PHYSICO-CHEMICAL PROPERTIES OF KERATIN DERIVED FROM VARIOUS COMMERCIAL BIRD FEATHERS AND ITS ANTIBACTERIAL EFFECTS

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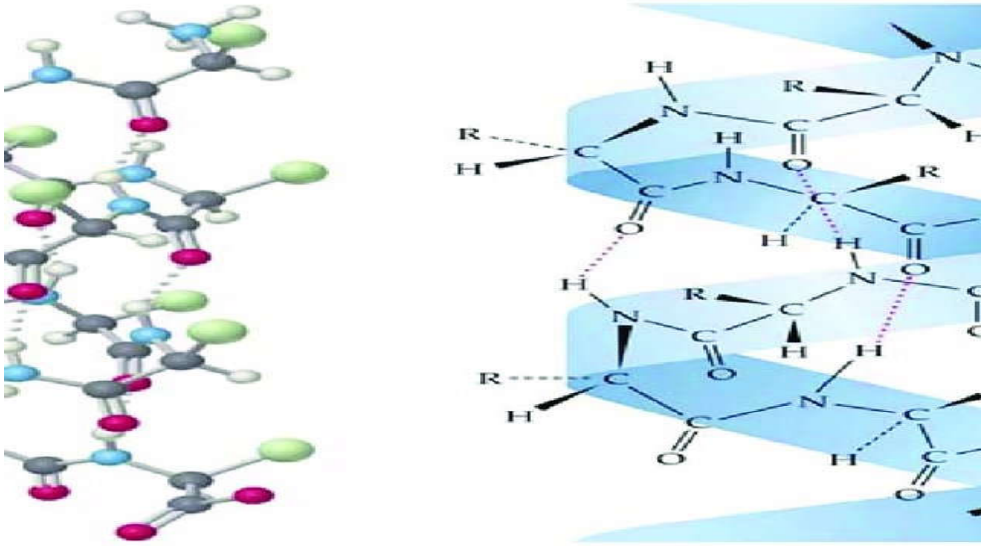
ABSTRACT:

Keratin is a fibrous structural protein that makes up a significant portion of both human and animal skin. Bird feathers are primarily composed of beta keratin, a specific type found in reptiles and birds, distinct from the alpha keratin present in mammalian hair and skin, as it is tougher and more rigid. The physical and chemical properties of keratin extracted from chicken feathers have been widely researched for potential applications. This keratin is structured into microfilaments, providing feathers with flexibility, resilience, and shape retention. Due to the high keratin content, feathers serve as an excellent protein source. The extraction process generally includes chemical treatments such as sodium sulfide and beta-mercaptoethanol, which also demonstrate antibacterial effectiveness. The extracted keratin retains most of its bonds and exhibits a porous surface morphology, as shown by scanning electron microscopy (SEM) analyses that highlight its distinct characteristics. Fourier transform infrared spectroscopy (FT-IR) confirms the presence of amide IIII bands, which offer valuable insights into the protein's conformation and backbone structure, thereby also validating the secondary structure of keratin. X-ray diffraction (XRD) analyzes the crystalline form, revealing a diffraction peak associated with the beta sheet configuration. Keratin nanoparticles have been investigated for biomedical applications, such as drug delivery and wound healing. The keratin obtained from bird feathers shows exceptional physico-chemical characteristics and antibacterial properties, establishing it as a promising biomaterial for various applications in cosmetics, pharmaceuticals, and biomedicine.

Keywords: keratin protein, bird feathers

INTRODUCTION:



Extracted keratin can be transformed into various Extracted like powder, films, and hydrogels, which are utilized due to keratin's excellent biocompatibility and biodegradability. This insoluble and scleroprotein is abundantly found in mammals, reptiles, and birds, serving as a key component of the intermediate filaments in the cytoskeleton. Keratin constitutes a significant part of the stratum corneum in the epidermis and its appendages, such as wool, feathers, and fingernails, providing protective functions for the body. It has diverse applications, including drug delivery, wound healing, tissue engineering, and serving as composite materials for enhancing cell adhesion and proliferation. The keratin in feathers is encased in a dense matrix of proteins and lipids, making extraction difficult; effective extraction methods generally involve breaking this malipidKeratin, with a highly conserved amino acid sequence, is present in all mammals and has shown to influence cell architecture and proliferation. This insoluble, fibrous structural protein in feathers has numerous industrial applications and consists of a threedimensional polymer interconnected through intermolecular disulfide bonds along with inter- and intramolecular interactions. bonding of nonpolar and polar amino acids which are the reason for their stability and distinctive physical properties .In food industry, near about 4X106ton per year are waste of chicken feathers found worldwide some of them are pretreated and used as animal feed and rest disposed

