



Harnessing the potential of feather keratin: Valorization of a wasted biomass.

Ashley Wambui Kihonge

Corresponding Author: [ashleykihonge@gmail.com](mailto:ashleykihonge@gmail.com);

**Received 9<sup>th</sup> February, 2024; Accepted 21<sup>st</sup> May 2024; Published online 30<sup>th</sup> June 2024**

**Citation:** Wambui Kihonge, A. Harnessing the Potential of Feather Keratin: Valorization of a Wasted Biomass. African Journal of Pure and Applied Sciences, 5(1). <https://doi.org/10.33886/ajpas.v5i1.497>

## Abstract

Dependence on the finite resource, fossil fuel, to obtain petroleum-based materials has caused adverse environmental decline both in the acquisition and disposal of the materials. To remedy this, a sustainable raw material stream is needed that is both eco-friendly and biodegradable. Feathers, a byproduct of poultry processing, emerge as a sustainable and renewable resource. Feathers have high keratin content and are biocompatible, non-toxic, and biodegradable. The current uses of the feathers are limited leading to a majority of the feathers being disposed of in an environmentally detrimental manner. To obtain the keratin, numerous extraction techniques are employed and consequentially the extracted keratin can be utilized in the creation of valorized products. This paper aims to review various keratin extraction techniques and provide a brief overview of current applications of extracted feather keratin.

## Keywords

feather keratin, valorization, keratin extraction, applications

## 1. Introduction

Over-reliance on petroleum-based materials has put a strain on the already finite resource and concurrently its use led to adverse environmental problems. This has necessitated the development of more sustainable materials that are also eco-friendly. The advent of this rapid development has seen the use of biopolymers as a sustainable and potential stream of materials to replace petroleum-based materials. One such biopolymer is keratin (Nuutinen *et al.*, 2021). Keratin is a fibrous structural protein forming the fundamental components of various parts of animals such as hooves, horns, hair, feathers, beaks, fur, wool, nails, skin, quill, and slime (Feroz *et al.*, 2020; Reddy, 2017). A number of studies have been carried out to evaluate and optimize the purification, extraction, characterization, and applications of keratin from various

sources (Chilakamarry *et al.*, 2021). Feather keratin in particular is a significant source of keratin.

In order to meet the high demand for meat and eggs, billions of poultry are reared each year. The processing of poultry generates a significant amount of feather waste, as feathers make up approximately 5% of a bird's body weight (Qin *et al.*, 2023). The majority of the feathers from poultry processing industries are discarded with only a small percentage being used as animal fodder, composting, or packaging. The feathers are often disposed of in landfills, by incineration, or in open-air dumps. These disposal methods pose health and environmental risks namely; leachate from landfills contaminates groundwater, zoonotic diseases borne from the discarded feathers, incineration requires large amounts of energy and causes carbon dioxide emissions, and composting causes the emission of hydrogen sulfide (Kamaraj and Vuppu, 2024; Sinkiewicz *et al.*, 2017). Therefore, there is need to consider alternative methods to deal with feather waste as it is a sustainable and renewable resource that can be used in the processing of soluble keratin for the development of valorized products.

Some of the feathers have been transformed into useful industrial and commercial products. Chicken feather peptone, for example, has been used in the production of adsorbents (Okoya *et al.*, 2020), construction material (Colunga-Sánchez *et al.*, 2019), computer parts (Wool and Sun, 2005), bio-fertilizers (Khalil *et al.*, 2020), pigment (Dwivedi and Kaneko, 2018), microbial cultivation (Akpore *et al.*, 2019), lactic acid (Taskin *et al.*, 2012), citric acid (Ozdal and Kurbanoglu, 2018), bio-surfactant (Ozdal *et al.*, 2017), xanthan gum (Ozdal and Kurbanoglu, 2019), and polyhydroxyalkanoates (Benesova *et al.*, 2017). Despite its application versatility, the feather waste is hard to recycle and its chemical inertness renders it barely usable.

The keratin composition renders the feathers recalcitrant to degradation by a majority of common proteolytic enzymes (proteases) such as papain and trypsin. To convert the feathers into soluble peptides for use in the creation of products the keratin has to be broken down chemically, physically, or through microorganisms (Pandey *et al.*, 2019).

