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COMPARATIVE YIELD OF KERATIN EXTRACTED FROM NATIVE AND BROILER FEATHERS USING CHEMICAL AND BIOLOGICAL DEGRADATION

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Abstract: Poultry feathers, a major waste product of the chicken industry, are often discarded despite being a rich source of keratin, a protein valued for its strength and range of commercial uses. Keratin's exceptional biodegradability and biocompatibility have made it extremely popular in recent years. The efficiency of keratin extraction from native and broiler chicken feathers was investigated in this study using both chemical and biological degradation. Feathers were treated with sodium hydroxide (NaOH) and hydrochloric acid (HCl) was used to precipitate the keratin during the chemical recovery process. The extraction time, acid treatment, and NaOH concentration were all optimized in order to boost yield. The best results were obtained with 3.0M NaOH and 3.0M HCL and 12 hours of treatment, which formed black, crystal-like keratin with highest recovery, but longer duration reduced yield. Comparative results showed that native feathers had superior structural qualities, as evidenced by their consistently higher keratin release when compared to broiler feathers. Microbial strains Penicillium and Bacillus Licheniformis were screened on skim milk agar as part of the biological degradation process. By creating hydrolysis zones, both showed that they could degrade keratin, but Penicillium was more successful. These findings show that native feathers are the best raw material for recovering keratin and using chicken feather waste sustainably through microbial degradation and chemical optimization

Key words: Waste degradation; Chicken feathers, Keratinase

INTRODUCTION

All domesticated birds, particularly those prized for their meat and eggs, like chickens, ducks, geese, turkeys, and guinea fowls, are considered poultry. Chickens make up 63% of all bird breeds (Nkukwana, 2018). The world's population is expected to grow by 2 billion people over the next 27 years due to its rapid expansion. By 2050, it is expected to have grown to an incredible 9.7 billion. As the population grows, so does the demand for and availability of food (Bist *et al*. 2017). The main sources of animal protein in people's diets around the world are eggs and poultry meat. Since waste is no longer usable, it must be disposed of. The poultry industry produces a variety of waste products, including solid waste and wastewater (Muduli *et al.* 2019).These days, the poultry industry produces a lot of solid waste, such as bedding, excreta, feed, feathers, hatchery trash, mortality waste, and waste water, such as urine, sawdust, faeces and leftover medication and pesticide residues, as well as waste from disinfecting chicken houses and abattoirs (Babuna *et al.*2005). Chicken waste also produces a lot of greenhouse gases, ammonia, aerosols, andother pollutants that could worsen environmental pollution and climate change. Furthermore, it could spread germs and diseases, endangering human health (Zhang *et al*., 2023).Worldwide; the poultry industry produces a lot of feather waste (Sinkiewicz and Staroszczyk, 2017). Feathers make up 5% to 10% of a bird's body weight, and the global poultry industry produces millions of tonnes of feather waste annually (Karuppannan *et al*. 2021). Because chicken feathers harbour a variety of diseases and pathogens, including Salmonella and Vibrio, feather waste is a significant problem that could lead to environmental contamination. The potential for feather waste to release a variety of pollutants, including ammonia, nitrous oxide, and hydrogen sulphide, has sparked worries about both environmental safety and human health (Zhang *et al*. 2017). There is no appropriate way to recycle chicken feathers. Therefore, it is crucial to devise a process for converting waste feathers into new materials that may be economical and efficient from an environmental and financial standpoint produces a lot of greenhouse gases, ammonia, aerosols, and other pollutants that could worsen environmental pollution and climate change. Furthermore, it could spread germs and diseases, endangering human health (Zhang *et al*., 2017).Worldwide; the poultry industry produces a lot of feather waste (Sinkiewicz and Staroszczyk, 2017). Feathers make up 5% to 10% of a bird's body weight, and the global poultry industry produces millions of tonnes of feather waste annually (Karuppannan *et al.* 2021). Because chicken feathers harbour a variety of diseases and pathogens, including