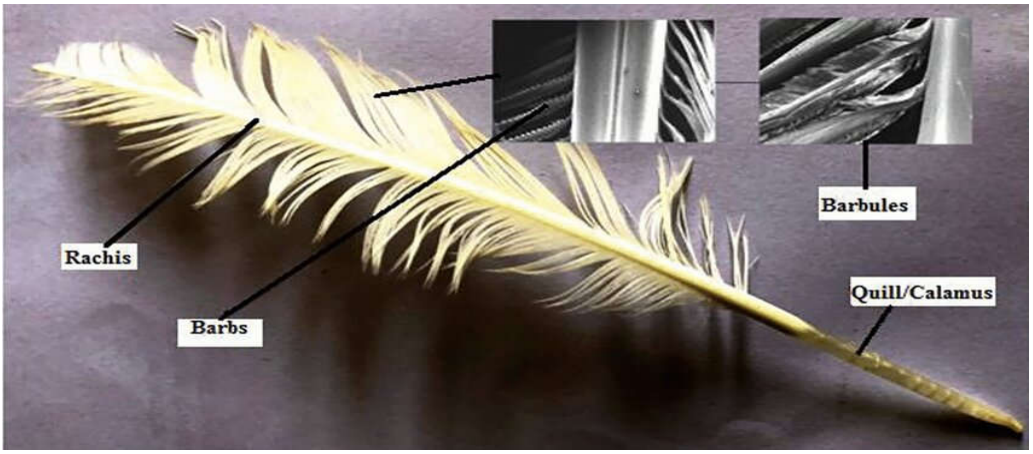
in the landfills which cause serious environmental problem. There are mainly three methods of extraction of keratin from thebiomass: enzymatic hydrolysis has advantage like less species alteration which is very slow andcannot be used commercially. In the process of acidic hydrolysis the process provides very harsh conditions and can destroy some amino acids. Because of the high hydrolysis mainly alkalinehydrolysis 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 hydrolysateischanged.Reduction hydrolysis can cleave disulfide bonds of protein fibers without any major peptide bond cleavage, thus the microstructure of keratin remain intact.Keratin is one of the most abundant proteins

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, FKP3respectively. Chicken feathers were hydrolyzed in sodium sulfide solution and after that by adjusting the pH value of hydrolyzed solution three kindof polypeptide were collected and characterized. The results showed the feasibility of extraction of keratin with different compositions of the polypeptide with applications.

Keratin was extracted from different segments of disposable waste chicken feathers (CF) including the whole feathers, calamus/rachis (β-sheet) and barbs/barbules (αhelix), 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 nontoxic. 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.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 . Keratin remains reactive however, because the cysteine units can be reduced, oxidized and hydrolyzed . The keratin structure is stabilized 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 formed from 19 amino acids connected in ladder-like polypeptide chains through peptide bonds. Its molecular arrangement is characterized by tightly packed alpha (α) helices or beta (β) sheets, which enhance its structural stability. Extracting keratin typically involves reduction processes due to their effectiveness. Reducing agents work by destabilizing the keratin fibers, breaking disulfide and hydrogen bonds, thus allowing proteases to access the polypeptide backbone and dissolve the protein. However, many of the reductive or oxidative agents, such as thiols and peroxides, are non-recyclable and can be toxic or challenging to handle. Both physical and chemical keratin extraction methods require significant energy expenditure. Research efforts are currently aimed at developing efficient and environmentally friendly methods for keratin dissolution. Enzymatic partial hydrolysis stands out as the most appealing option from an ecological perspective because it uses milder conditions and helps maintain the functional properties of the end products.

