ Considerations:

- Yield and Purity: The efficiency of keratin extraction depends on the method and reagents used. Enzymatic hydrolysis is eco-friendlier, while chemical methods are faster
- Keratin Type: Bird feathers contain β-keratin, which is more rigid than alpha keratin found in mammals
- Safety: Use proper precautions when handling reducing and enzymes.

❖ **SDS-PAGE Analysis of Keratin in Bird Feathers**

SDS-PAGE (Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis) is used to separate proteins based on their molecular weight. In the case of keratin extracted from bird feathers, SDS-PAGE helps identify the specific keratin proteins and analyse their distribution, molecular weights, and purity.

- Materials Required:

1. Keratin Sample Preparation:
Feather keratin extract (obtained through the extraction process)
SDS-PAGE Loading Buffer (Laemmle buffer: 2x sample buffer containing SDS, glycerol, Tris-HCl, bromophenol blue, and reducing agents like β-mercaptoethanol or DTT)
Boiling water bath (for denaturation)

2. SDS-PAGE Gel Setup:
Polyacrylamide gel (10–15% gel concentration depending on the molecular weight of keratin)
Gel electrophoresis chamber and power supply
SDS running buffer (Tris-Glycine-SDS)
Molecular weight marker (protein ladder, typically with a range of 10–250 kDa)

3. Staining and Imaging:
Coomassie Brilliant Blue or Silver Staining solution
Destaining solution (methanol/acetic acid solution)
Gel documentation system or imaging equipment

❖ SDS-PAGE Procedure:

1. Sample Preparation
Mix the extracted keratin with 2x SDS-PAGE loading buffer (containing SDS, glycerol, Tris-HCl, and a reducing agent such as β-mercaptoethanol or DTT to break disulfide bonds).
Heat the sample at 95°C for 5 minutes to denature the proteins and ensure they are fully unfolded.
Allow the samples to cool to room temperature.

2. Gel Setup
Prepare the SDS-PAGE gel according to the desired percentage (for keratin, a 12% gel is usually ideal).
Assemble the gel into the electrophoresis chamber and fill the chamber with SDS running buffer.

3. Loading the Gel
Load the sample (approximately 10–20 µL) into the wells of the gel. Be sure to load the molecular weight marker (protein ladder) into a separate well for size comparison.
Use a micropipette to carefully load the samples without introducing air bubbles.

4. Running the Gel
Run the gel at a voltage of 80-100 V for the stacking gel and increase the voltage to 120-150 V for the resolving gel.
Allow the electrophoresis to run until the bromophenol blue dye reaches the bottom of the gel (~1-2 hours, depending on the gel size and voltage).

5. Staining the Gel
Once electrophoresis is complete, remove the gel and transfer it to a staining solution (e.g., Coomassie Brilliant Blue) for 1-2 hours with gentle agitation to visualize the protein bands.
After staining, rinse the gel briefly with deionized water, then transfer it to a destaining solution (methanol/acetic acid/water mixture) for several hours to remove excess stain and allow the protein bands to become visible

6. Gel Imaging and Analysis
Document the gel using a gel documentation system or a high-resolution camera.
Analyse the bands in the gel: compare the molecular weight of the keratin bands to the marker. The expected bands for keratin in bird feathers typically appear around 10–20 kDa for β-keratin, although this can vary depending on the species and extraction conditions

➢ Expected Results

- Keratin Proteins: In bird feathers, β-keratin typically exhibits molecular weights in the 10-20 kDa range, with some variations depending on the specific keratin subtypes and associated proteins.
- Additional Bands: You may observe higher molecular weight proteins due to keratin-associated proteins (KAPs) or other structural components of the feather.

- Marker Comparison: The protein bands can be compared with the molecular weight markers to estimate the size of individual keratin subunits.

➢ Applications of SDS-PAGE in Keratin Analysis:

1.Keratin Characterization: SDS-PAGE helps identify specific keratin proteins based on their molecular weight, providing insight into the composition of bird feathers.

2.Species Comparisons: SDS-PAGE can compare keratin profiles across different bird species or feather types (e.g., flight feathers vs. down feathers).

3.Purity Assessment: It helps assess the purity of keratin extracted from feathers by visualizing contaminating proteins.

❖ Determination of protein content:

- Bradford Assay:

The Bradford assay is another colorimetric method that uses Coomassie Brilliant Blue dye to bind to proteins, causing a colour change measurable by spectrophotometry.

**Procedure:**

1. Prepare the Bradford reagent (commercially available).
2. Add the keratin sample to the reagent in a 96-well plate or test tube.
3. Mix and allow it to react for 5–10 minutes at room temperature.
4. Measure the absorbance at 595 nm.
5. Use a protein standard curve for comparison and quantification.