Salmonella and Vibrio, feather waste is a significant problem that could lead to environmental contamination. The potential for feather waste to release a variety of pollutants, including ammonia, nitrous oxide, and hydrogen sulphide, has sparked worries about both environmental safety and human health (Zhang *et al*. 2017). There is no appropriate way to recycle chicken feathers. Therefore, it is crucial to devise a process for converting waste feathers into new materials that may be economical and efficient from an environmental and financial standpoint (Wang and Cao, 2012). 90% of bird feathers are made of keratin, an insoluble, fibrous, recalcitrant protein that is also a necessary auxiliary material in many organs. Keratin has a fibrous structure and is found in many ecosystems (Bomou *et al*. 2016). Keratin protein has many uses in leathering, textiles and agriculture. It can also be used to make wood adhesives, biomaterials, biomedicines, bio-remediation, flame retardants, and bio composites (Zhang *et al.* 2017). Keratin is a fibrous structural protein that can be broadly divided into two types: α-keratin and β-keratin. Feathers have a complex keratin structure consisting of barbs, barbules, and a central rachis. Barbules grow from barbs, which branch from the rachis. The rachis is rich in β-sheet structures, while barbs and barbules have a higher percentage of α-helices (Thanakorn *et al*. 2025). Chicken feathers typically have 14.2% random coils or turns, 32.2% α-helix, and 53.6% β-sheet (Joanna *et al.* 2012). Feathers, hair, and nails are examples of keratin wastes that are challenging to manage due to their robust, unbreakable structure. These wastes are typically disposed of by incineration, landfilling, composting, and mechanical grinding (Feroz *et al.* 2020). However, the environment is severely harmed by these traditional methods. Both aquatic and terrestrial ecosystems may suffer as a result of their frequent releases of dangerous chemicals and pollutants into the atmosphere (Ujjwal *et al*. 2021). Finding sustainable and eco-friendly solutions has become more important as a result of these issues. A workable alternative is to use keratin-degrading microorganisms, also known as keratinophilic bacteria. These organisms have the ability to spontaneously break down keratin and convert it into simpler, more useful compounds (Feroz *et al*. 2020). To guarantee the proper use of keratin protein for a range of industrial applications, numerous attempts have been made to degrade keratinous wastes. Chemical, biological
enzymatic, and hydrothermal processes are frequently used for hydrolysing keratin wastes. The phrase "circular bio-economy" has been used to describe bio economy activities that are integrated into the more recent policy emphasis on circularity. A circular economy's two guiding concepts are to reduce service loss over time and maximize the service provided by the resources used to make things. Keratin extraction from chicken feathers using chemical and microbiological techniques has been the subject of recent research and small-scale projects (Murtaza and Megha, 2022).
Using bacteria that generate keratinase, researchers have investigated the microbial breakdown of feathers, allowing for environmentally acceptable recycling (Faisal *et al.,* 2020).

MATERIALS AND METHODS

Sample collection

Native and grill chicken feathers were gathered from poultry farms and cleaned for future use. The feathers were washed four times using clean water and detergent. This assisted in eliminating all impurities, such as dust, grime, bloodstains, and any leftover particles. After that, the feathers are spread out gently and left in the sun for three days to dry. Once the feathers are completely dry, place them in clean plastic bags and store them at room temperature.

Extraction of Keratin from Chicken Feathers

Feathers (10 gm) were dissolved in an alkaline solution (10 gm of sodium hydroxide (NaOH) and 250 ml of distilled water).To neutralize the alkali and induce keratin to precipitate, 50 ml HCl were gradually added to the mixture after it had been left at room temperature for 24 hours. The precipitate that formed at the bottom of the flask was proof that the keratin had been successfully recovered. The resulting keratin was appropriately dried in a hot air oven after this precipitate was filtered through filter paper. After drying, the keratin was carefully scraped off the Petri plate and stored for later use.

