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BIOCHEMICAL RECOVERY OF KERATIN FROM POULTRY FEATHERS USING HYDROLYSIS TECHNIQUES AND PROTEOLYTIC MICROORGANISMS

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Abstract: Chicken feathers are a major source of environmental waste because of their high keratin content and resistance to natural degradation. The purpose of this study was to use chemical hydrolysis to extract keratin from Broiler and Native chicken feathers and its biodegradability was assessed using microbial proteolytic activity. Feathers were gathered, cleaned, allowed to dry in the sun, and then treated with both acidic (HCl) and alkaline (NaOH) solutions. By adjusting the HCl concentration, the hydrolysis time (12, 18, and 24 hours), and the NaOH molarity (2.0M and 3.0M), the extraction procedure was optimized. The highest keratin recovery (10.8%) was obtained by alkaline hydrolysis with 3.0M NaOH after 12 hours of incubation, whereas the highest recovery of 15% was obtained by acid hydrolysis with 3 M HCl, which produced a protein content of 274.107 mg/ml. Effective keratin recovery was confirmed by protein quantification using the Lowry assay and absorbance. Microbial isolates with keratinolytic potential were found in soil samples from Shaheed Benazir Bhutto University, particularly Aspergillus niger and *Bacillus licheniformis*. Proteolysis zones were evident in skim milk agar assays; A. Niger produced a larger and more persistent zone than bacteria. The effectiveness of alkaline hydrolysis in removing keratin from white poultry feathers is confirmed by the results. Assessment of microbial biodegradability promotes sustainable waste valorization and supports biotechnology and environmental control applications.

Key words: Poultery feather waste, Alkaline hydrolysis, Keartin extraction, Keratinolytic micro-organisms, Keratinase, Protein hydrolysates, Circular Bioeconomy.

INTRODUCTION

With over 102 million tons of chicken meat and 1627 billion eggs produced globally in 2022, the demand for poultry products is steadily rising due to population growth and rising protein consumption (Khiewwijit *et al*., 2024). Among the main wastes produced by the poultry industry's explosive expansion are feathers, which make up 5-10% of a bird's body weight and generate millions of tonnes of keratin-rich waste each year, along with manure, litter, and slaughterhouse byproducts (Qingxin Li, 2019; Parrado *et al*.,2014). Due to greenhouse gas emissions, contaminated soil and water, and risks to human health like chlorosis and other diseases like mycoplasma and fowl cholera, conventional disposal techniques like landfilling, incineration, and open dumping are not environmentally sustainable. (Abah *et al*., 2019; Prasanthi *et al*., 2016; Sharma & Gupta, 2016). Structurally, Chicken feathers consist of up approximately 91% keratin, 8% water, and 1% lipids by mass, thus representing a renewable source of livestock biowaste. Keratin is the primary structural protein in feathers and is highly stable, insoluble, and resistant to natural degradation (Aradoaei *et al*., 2024; Mishra *et al*., 2023).It is composed of polypeptide chains that can adopt α-helical or β-pleated sheet conformations. α-keratin forms the primary structural component of mammalian hair, nails, hooves, horns, quills, and skin, whereas the more resilient β-keratin is found in bird and reptilian feathers, claws, scales, and beaks, as well as in the setae of gecko feet, which provide strong surface adhesion (McKittrick *et al*., 2012). A common scaffold material in tissue engineering and regenerative medicine, keratin supports cell adhesion, proliferation, and differentiation due to its highly cross-linked polypeptide structure, biocompatibility, and biodegradability. Additionally, it has applications in environmental cleanup, cosmetics, bone and nerve regeneration, wound healing, drug delivery, and ocular restoration (Senthilkumar *et al*., 2022; Lin *et al*., 2019; Borrelli *et al*., 2015; Tomblyn *et al*., 2016).

The possibilities of chemical and biological methods for repurposing feather waste have been highlighted by recent studies. Strong disulphide bonds in keratin can be effectively broken down by microbial keratinases, which are produced by bacteria like Streptomyces and *Bacillus*. This process turns feathers into protein-rich hydrolysates that can be used to make biomaterials, animal feed, biofertilizers, and medications (Bhari *et al*., 2021; Nad *et al*., 2024).The sustainable utilization of poultry by-products is further supported by circular economy approaches that priorities resource recovery and waste reduction (Alhawari *et al*., 2021).Pakistan's poultry sector, which produces more than 18 billion eggs and 1.5 million tons of chicken meat yearly, makes a substantial economic and food security contribution (Umair *et al*., 2021). Despite this expansion poor waste management, especially with regard to feathers and litter, notwithstanding this expansion (Hussain *et al*., 2015; Akhtar *et al*., 2024).In this study, keratin is biochemically recovered from chicken feathers using keratinolytic bacteria and optimized hydrolysis, converting low-value waste into high-value keratin-based biomaterials and promoting sustainable bioeconomy practices.