This study will confirm that chicken feathers are composed of 50% (w/w) fiber (including barbs and barbules) and 50% (w/w) quill (calamus and rachis), aligning with existing literature. The quill is primarily made up of β-sheets, while the feather fiber predominantly contains α-helices. Multiple attempts to extract keratin from chicken feather fiber (CFF) have been made, utilizing hydrothermal, chemical methods, ionic liquids, and enzymatic hydrolysis. However, few studies have analyzed α-helix and β-sheet keratin from CFF individually or compared them to whole CFF keratin, which is a key goal of this research. This study aims to identify a practical and environmentally conscious procedure for keratin extraction from CFF, comparing the use of sodium sulfide with a green chemical processing method involving l-cysteine/urea solution. The resulting regenerated keratin will be characterized using techniques such as SDS gel electrophoresis, LC–MS, FTIR spectroscopy (including ATR-FTIR), Raman spectroscopy, NMR, and thermogravimetry. The advancements in extraction, purification, and characterization of keratins may lead to new derivatives and applications, thereby adding value to what is currently considered waste material.

White chicken feathers (approximately 3–20 cm long) from freshly slaughtered adult Leghorn chickens were sourced from Baiada Poultry Pty Ltd in Melbourne, Australia. Sodium sulfide (AR hydrated, Na2S·xH2O) and various other chemicals were procured from Chem-Supply Pty Ltd. Keratin is insoluble in water and has low chemical reactivity; however, its solubility increases in

high-temperature conditions and when reducing agents are present. The reduction breaks down disulfide bonds and modifies the keratin structure, facilitating its solubilization.

Electrospun nanofibers are of significant interest for biomedical applications, such as tissue engineering, drug delivery, and wound healing, as they mimic the natural extracellular matrix. This study emphasizes the electrospinning preparation and characterization of polyacrylonitrile integrated with chicken feather keratin. Keratin was extracted from feather waste using an ecofriendly method to reinforce polymeric nanofiber mats. Techniques like scanning electron microscopy, energy dispersive spectroscopy, and transmission electron microscopy were employed to assess the morphology and structure of the nanofiber mats. The research explores how keratin impacts the porosity and tensile strength of the nanofibers. The porosity of the nanofiber mats increased from 24.52 ± 2.12% for plain polyacrylonitrile (PAN) to 90.89 ± 1.91% for PAN with 0.05 wt% keratin. Additionally, the incorporation of keratin enhances the mechanical properties of the nanofibers, making them suitable for wound dressings, while also demonstrating antibacterial properties with minimal hemolysis (<2%). The best antibacterial effectiveness was recorded against Pseudomonas aeruginosa and Staphylococcus aureus for the PAN/0.05% K sample, indicating a promising potential for feather keratin-based nanofibers in wound healing applications.

LITERATURE REVIEW

(Pour Javaheri 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 characterized 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.

(EmanSerag et al 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 good potential to broaden the use of feather keratin-based nanofibers in wound healing

(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. On the other hand, feathers are cheap, eco-friendly as an alternative available abundantly as a natural source of protein (keratin) that 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

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, it 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

(Swati Sharma et al 2018)

Keratin is present in all mammals having a highly conserved amino acid sequence. 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. Keratins 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 NeeharikaSenthilkumar et al (2022) Keratin is a fibrous structural protein naturally

NeeharikaSenthilkumar et al (2022)

Keratin is a fibrous structural protein naturally present in the appendages of animals such as hair, wool, feathers, hooves and hides. Currently millions of tons of these appendages are discarded as waste products by the meat, poultry, textile and leather industries. These keratin-rich wastes lack environmentally friendly disposal methods and are often dumped in landfills or incinerated. Over the last few years, several studies have developed various methods to repurpose these wastes as a potential source for obtaining keratin. Keratin has recently been demonstrated to have applications within the biomedical field (as a scaffold material in tissue engineering, for drug delivery and wound healing), cosmetic products

SandleenFeroz et al (2020)

Keratin constitutes the major component of the feather, hair, hooves, horns, and wool represents a group of biological material having high cysteine content (7– 13%) as compared to other structural

proteins. Keratin -based biomaterials have been investigated extensively over the past few decades due to their intrinsic biological properties and excellent biocompatibility. Unlike other natural polymers such as starch, collagen, chitosan, the complex three-dimensional structure of keratin requires the use of harsh chemical conditions for their dissolution and extraction.