### ***1.1 Keratin composition and structure***

The stability of keratin is ensured by the dense arrangement of peptide chains and the presence of cross-links formed by disulfide bonds, hydrophobic interactions, and

hydrogen bonding. In the polypeptide chain, polar amino acids can create a molecular potential that helps stabilize keratin by electrostatic interaction and interconnections between hydrogen bonds (Alashwal *et al.*, 2020; Oluba *et al.*, 2022). Plate 1 shows the bonds in keratin structures that maintain their stability and contribute to the toughness and strength of keratin. The three main sub-types of keratin, namely  $\alpha$ -keratins,  $\beta$ -keratins, and  $\gamma$ -keratins.  $\alpha$ -keratins are fibrillar and comprised of polypeptide chains in an alpha helical structure, whereas, the polypeptide chains in  $\beta$ -keratins are in the form of pleated  $\beta$ -sheets, and those of  $\gamma$ -keratins are globular in shape.  $\beta$ -keratins are synthesized in avian feathers, claws, and beaks (Feroz *et al.*, 2020; Hill *et al.*, 2010).

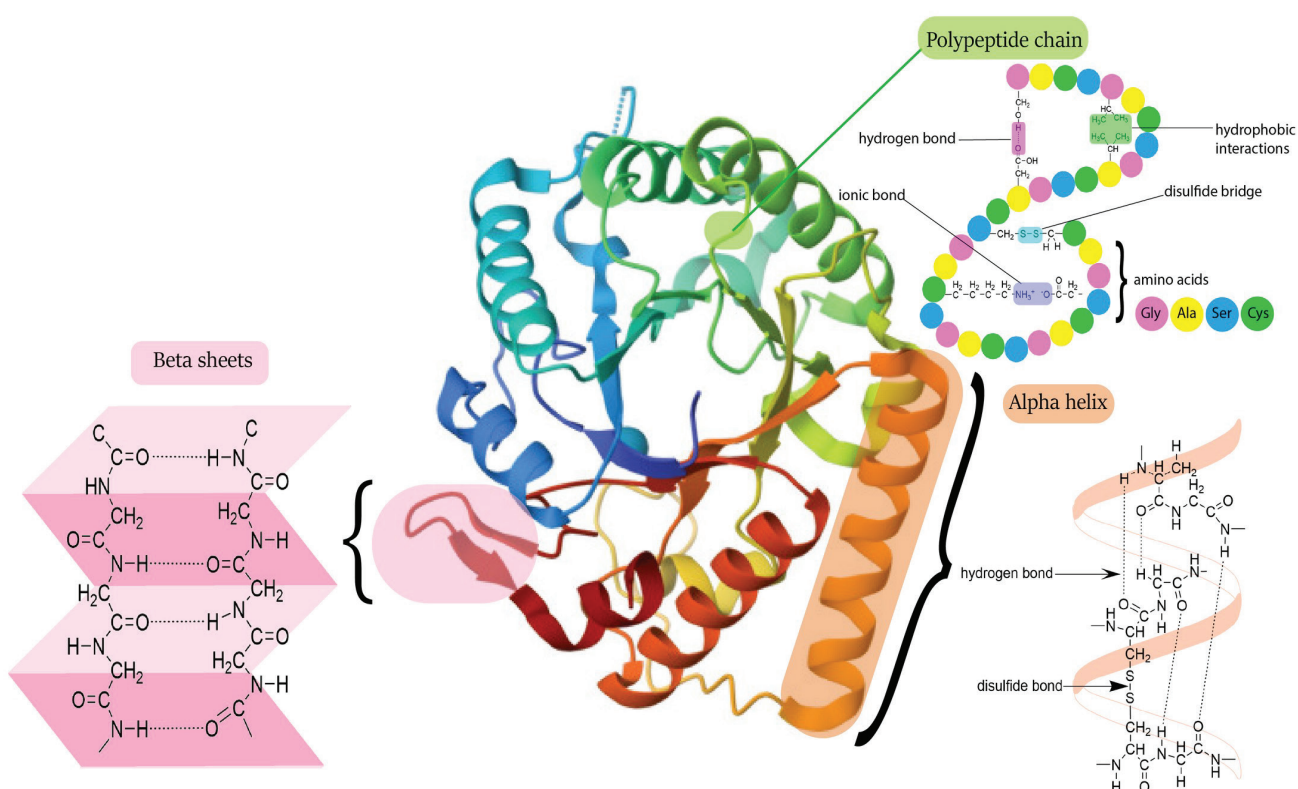


Plate 1: Bonds in keratin structures that maintain their stability. Image of 3D structure from the Research Collaboratory for Structural Bioinformatics Protein Data Bank ([RCSB.org](https://www.rcsb.org)) of PDB ID 6UJF (Scott *et al.*, 2020).