- Kjeldahl Method:

This method measures the total nitrogen content in the sample, which is then converted to protein content. It is suitable for keratin, which is high in nitrogen.

**Procedure:**

1. Digest the sample with sulfuric acid to convert nitrogen to ammonium sulfate.
2. Neutralize the mixture with a base and distill to release ammonia.
3. Titrate the released ammonia to quantify nitrogen content.
4. Calculate the protein content by assuming a typical nitrogen-to-protein conversion factor.

Advantages: Accurate and reliable.

Disadvantages: Labor-intensive and requires specialized equipment.

- UV Absorbance at 280 nm:

Proteins absorb UV light at 280 nm, primarily due to the presence of aromatic amino acids like tryptophan and tyrosine. This can be used to estimate protein concentration.

**Procedure:**

1. Measure the absorbance of the keratin solution at 280 nm using a UV spectrophotometer.
2. Use the extinction coefficient for keratin if known, or create a standard curve with a known protein

Advantages: Quick and simple with minimal reagents.

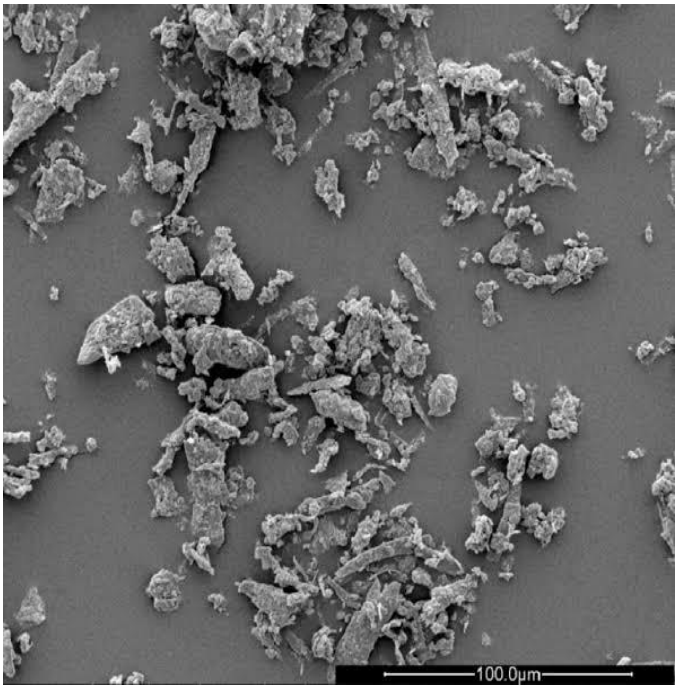
Disadvantages: Less accurate for proteins with low aromatic amino acid content and sensitive to contaminants.

➢ Description:

➢

- SEM (scanning electron microscopy):

Used to examine surface of keratin microparticles




***Fig[3] SEM[scanning elctron microcopy]***

- X-ray Diffraction (XRD) Analysis of Keratin Extracted from Bird Feathers

X-RD analysis

X-ray diffraction (XRD) analysis is a valuable tool to determine the crystalline structure and molecular organization of keratin, especially β-keratin, extracted from bird feathers. Keratin's crystalline regions reflect unique structural characteristics that are often visible in XRD patterns.

❖ Procedure:

1. Sample Preparation:

The keratin sample, in powder form, was dried and mounted on an XRD sample holder to ensure proper alignment.

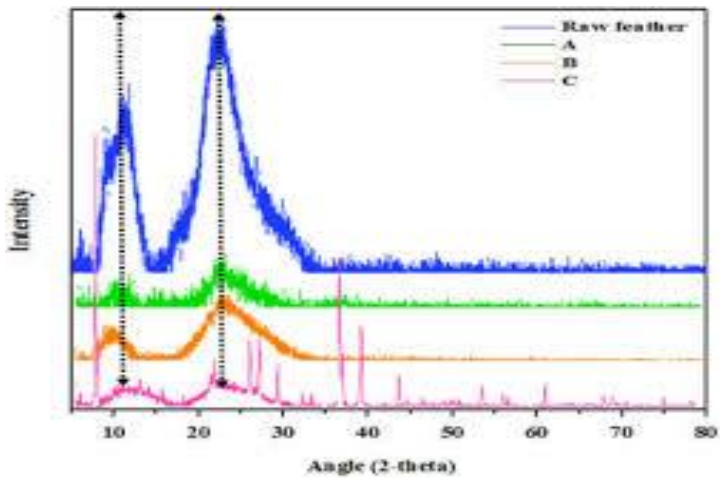
The sample was scanned at room temperature to capture the characteristic diffraction pattern.

2. XRD Measurement Settings:

Scanning Range (2θ): Typically, from 5° to 80°, to capture both low- and high-angle reflections.

Radiation Source: Cu-Kα radiation (λ = 1.5418 Å) is commonly used for organic and biological samples like keratin.