(Shyam Dev Maurya et al)2023

: The current study was to extract the keratin protein from waste broiler chicken feathers. Due to their high keratin content, chicken feathers offer a valuable source of protein. The two steps involved are treating sodium sulfide (Na2S) and then extracting the protein from the mixture. After the feathers have been broken down using reducing agents, dilute hydrochloric acid (HCl) is added to the solution to precipitate the protein. The precipitated protein was repeatedly rinsed with distilled water and dissolved in a sodium hydroxide (NaOH) solution to form a solution. The results indicate that a chicken feather has unique features. Keratin obtained from broiler chicken feathers had a yield of 81.1%, which is relatively high in cases of waste raw material conversion into value-added products. The prepared keratin was characterized by Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and thermogravimetric analysis (TGA) to investigate the chemical composition, thermal properties, crystallinity properties, and also physico-mechanical properties, including density. These findings suggest that chicken feathers can be used to extract protein

(**A.D. Mahmud et al)2023**

This study investigated the extraction of keratin protein from chicken feathers through alkaline hydrolysis using sodium sulphide as a digesting agent. The protein was precipitated using hydrogen chloride and confirmed through biuret test, solubility test, sulphur test, and FT-IR analysis. The effect of varying sodium sulphide concentrations (0.5M, 0.75M, and 1M) on the extracted keratin was evaluated. Results showed that a higher concentration of sodium sulphide produced a higher yield of keratin, with 1M producing 65.8% yield. However, the shelf-life of wet keratin extracted using 1M concentration was four weeks, compared to six weeks for 0.5M and 0.75M concentrations. The dried keratin was unaffected after six weeks. The study suggests that a higher concentration of the reducing agent produced a higher yield of keratin protein but with a shorter shelf-life if drying was not carried out. The utilization of abundant waste generated by

poultry industries is crucial in reducing pollution and creating opportunities for valuable product development. The extraction of keratin from chicken feathers provides an eco-friendly approach to waste management and creates opportunities for product development

METHODOLOGY:

PROCESS OF EXTRACTION:

- **MATERIALS AND REAGENTS:**

Chicken feathers (clean, dry)

Sodium hydroxide (NaOH), analytical grade

Distilled water

Hydrochloric acid (HCl), 1 M (for pH adjustment)

Ethanol (70%, v/v)

Filter paper (Whatman No. 1 or equivalent) pH
meter or indicator strips
Magnetic stirrer and hot plate

Beakers (500 mL, 1000 mL)

Glass rod

Buchner funnel and vacuum filtration setup

Centrifuge and centrifuge tubes (optional)

Oven or drying chamber Procedure:
1. **PRE-TREATMENT OF FEATHERS:**

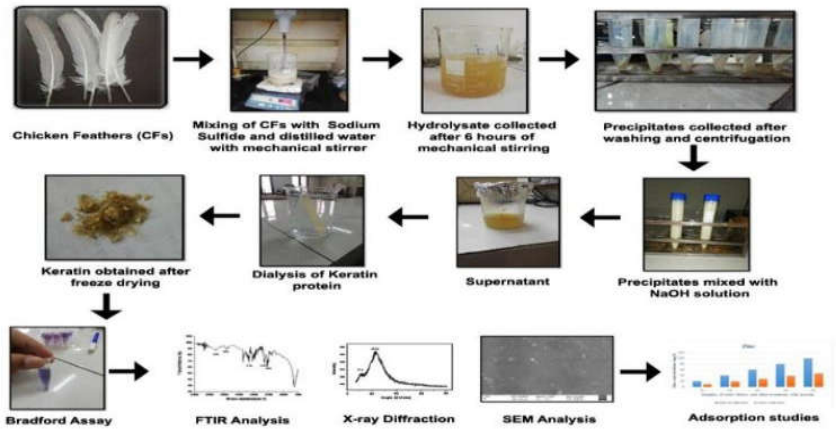
Collect fresh chicken feathers and wash thoroughly with distilled water to remove dirt, blood, and other impurities.

Boil feathers in water for 30 minutes to denature superficial contaminants.

Dry the cleaned feathers in a hot air oven at 60°C for 24 hours.

Grind the dried feathers into small pieces or coarse powder using a mechanical grinder.