## 1.2 Feather keratin

Feather fibers have a crystal structure giving them their natural longevity and stability. The main components of the feather are the rachis, barbules, and barbs. Each part of the feather contains keratin. Plate 2 shows the general anatomy of the bird feather. A feather is made up of 90% keratin with an approximate molecular weight of 10.5 kDa. The main amino acids present are alanine, cysteine, glycine, serine, and valine. The feather keratin molecules' structure has  $\beta$ -pleated sheets in the ordered regions of the polypeptide chain and other disordered structures with the feather fiber containing more  $\alpha$ -helix structures than  $\beta$ -pleated sheets. The strength of the feather is derived from disulfide bonds with cystine molecules of which 7~13% of the feather keratin is cystine, hydrogen bonds, and hydrophobic interactions (Azmi *et al.*, 2018; Maurya and Singh, 2023). Costa *et al.*, (2012), did a study and found that the feather contains  $99\pm 1.4\%$  worth of volatile solids of which  $92.0\pm 0.48\%$  represents the possible degradable matter which in turn  $88\pm 0.51\%$  constitutes keratin according to a study by Xia *et al.*, (2012).

Feathers are abundant and possess properties that make them exceptional as a feedstock for their valorization into value-added products such as biocompatibility, biodegradability, absorbability, non-toxicity, and bioactivity, which allow them to be utilized across a wide range of industries (Khalilipourroodi *et al.*, 2022; Qin *et al.*, 2023). Due to strong internal interactions within the keratin structure, it is difficult to process and extract keratin from feathers and as such would require techniques to destabilize the structure of keratin to be able to valorize its basic components.