MATERIALS AND METHODS

Sample collection

Local poultry vendors and slaughterhouses in Peshawar, Khyber Pakhtunkhwa (KPK), Pakistan, provided the discarded chicken feathers. There were two different kinds of feathers: white broiler feathers and indigenous native feathers. After being collected, samples were carefully separated from any leftover skin tissues, put in sterile polyethylene bags, and quickly taken to the lab under controlled conditions for further processing.

Feather preparation

Cleaning and drying

The collected feathers were thoroughly rinsed two or three times under running tap water using a mild detergent to remove surface impurities such as blood, dirt, and remaining organic matter. The cleaned feathers were then exposed to the sun for 48 hours to ensure that all the moisture had been removed.

Prior to treatment

Feathers were manually divided into uniform pieces (about 1-2 cm) after drying in order to increase surface area and make keratin extraction more effective.

Alkaline-aided keratin extraction technique

Process of hydrolysis

For each experiment, 250 millilitres of a 10g sodium hydroxide (NaOH) solution were used to suspend 10g of pre-treated feather material. The suspension was incubated for 24 hours at room temperature under static conditions in order to promote alkaline hydrolysis and facilitate keratin release.

Precipitation and filtration

After hydrolysis, the reaction mixture was filtered through sterile gauze to remove any remaining feather residues. The filtrate was gradually acidified by adding 100 mL of hydrochloric acid (HCl) drop wise while continuously stirring it after it had been enriched with solubilised keratin. Acidification persisted until protein precipitation appeared, indicating that the keratin had successfully recovered.

Collecting and casting native keratin

Filtration was used to gather the precipitated keratin, which was then carefully moved to sterile Petri dishes. After that, samples were dried for two hours at 70°C in a hot air oven to eliminate any remaining moisture. After drying, the keratin was carefully scraped off with a sterile spatula, precisely weighed, and sealed in vials to maintain its integrity for further examinations.

Parameters for optimization

Important process parameters were thoroughly optimized to get maximum keratin recovery. Firstly, the effect of alkalinity was studied by comparing treatments with 2.0 M and 3.0 M NaOH. Secondly, the effect of the hydrolysis period in the range 12, 18, and 24 hours was tested to determine the optimum extraction time. Finally, 3 M hydrochloric acid (HCl) was tested for protein precipitation efficiency.

Quantification of proteins

The total protein concentration was determined using the Lowry technique. After preparing Bovine serum albumin (BSA) standards to build a calibration curve, absorbance readings at 650 nm were recorded using a UV-visible spectrophotometer. Each measurement was performed three times to ensure accuracy and consistency.

Keratinolytic microorganism isolation

Sampling of soil

Samples of soil were collected from fields near Shaheed Benazir Bhutto University. Samples were inoculated onto Potato Dextrose Agar (PDA) for fungal isolation and Nutrient Agar (NA) for bacterial isolation after being serially diluted up to 10^{-5}.

Identification and isolation of fungi

Different fungal colonies were purified by sequential sub-culturing on potato dextrose agar (PDA) slants in order to ensure axenic cultures. Both morphological and microscopic identification were accomplished using lactophenol Cotton Blue staining, and examination was conducted using a light microscope set to 100× magnification.

Screening for proteolytic activity

The Riffel and Brandelli methodology was used to evaluate the keratinolytic capability of fungal isolates on skim milk agar (SMA). The SMA medium consisted of 12 g/L agar, 100 mL/L skim milk, 3 g/L yeast extract, and 5 g/L peptone. At 28–32 °C, infected plates were incubated at intervals of 3, 6, 9, and 12 days.

An indication of proteolytic activity was the formation of clear zones surrounding fungal colonies. The diameter of these hydrolysis foci was quantified in order to assess enzymatic efficiency.

RESULTS AND DISCUSSION

Selection of sources

Out of the two types of feathers tested, white feathers were selected because they were more soluble and produced more keratin than black feathers. Although the greater melanin content of black feathers impedes their solubility and lowers the efficiency of the processing, the lesser pigmentation of white feathers facilitates greater accessibility of keratin. The present results agree with other studies that have demonstrated white feathers are more suitable for obtaining keratin by alkaline and acid methods (Sharma *et al*., 2018; Babuaro *et al*., 2025).

Extraction of keratin

Keratin from white feathers was successfully extracted using alkaline hydrolysis with NaOH, followed by acid precipitation with HCl. According to earlier studies showing colour changes under harsh chemical treatment, the extracted keratin appeared black and porous (Dąbrowska *et al*., 2022; Belarimino *et al*., 2012).