2. **ALKALINE HYDROLYSIS:**

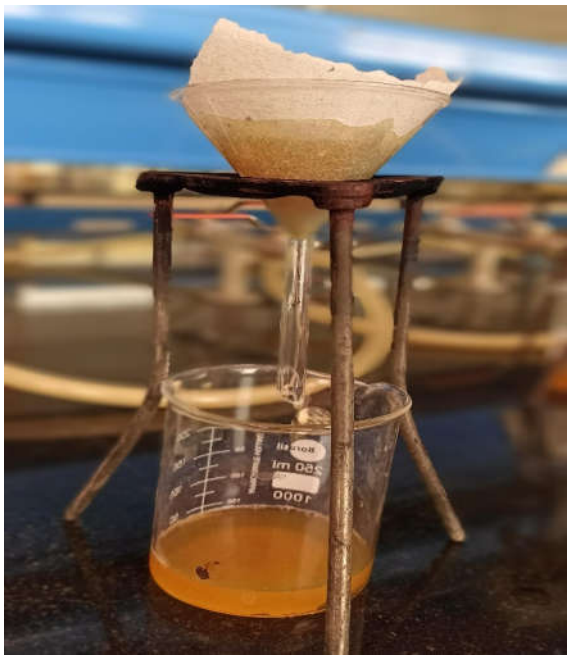


Prepare a 0.5 M NaOH solution in a beaker.

Add 10 g of ground feathers to 100 ml of the NaOH solution.

Heat the mixture at 70–80°C while stirring continuously on a magnetic stirrer for 2–4 hours.

Monitor the reaction and ensure the feathers are progressively solubilized.



3. **FILTRATION AND CLARIFICATION**:

Cool the solution to room temperature.

Filter the hydrolysate using a Buchner funnel and Whatman filter paper to remove undigested residues.

Optionally, centrifuge the filtrate at 5000 rpm for 10 minutes to further clarify the solution.

4. **PRECIPITATION OF KERATIN:**

Adjust the pH of the filtrate to 4.0–4.5 using 1 M HCl to precipitate keratin.

Allow the solution to stand undisturbed at room temperature for 4–6 hours to complete precipitation.

Collect the precipitated keratin by filtration or centrifugation.

5. Washing and Purification:

Wash the keratin precipitate with 70% ethanol to remove residual salts and impurities.

Repeat washing twice, then re-suspend in distilled water and filter again.

6. Drying:

Transfer the purified keratin to a Petri dish or drying tray.

Dry in a vacuum oven at 40–50°C until a constant weight is achieved.

Store the keratin in an airtight container under desiccated conditions.

Notes:

Optimize hydrolysis time and NaOH concentration based on desired molecular weight range of extracted keratin.

All steps should be performed with appropriate safety measures, including gloves and eye protection when handling NaOH and HCl.

The yield and purity of the extracted keratin can be assessed using SDS-PAGE, FTIR, or UV–Vis spectrophotometry.

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, TrisHCl, 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

Distaining 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 (~12 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

1. 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.
2. 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 analyzed alongside molecular weight markers to approximate the sizes of individual keratin subunits.

Applications of SDS-PAGE in Keratin Analysis:

1. Keratin Characterization: SDS-PAGE is utilized to identify specific keratin proteins by their molecular weight, which aids in understanding the makeup of bird feathers.

2. Species Comparisons: This technique allows for the comparison of keratin profiles among various bird species or types of feathers (e.g., flight feathers versus down feathers).

3. Purity Assessment: SDS-PAGE can be employed to evaluate the purity of keratin extracted from feathers by visualizing any contaminating proteins.

Protein Content Determination:

- Bradford Assay: This is a colorimetric method involving Coomassie Brilliant Blue dye that binds to proteins, resulting in a color change that can be measured spectrophotometrically.

Procedure:

1. Prepare the commercially available Bradford reagent.

2. Add the keratin sample to the reagent in a 96-well plate or test tube.

3. Mix and allow to react for 5-10 minutes at room temperature.

4. Measure the absorbance at 595 nm.

5. Compare and quantify using a protein standard curve.

- Kjeldahl Method: This technique measures total nitrogen in the sample, which is then translated into protein content, making it suitable for high-nitrogen keratin.