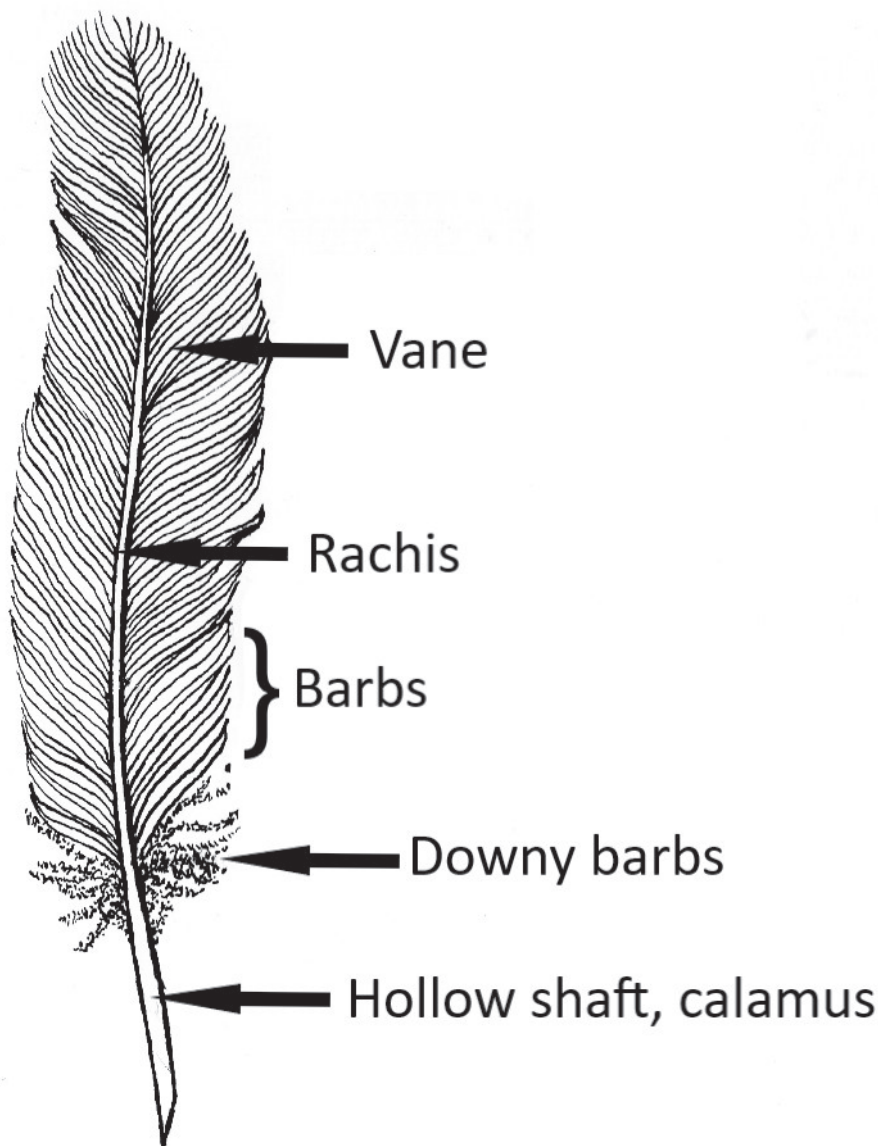


Plate 2: General anatomy of a bird feather (Foresman, 2019).

## 2. Extraction techniques for the valorization of feather keratin

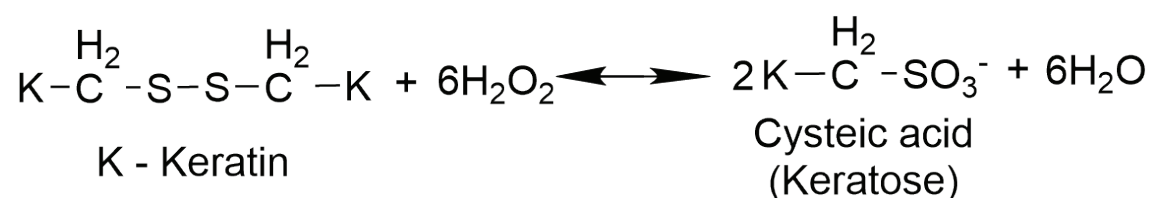
Several factors come into play when dissolving and extracting keratin from various biomasses. Its strong physical and mechanical properties render it difficult to process without the aid of external intervention be it chemical, physical, or even microbial. Techniques have been developed to extract and/or solubilize keratin. These techniques target the various bonds and structural components that stabilize keratin (Sinkiewicz *et al.*, 2017). Each method of keratin extraction has its own advantages and drawbacks, and the choice of method should be based on factors such as potential applications, desired yield, quality of keratin, environmental impact, time, and cost considerations.

## 2.1 Chemical methods of keratin extraction

### 2.1.1 Oxidative extraction

Oxidative methods employ oxidants leading to the formation of Keratoses. The most commonly used chemical oxidants include hydrogen peroxide, potassium permanganate, sodium hypochlorite, and organic peracids. Keratoses are characterized by a non-covalent cross-linking within the structure where in place of disulfide bonds there are sulfonic acid groups and cysteic acid (Martinez *et al.*, 2020; Sinkiewicz *et al.*, 2017).

Fernández-d'Arlas (2019) conducted a study on the preparation of a novel bioplastic film derived from chicken feather keratin by use of oxidative keratin extraction method. Hydrogen peroxide was employed as a bisulfide splitting agent with the proposed reaction being as shown, K representing the rest of the keratin polypeptide chain:



The extraction method shows that no toxic by-products were generated where the only products were hydroxide, oxygen, and water and the yielded keratin was at 87.1% (Fernández-d'Arlas, 2019).