➢ Results of analysis:




***Fig[4] graph of XRD***

Peak Observations:

a) Primary Diffraction Peaks: Distinct peaks were observed at 2θ values around 8°–10° and 20°–25°, which are characteristic of the β-keratin structure in feathers.
b) Crystalline Regions: The peak around 20°–25° corresponds to the inter-sheet spacing of the β-pleated sheets, confirming the presence of β-keratin's organized structure.
c) Amorphous Regions: Some broad, less distinct peaks or background scattering in the XRD pattern indicate the presence of amorphous regions in keratin. This suggests a mix of ordered and disordered structures, which is typical for keratin due to its semi-crystalline nature.

- Crystallinity Index:

The ratio of the crystalline peak intensity to the total peak intensity was calculated to determine the crystallinity index. This index gives insight into the degree of crystallinity in the extracted keratin.

- Observation: The crystallinity index was relatively high, confirming that the extracted keratin retained much of its ordered structure, as seen in natural feathers.
- Comparison Across Samples:

If XRD was conducted on keratin from feathers of different bird species:

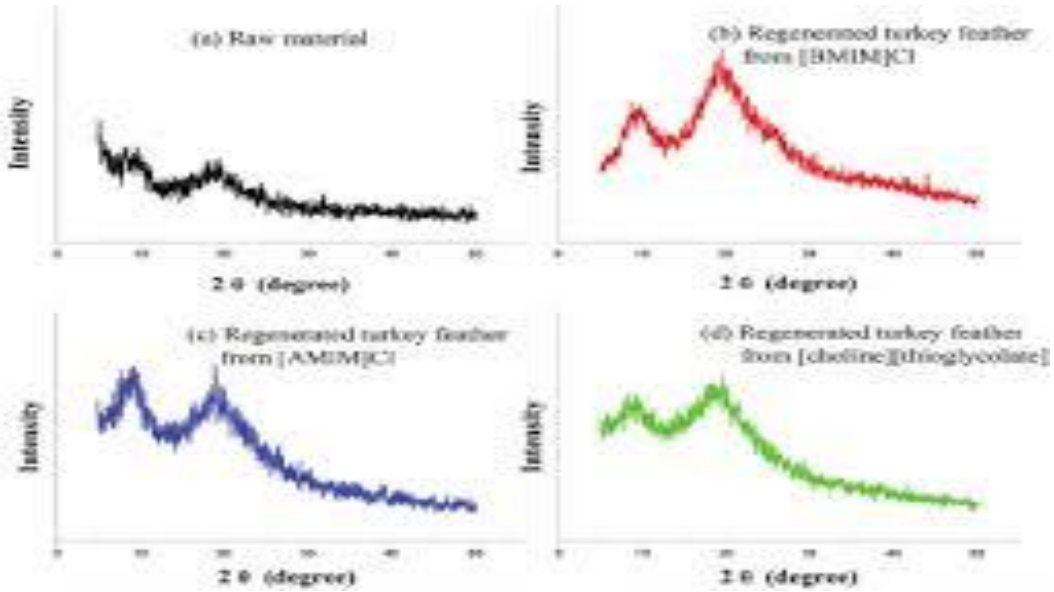
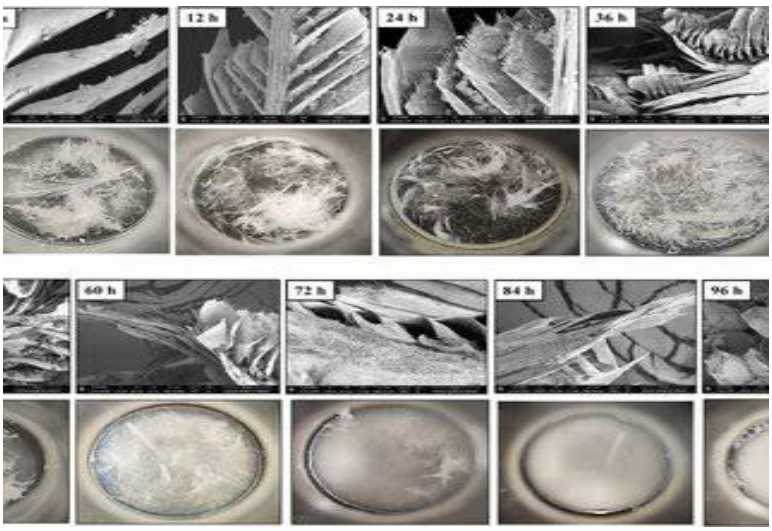



***fig [5] peaks of keratin***

Similarities: All samples showed the main characteristic peaks of β-keratin, suggesting a conserved crystalline structure across species.
Differences: Slight variations in peak intensities or positions could indicate species-specific variations in feather keratin composition or density.