- **Procedure**:

1. Digest the sample with sulfuric acid to convert nitrogen into ammonium sulfate.

2. Neutralize the mixture with a base and distill to release ammonia.

3. Titrate the released ammonia to measure nitrogen content.

4. Calculate protein content using a specific nitrogen-to-protein conversion factor.

- Advantages: Provides accurate and reliable results.

- Disadvantages: It is labor-intensive and requires specialized equipment.

- UV Absorbance at 280 nm: Proteins absorb UV light at 280 nm, mainly due to aromatic amino acids like tryptophan and tyrosine, which can help estimate protein concentration.

- **Procedure**:

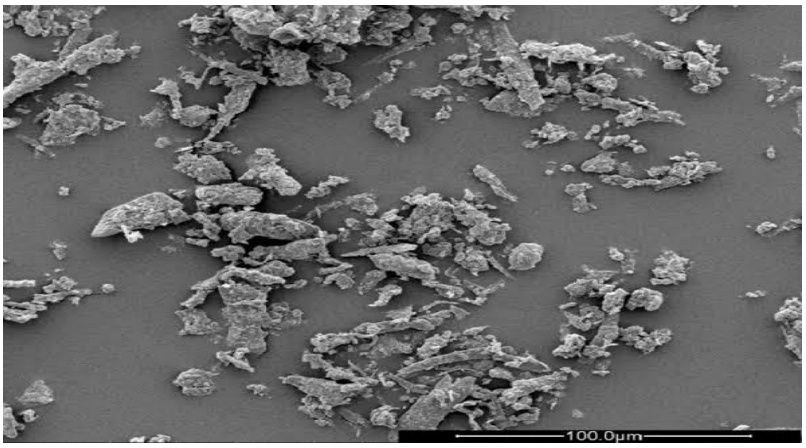
1. Measure the absorbance of the keratin solution at 280 nm using a UV spectrophotometer.

2. Utilize the known extinction coefficient for keratin or create a standard curve using a known protein.

- Advantages: This method is quick and straightforward with minimal reagent requirements.

- Disadvantages: It may be less accurate for proteins low in aromatic amino acids and can be affected by contaminants.

- SEM (Scanning Electron Microscopy): This technique is used to analyze the surface structure of

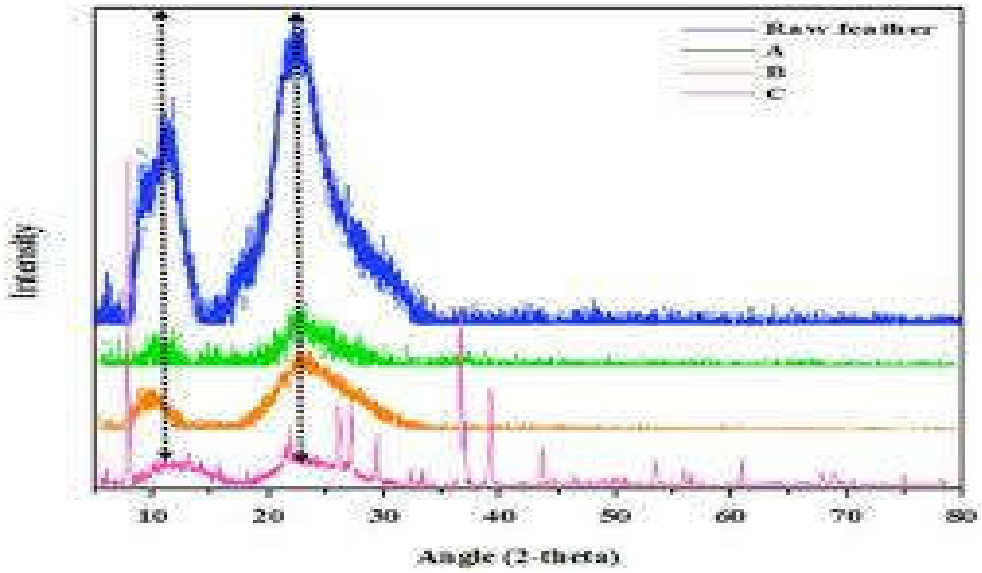


keratin microparticles.