Determination of total Protein content

A total protein test was performed to determine the amount of protein obtained after the keratin was extracted from the chicken feathers (Lowry *et al*., 1951).

$$\text{Total protein (mg/ml)} = \frac{\text{OD of test}}{\text{OD of standard}} \times \text{Concn. of standard}$$

Optimization of keratin yield

Three factors were changed in order to optimize the keratin yield: extraction time, diluted hydrochloric acid (HCl), and sodium hydroxide (NaOH) concentration (2.0 and 3.0 M solution).Following alkaline extraction, which produced keratin precipitation, the function of diluted HCl was investigated. Keratin was extracted from feathers using three distinct time intervals: 12, 18, and 24 hours.

Biological degradation of Keratin

Microbial strains were used to study the biological breakdown of keratin and evaluate how well they degraded it. To test for proteolytic and keratinolytic activity, two organisms, the bacterium Bacillus Licheniformis and the fungus Penicillium were cultivated on skim milk agar. The microbe's capacity to break down keratin was demonstrated by the clear zones of hydrolysis surrounding the colonies, which verified the enzymatic breakdown of proteins. Although both strains were effective, according to comparative results, Penicillium produced larger zones and showed a stronger capacity to degrade keratin than bacteria. This implied that compared to bacterial strains, fungal strains might affect feather keratin more quickly and effectively.

RESULTS AND DISCUSSION

Selection of Source

The results demonstrated that native feathers produced more keratin and had a higher initial feather weight than broiler feathers. Additionally, native feathers appeared to have a denser, harder texture that would help them withstand chemical treatment without degrading. They were therefore more successful in keratin recovery. According to Karthikeyan *et al*. (2007), broiler chickens and native chickens have very different feather structures. The keratin content and processing endurance of native birds are increased by their thicker, more fibrous feathers. According to Onifade *et al*. (1998), native breeds typically have stronger, more resilient feathers that are better suited for keratin extraction. The physical characteristics of feathers, such as density and thickness, are also influenced by the species, age, and rearing conditions.

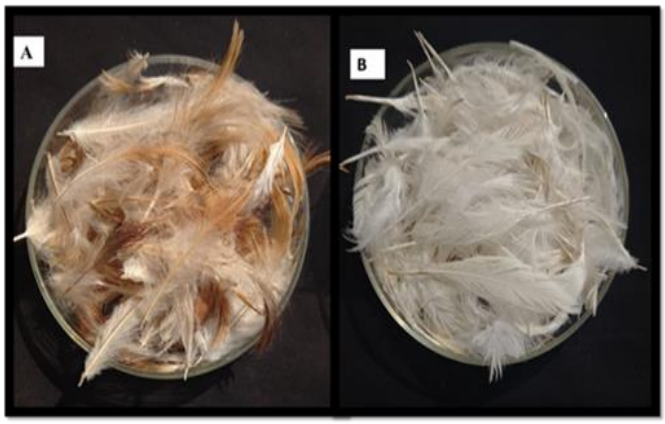

Fig 1. (A) Native feathers (B) Broiler feathers

Extraction of Keratin

From Native chicken feathers 1.0 gm of keratin were extracted by using 10 gms of NaOH and 50 ml of HCl, producing a porous, black product.

Keratin yield optimization using different conditions

A number of parameters, including variations in NaOH concentration, acid precipitation, and extraction time, were changed to achieve the highest keratin production. After adjusting these parameters, different outcomes were seen in terms of texture, color and keratin yield.

Optimization of NaOH for Keratin Extraction

Two different concentrations of sodium hydroxide, 2.0 M and 3.0 M were used to observe the effectiveness of keratin extraction (Table 1). It was observed that when 2.0 M sodium Hydroxide was used, finer powder keratin was extracted. On the other hand, keratin yield black in color and final weight were considerably higher after extraction with 3.0 M NaOH.