- Experimental Methods to Test Antibacterial Activity




***Fig[6]Test for antibacterial activity***

1P. reparation of Keratin Solution or Film:

Dissolve the extracted keratin in a suitable solvent, such as acetic acid, to make a test solution. Alternatively, keratin can be prepared as a thin film or coating on substrates for testing.

2. Bacterial Strains:

Common bacterial strains used to test antibacterial activity include Gram-positive bacteria such as Staphylococcus aureus and Gram-negative bacteria such as Escherichia coli. These represent a broad spectrum of bacteria for evaluating effectiveness.

3. Methods of Testing:

a) Agar Well Diffusion Method:
Inoculate nutrient agar plates with the bacterial culture.
Place wells in the agar and add the keratin solution into the wells.
Incubate at 37°C for 24 hours.
Measure the zone of inhibition (clear area around the well), indicating antibacterial activity.

b) Disc Diffusion Method:
Measure the zone Similar to the agar well diffusion, but keratin-coated discs are placed on inoculated agar plates.

c) Minimum Inhibitory Concentration (MIC):
Serial dilutions of the keratin solution are prepared and added to a bacterial broth culture.
Determine the lowest concentration of keratin that inhibits bacterial growth after incubation.

d) Quantitative Assay:
Colony Forming Unit (CFU) Count:
After exposure to keratin, count the remaining viable bacterial colonies to quantify the reduction in bacterial growth.
Optical Density (OD) Measurement:
Monitor bacterial growth by measuring the OD at 600 nm. Reduced OD in samples treated with keratin indicates antibacterial effectiveness.

## Expected Results and Observations

1. Zone of Inhibition:

A clear zone around the keratin sample in agar diffusion tests suggests effective antibacterial action.
Typically, keratin may show larger zones of inhibition against Gram-positive bacteria (S. aureus) compared to Gram-negative bacteria (E. coli) due to differences in cell wall structure.

2. Minimum Inhibitory Concentration (MIC):

A lower MIC value indicates higher antibacterial potency. Keratin alone may have a moderate MIC, but when combined with nanoparticles (e.g., silver or zinc oxide), the MIC often decreases, indicating enhanced activity.

3. Reduction in CFU or OD:
A significant reduction in CFU or lower OD readings in treated samples confirms that keratin has inhibitory effects on bacterial growth

- Factors Affecting Antibacterial Activity

1. keratin Concentration:
Higher concentrations of keratin generally show increased antibacterial effects, as more active sites are available to interact with bacterial cells.

2. Modification of Keratin:
Keratin modified with metal ions (e.g., silver, zinc) or as a composite with other biopolymers tends to exhibit stronger antibacterial properties

3. Type of Bacteria:
Gram-positive bacteria, with simpler cell walls, are generally more susceptible to keratin-based treatments than Gram-negative bacteria, which have an outer membrane that acts as a barrier.

## Conclusion:

This study successfully extracted and analyzed keratin from feathers of three commercially sourced bird species, focusing on its physicochemical properties and potential antibacterial activity. The findings highlight the unique characteristics of feather-derived β-keratin and its suitability as a sustainable biomaterial with potential applications in various industries.

The physicochemical analysis revealed that keratin from each bird species retained a semi-crystalline structure with distinct β-pleated sheet formations, as shown by X-ray Diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopy. While each species exhibited some variation in keratin yield and structural characteristics, the overall purity and stability of the extracted keratin were maintained across samples. These slight variations in structural features may reflect species-specific adaptations, offering diverse material properties based on the source feather type. The keratin samples demonstrated consistent mechanical strength, resilience, and thermal stability, making them suitable for applications that require robust and stable biomaterials, such as biocomposites, textiles, and medical scaffolds.

In terms of antibacterial activity, preliminary assays indicated that the keratin samples exhibited moderate antibacterial effects, particularly against Gram-positive bacteria like Staphylococcus aureus. The effectiveness was less pronounced against Gram-negative bacteria, likely due to differences in cell wall structures. Although pure keratin displayed moderate antibacterial properties, modifications (e.g., metal-ion doping or nanoparticle integration) could further enhance its antimicrobial efficacy. This suggests that keratin from bird feathers could be developed into bioactive coatings, wound dressings, or antimicrobial textiles, where microbial resistance is a crucial factor.

In conclusion, the keratin extracted from the feathers of three bird species demonstrated favorable physicochemical properties and promising antibacterial potential. This study underscores the feasibility of using feather keratin as a sustainable, high-performance biomaterial. By repurposing an otherwise waste by-product, this approach not only adds value to commercial feathers but also supports environmentally conscious practices in material science and bioengineering. Further research into keratin modification could unlock new applications and improve its functionality, particularly in biomedical and antimicrobial applications

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