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

XRD Analysis Overview

X-ray diffraction (XRD) serves as an important method for analyzing the crystalline structure and molecular arrangement of keratin, particularly β-keratin, sourced from bird feathers. The crystalline regions of keratin exhibit distinct structural features that can be identified in XRD patterns.



Procedure:

1. Sample Preparation:

The keratin was processed into a dry powder and placed onto an XRD sample holder to ensure it was properly aligned. The sample was then scanned at room temperature to obtain the specific diffraction pattern.

2. XRD Measurement Settings:

- Scanning Range (2θ): Generally set from 5° to 80° to include both low- and highangle reflections.

- Radiation Source: Cu-Kα radiation (λ = 1.5418 Å) is typically employed for analyzing organic and biological samples, such as keratin.

Analysis Results: Peak

Observations:

a) Primary Diffraction Peaks: Clear peaks were detected at 2θ values between 8°–10° and 20° –25°, which are indicative of the β-keratin structure found in feathers.

b) Crystalline Regions: The peak in the 20°–25° range is associated with the spacing between β-pleated sheets, confirming the ordered structure of β-keratin.

c) Amorphous Regions: The presence of broad, less defined peaks or background scattering in the XRD pattern suggests there are amorphous regions within the keratin. This indicates a combination of ordered and disordered structures, as is typical for keratin due to its semi-crystalline nature.

Crystallinity Index:

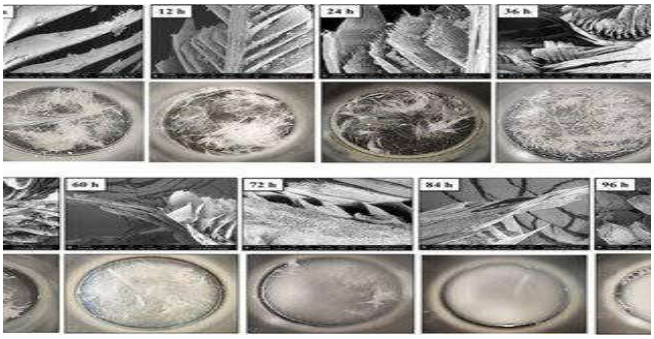
The crystallinity index was calculated by analyzing the ratio of crystalline peak intensity to total peak intensity, providing insights into the crystallinity level of the extracted keratin.

1. Observation: The crystallinity index was relatively high, indicating that the extracted keratin maintained much of its organized structure as observed in natural feathers.
2. Comparison Across Samples:

If X-ray diffraction (XRD) analysis was performed on keratin sourced from feathers of various bird species:

Similarities: All samples exhibited the primary characteristic peaks of β-keratin, suggesting a maintained crystalline structure across different species.

Differences: Minor variations in peak intensities or positions could reflect species-specific differences in feather keratin composition or density.



Experimental Methods to Assess Antibacterial Activity.

1. Preparation of Keratin Solution or Film:

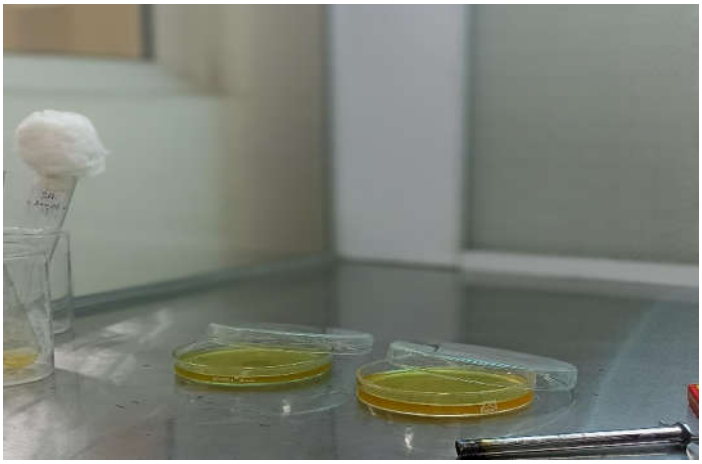
Dissolve the isolated keratin in an appropriate solvent like acetic acid to create a test solution. Alternatively, keratin can be formed into a thin film or coating on various substrates for testing purposes.

2. Bacterial Strains:

Common bacterial strains utilized to assess antibacterial activity include Gram-positive bacteria such as Staphylococcus aureus and Gram-negative bacteria like Escherichia coli, providing a comprehensive range of bacteria for effectiveness evaluation.