Fig 2: keratin extracted from native chicken feathers

The keratin that was formed is in dark black color and it's in a shiny powder texture. These results are consistent with those of (Yeo *et al.* 2018), who assessed various NaOH molarities and discovered that keratin solubilization was much enhanced by higher concentrations.

Table 1: Effect of Sodium Hydroxide on keratin yield

Sodium Hydroxid	Feathers weight (gm)	Keratin weight (gm)	Keratin Yield %
2.0 M	2.0	0.071	3.6
3.0 M	2.0	0.300	15

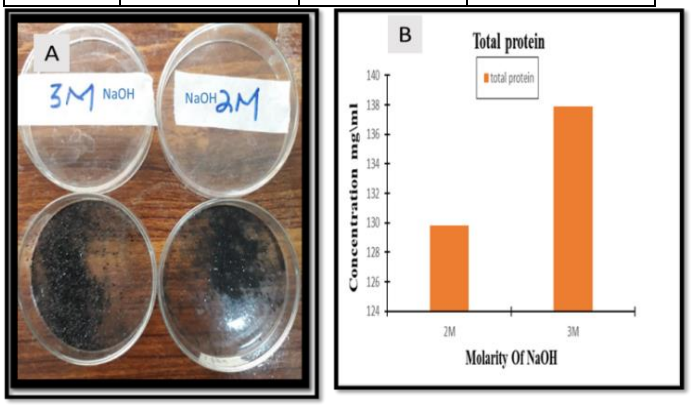

Fig 3. Keratin from optimization of NaOH (A), Effect of NaOH on Keratin yield (B).

Optimization of HCl for keratin extraction

In order to achieve a higher keratin yield in the initial optimisation, we used 3.0 M NaOH; consequently, we used this concentration for the subsequent experiment. To see how acid precipitation might impact the keratin extraction process and keratin yield, we use 3.0 M diluted hydrochloric acid (HCl) rather than concentrated HCl (Table 2).

Table 2: Effect of HCl on keratin yield

Molarities	Feathers weight (gm)	Keratin weight (gm)	n yield %
3.0 M	2.0	0.100	5.0

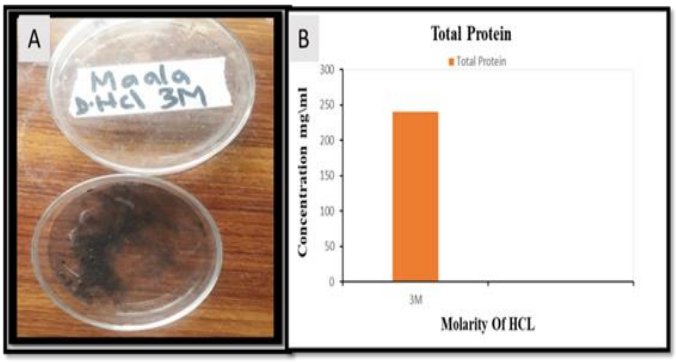

Fig 4. Keratin from optimization of HCl (A), Effect of HCl on Keratin yield (B).

The output of keratin was found to be low. Furthermore, less greyish black precipitate was generated, and the final keratin powder had a rough, greyish colour (Fig 4). This outcome is consistent with studies on feather keratin that employed pre-treatments with ethanol and HCl (Zhou *et al.* 2013). They discovered that excessive HCl significantly disrupted peptide bonds, lowering yield and molecular weight and producing darker, damaged keratin forms.

Table 3: Effect of HCl Optimization on keratin yield

e Interval (Hours	thers weight (gm)	tin weight (gm	ratin Yield %
12	2.0	0.25	15.0
18	2.0	0.30	12.5
24	2.0	0.64	32.0

These results are in line with a previous study by Dąbrowska *et al.* (2022), who enhanced the alkaline hydrolysis of feather keratin and found that longer reaction times increase protein solubilisation, but excessive hydrolysis reduces overall yield and integrity. In their study, yields were 24%, 37%, and 41% at 16, 24, and 32 hours, respectively; however, longer times resulted in lower-quality protein and more fragmentation.