Optimization of Sodium Hydroxide concentration

Keratin extraction was assessed using 2.0M and 3.0M NaOH. The 3 M solution produced higher keratin recovery and solubility, which was attributed to better disulphide bond cleavage under more alkaline conditions. Both Shavandi *et al*. (2017) and Vineis *et al*. (2019) reported better extraction with higher NaOH concentration, despite the fact that excessive alkalinity may compromise protein integrity. For further processing, 3.0 M NaOH was selected**.**

Table 1. Sodium Hydroxide Impact on Keratin Extraction

NaOH (M)	Concentration of protein (mg/ml)
2.0	7.07
3.0	99.04

Optimization of HCl concentration on Keratin Extraction

The current study used 3.0 M HCl to achieve the highest protein recovery, demonstrating the effectiveness of the keratin extraction method. This yield exceeds the earlier findings of Tursunova and Maksumova (2024), who discovered that recovery with 5% HCl (roughly 1.4 M) was only moderate. The significance of the current quantitative approach is further highlighted by the findings of Faraon *et al.* (2023), who examined aqueous extraction methods without yield quantification.

Table 2. Total Protein Yield using 3.0M HCl

HCl (M)	Concentration of protein (mg/ml)
3.0	274.18

Time-dependent variability in keratin extraction

After 12, 18, and 24 hours, the keratin yield was evaluated. After 12 hours, when the maximum recovery of 15.5% was attained, the yield slightly decreased. Extensive extraction resulted in minor colour changes and the formation of crystalline structures, possibly due to protein rearrangement during extended treatment (Polesca *et al*., 2023; Shavandi *et al*., 2017).

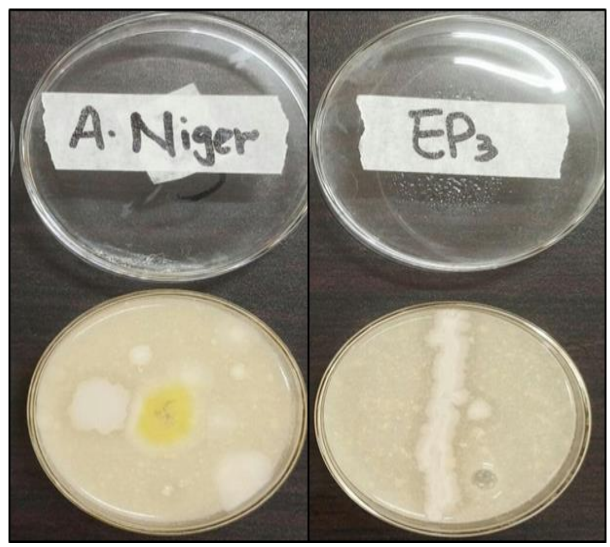
Incubation time (hours)	Protein (mg/ml)
12	216.07
18	139.75
24	173.29

Microorganism isolation and identification

The bacterial strain and the fungal isolate *Aspergillus niger* were among the keratinolytic microorganisms that were isolated from soil samples. *Aspergillus niger* was confirmed by morphological and microscopic characterisation based on conidiophore structure and colony morphology. According to numerous reports, *Aspergillus niger* is a strong keratinolytic fungus. Mazotto *et al*. (2013) used feather-based media to screen several A. niger strains for the production of keratinase. Under solid-state fermentation, strain 3T5B8 demonstrated the highest keratinolytic activity (172.7 U/ml), with distinct hydrolysis zones on keratin agar.The potential of genera like *Bacillus*, *Stenotrophomonas* and *Exiguobacterium* in sustainable waste biodegradation was highlighted by Gerlicz *et al*. (2024), who isolated 113 bacterial strains from keratin-rich environments. These strains exhibited strong keratinolytic activity, particularly against β-keratin substrates.

Keratinolytic potential screening

Aspergillus niger demonstrated a greater zone of clearance on skim milk agar than bacteria, indicating greater proteolytic activity and a greater ability to degrade keratin. These results are in line with those of Lopes *et al*. (2011) and Ogbonna & Ogbonna (2019), who discovered that *A. niger* produced a significant amount of extracellular protease in 48-72 hours.



Figuer 1 *:* Aspergillus niger alongside EP3 proteolytic efficiency comparison based on zone of clearance diameter.

CONCLUSIONS

According to this study, poultry feathers especially white feathers from broilers are a good source of keratin. The highest protein recovery was obtained with optimized chemical treatments that included NaOH and HCl (3.0M) and after 12-hour incubation period. *Aspergillus niger* was found to be the most effective strain through microbial screening, demonstrating exceptional proteolytic activity. The efficiency of keratin recovery was increased by combining chemical and microbiological methods. All things considered, these results are in line with the goals of the circular economy and promote the sustainable valuation of feather waste.

Conflict of interest

Authors declare no conflict of interest.

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