3. Testing Methods:

a) Agar Well Diffusion Method:



Inoculate nutrient agar plates with the bacterial sample. Create wells in the agar and introduce the keratin solution into these wells. Incubate at 37°C for 24 hours, then measure the zone of inhibition (the clear area surrounding the well) to assess antibacterial effectiveness. b) Disc Diffusion Method:

Similar to the agar well diffusion, but keratin-coated discs are placed on inoculated agar plates.

c) Minimum Inhibitory Concentration (MIC):

Prepare serial dilutions of the keratin solution and add them to a bacterial broth culture. Identify the lowest concentration of keratin that prevents bacterial growth after incubation. d) Quantitative Assay:

Colony Forming Unit (CFU) Count: After exposure to keratin, count the surviving bacterial colonies to evaluate the reduction in bacterial growth. Optical Density (OD) Measurement: Assess bacterial growth by measuring the OD at 600 nm; a reduced OD in keratin-treated samples indicates effective antibacterial properties.

Expected Results and Observations:

1. Zone of Inhibition:

A clear area surrounding the keratin sample in agar diffusion tests indicates effective antibacterial activity, typically showing larger inhibition zones against Gram-positive bacteria (S. aureus) compared to Gram-negative bacteria (E. coli) due to cell wall structural differences.

2. Minimum Inhibitory Concentration (MIC):

A lower MIC value signifies greater antibacterial efficacy. While keratin may have a moderate MIC alone, its combination with nanoparticles (such as silver or zinc oxide) often results in a decreased MIC, demonstrating enhanced activity.

3. Reduction in CFU or OD:

A significant decline in CFU or lower OD measurements in treated samples confirms the inhibitory effect of keratin on bacterial growth.

Factors Influencing Antibacterial Activity:

1. Keratin Concentration:

Higher keratin concentrations generally enhance antibacterial effects due to more active sites available for interaction with bacterial cells.

2. Keratin Modification:

Keratin modified with metal ions (e.g., silver, zinc) or combined with other biopolymers tends to exhibit improved antibacterial properties.

3. Type of Bacteria:

Gram-positive bacteria, having simpler cell walls, are typically more vulnerable to keratin-based treatments than Gram-negative bacteria, which possess an outer membrane acting as a protective barrier.

Conclusion:

This study effectively extracted and analyzed keratin from the feathers of three commercially sourced bird species, examining its physicochemical properties and potential antibacterial activity. The results emphasize the distinctive features of feather-derived β-keratin and its viability as a sustainable biomaterial with potential uses across various industries.

The physicochemical analysis demonstrated that keratin from different bird species maintained a semi-crystalline structure with specific β-pleated sheet formations, as evidenced by X-ray Diffraction (XRD) and Fourier Transform Infrared (FTIR) spectroscopy. Although variations in keratin yield and structural characteristics were observed among species, the purity and stability of the extracted keratin remained consistent across the samples. These minor differences in structural properties may indicate species-specific adaptations, providing varying material qualities depending on the type of feather. The keratin samples showed reliable mechanical strength, resilience, and thermal stability, making them suitable for applications that require durable and stable biomaterials, such as biocomposites, textiles, and medical scaffolds.

In terms of antibacterial properties, initial tests revealed that the keratin samples had moderate antibacterial effects, especially against Gram-positive bacteria like Staphylococcus aureus, while the effect was weaker against Gram-negative bacteria, likely due to differences in cell wall structures. While pure keratin exhibited moderate antibacterial properties, enhancements through modifications such as metal-ion doping or nanoparticle integration could improve its antimicrobial effectiveness further. This suggests that keratin from bird feathers could be adapted for creating bioactive coatings, wound dressings, or antimicrobial textiles, where microbial resistance is essential.

In summary, the keratin extracted from the feathers of three bird species displayed favorable physicochemical properties and antibacterial potential. This study highlights the practicality of utilizing feather keratin as a sustainable, high-performance biomaterial. By repurposing waste feathers, this approach not only adds value to a commercial byproduct but also promotes environmentally friendly practices in material science and bioengineering. Future research into modifications of keratin could lead to new applications and enhanced functionality, particularly in biomedical and antimicrobial fields.

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