Screening of Keratinase activity on milk agar plates

After screening fungal isolates on milk agar plates for 24 hours, the clear zones surrounding the fungal colonies showed evidence of proteolytic activity. Both *B. licheniformis* and *Penicillium* grew on milk agar plates. Nevertheless, compared to bacteria, Penicillium exhibited larger clear zones surrounding the colony, indicating higher keratinase activity

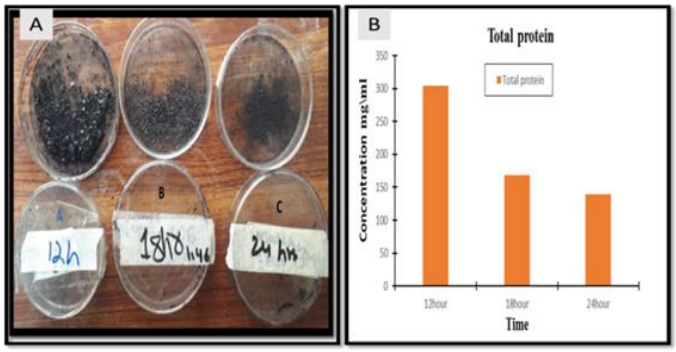

Fig 5. Keratin from HCl at different time interval (A), Effect of time on Protein content of extracted Keratin (B).

Penicillium breaks down more keratin than bacteria, which have smaller, less transparent zones (Fig 6). Ogbonna and Ogbonna's (2019) study found that *Penicillium purpurogenum* produced notable clear zones on milk agar, a sign of high proteolytic and keratinolytic activity. This outcome is consistent with what they discovered. Filamentous fungi such as Penicillium are more versatile in their ability to degrade complex proteins like keratin than bacterial isolates, which may account for these differences in enzyme synthesis.

Fig.6: Inhibition of keratin by *Bacillus licheniformis* (A), and *Penicillium* (B)

CONCLUSIONS

This study looked into poultry feathers, a major waste product of the poultry industry, as a potential source of keratin. Keratin was extracted from Native and Broiler chicken feathers under ideal incubation, HCl, and NaOH treatment conditions. Desi feathers consistently yielded higher yields, so they were selected for further work. *Penicillium* and *B. licheniformis*, two soil-derived microbial strains, were also isolated and their keratinase activity examined. Because of its greater capacity for proteolysis and ability to form larger clear zones, *penicillium* was chosen for advanced keratin degradation research.

Conflict of interest

Authors declare no conflict of interest.

REFERENCES

Babuna, F. G., S. Dogruel, and D. Orhon, (2005) Poultry waste management. Food Sci. Tech. Eng. Handb, 7, 2608–2614.

Bist, R. B., K. Bist, S. Poudel, D. Subedi, X. Yang, B. Paneru, S. Mani, D. Wang and L. Chai (2024) Sustainable poultry farming practices: A critical review of current strategies and future prospects. *Poult Sci.*,103 104295. doi: 10.1016/j.psj.2024.104295

Bomou, M., X. Qiao, X. Hou, and Y. Yang, (2016) Pure keratin membrane and fibers from chicken feather. *Int. J. Biol. Macromol.* 89, 614-621.

Dąbrowska, M., A. Sommer, I. Sinkiewicz, A. Taraszkiewicz, and H. Staroszczyk, (2022) An optimal designed experiment for the alkaline hydrolysis of feather keratin. Environ *Sci Pollut Res Int*, 29, 24145-24154.

Faisal J., M. G. Shahid and A. Javed (2020) Effect of Different Operational Parameters on Bio-degradation of Chicken Feathers by Aspergillus niger: Investigation Under Submerged Fermentation Process. *Pak. J. Sci. Ind. Res. Ser. B: Biol. Sci.* 63, 71-76.

Feroz, S., S. Sumaira, M. N. Aftab, and M. J. Asad, (2020) Biodegradation of keratin waste by keratinolytic microorganisms and its application. *Waste Biomass valori,*, 11, 1963–1977.

Joanna, M., P-Y. Chen, S. G. Bodde and W. Yang (2012) The Structure, Functions, and Mechanical Properties of Keratin. J. Minerals, Metals & Materials Soc. 64, 449-468.

applications of keratin-degrading microorganisms and their enzymes for nutritional improvement of feathers and other keratins as livestock feed resources. *Bioresour. Technol.*, 66, 1–11.

Sinkiewicz, I., A. Śliwińska, H. Staroszczyk, and I. Kołodziejska, (2017) Alternative methods of preparation of soluble keratin from chicken feathers. *Waste Biomass valori,* 8, 1043-1048.

Thanakorn M., L. Salaipeth, A. E. Cope, M. J. Taherzadeh , T. Watanabe and P. Phitsuwan (2025) Current Understanding of Feather Keratin and Keratinase and Their Applications in Biotechnology. *Biochem Res Int.* 22;2025:6619273. doi: 10.1155/bri/6619273

Ujjwal S., N. Gupta, K, K. Saxena (2021) Comparative study on the effect of industrial by-products as a replacement of cement in concrete. *Mater. Today Proc*. 44, 45-51.

Wang Y. X., and X. J. Cao (2012) Extracting keratin from chicken feathers by using a hydrophobic ionic liquid. *Process Biochem*. 47, 896-899.

Zhang, L., J. Ren, and W. Bai (2023) A Review of Poultry Waste-to-Wealth : Technological Progress, Modeling and Simulation Studies, and Economic- Environmental and Social Sustainability. *Sustainability*, 15. 5620; https://doi.org/10.3390/su15075620.

Zhao, Y., Q. Liu, and X. Zheng, (2020) Feather keratin: Structure, function, and application. Materials Science and Engineering: C, 107, 110289

Karthikeyan, R., B. Srinivasan, and P. K. Sehgal (2007) Industrial applications of keratins – A review. *J. Sci. & Ind. Res*., 66, 710-715.

Karuppannan, S. K., M. J. H. Dowlath, G. I. D. Raiyaan, S. Rajadesingu, and K. D. Arunachalam (2021) Application of poultry industry waste in producing value-added products—A review. Concepts of advanced zero waste tools, 91-121.

Li, Q. (2019). Progress in microbial degradation of feather waste. Front. Microbiol. 10:2717. doi: 10.3389/fmicb.2019.02717

Lowry, O. H., N. J. Rosebrough, A. L. Farr and R. J. Randall (1951) Protein measurement with the Folin phenol reagent. *J. Biol. Chem*.193, 265-275.

Muduli, S., A. Champati, H. K. Popalghat, P. Patel, and R. K. Sneha, (2019) Poultry waste management: An approach for sustainable development. *Int. J. Adv. Sci. Res*., 4, 8–14.

Murtaza, H. and, G. Megha (2022) Microbial degradation of keratin from poultry waste. *Int. J. Curr. Sci*. 12, 337-346.

Nkukwana, T. T. (2018) Global poultry production: Current impact and future outlook on the South African poultry industry. *S. Afr. J. Anim. Sci*., 48, 869-884.

Ogbonna, A. I., and C. I. C. Ogbonna, (2019) Keratin degradation by *Aspergillus niger* and *Penicillium purpurogenum* isolated from Nigerian soil. *Food Sci Nutr Technol*, 4, 1-5.

Onifade, A. A., N. A., Al-Sane, A. A., Al-Musallam, and S. Al-Zarban (1998) review: Potentials for biotechnological

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