Keratin, a fibrous protein, is especially vulnerable to damage from strong chemical treatments like oxidation. When feathers are exposed to harsh oxidizing agents, the keratin molecules can break down, reducing the quality and functionality of the extracted keratin. Extraction by oxidation using peracetic acid and performic acid has drawbacks, including incomplete oxidation of cystine to cysteic acid and the loss of some amino acids (Feroz *et al.*, 2020). Additionally, oxidizing agents can introduce impurities into the extracted keratin, affecting its suitability for various applications. Furthermore, oxidizing agents such as bromine, permanganate, and hydrogen peroxide tend to break disulfide bonds slowly, thus slowing down the protein extraction process. In contrast, reducing agents act quickly and dissolve keratin efficiently (Gupta *et al.*, 2012; Khumalo, 2021).

### 2.1.2 Reductive extraction

Extraction of keratin by reduction involves the keratin being reduced into kerateines. The most common chemicals used in the reduction process are thiols and phosphines. In terms of stability, kerateines are superior in stability when compared to keratoses (Martinez *et al.*, 2020; Vineis *et al.*, 2019). The general reductive process involves the incorporation of an ionizable carboxylic group from the reducing agent which will favor keratin solubilization. The bisulfide bond in alkaline conditions makes it labile thus allowing substitution (Fernández-d'Arlas, 2018). A summary of the global reaction is as follows, K representing the rest of the keratin polypeptide chain and R representing the reducing agent:



K - Keratin

R - Reducing agent

Nur-E-Alam *et al.*, (2019) conducted a study to establish the optimal concentration of sodium sulfide for the extraction of keratin from chicken feathers. The optimal conditions were concluded to be at 30°C, pH of 10-13 with 0.5M sodium sulfide which had the highest yield of 63.25%. The reduction reaction involves the cleavage of disulfide bonds, hydrogen bonds, and salt linkages making keratin fibers dissolve (Nur-E-Alam *et al.*, 2019). A research study by Ge *et al.*, (2021) on the fabrication and characterization of a keratin hydrogel extracted by reduction with L-cysteine from rabbit hair. The rabbit hair was cleaned with ethanol, oven-dried, cut up, and put into the extraction solution which contained urea, L-cysteine, and NaOH. After drying and grinding to a powder, the extraction yield was 61% (Ge *et al.*, 2021). Table 1 is a summary of other studies and researches done on keratin extraction by reduction.

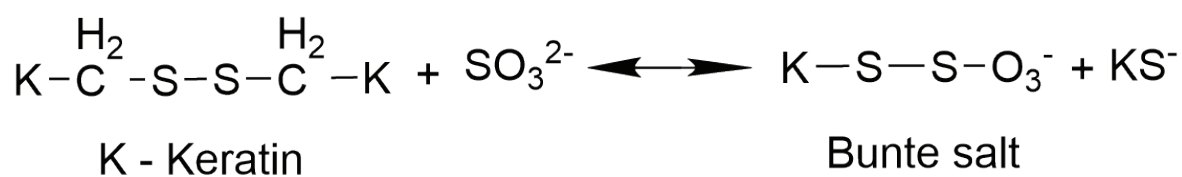
| Keratin source  | Reducing agent | Reaction Conditions   | Yield % | Reference                       |
|-----------------|----------------|-----------------------|---------|---------------------------------|
| Chicken Feather | Sodium Sulfide | pH 10-13,<br>6h, 30°C | 63.3    | Nur-E-Alam <i>et al.</i> , 2019 |

|                 |                                                                                                              |                                                |                                                                      |                                 |
|-----------------|--------------------------------------------------------------------------------------------------------------|------------------------------------------------|----------------------------------------------------------------------|---------------------------------|
| Chicken Feather | i. 2-mercaptoethanol<br>ii. Dithiothreitol (DTT)<br>iii. Sodium- <i>m</i> -bisulfite<br>iv. Sodium bisulfite | 2h, 50°C                                       | i. 83.8 ± 0.3<br>ii. 77.6 ± 1.4<br>iii. 62.9 ± 1.0<br>iv. 82.4 ± 0.1 | Sinkiewicz <i>et al.</i> , 2017 |
| Chicken Feather | L-Cysteine                                                                                                   | pH 10.5,<br>12h, 70°C                          | 60.0                                                                 | Ma <i>et al.</i> , 2016         |
| Chicken Feather | Sodium Sulfide Nonahydrate (Na <sub>2</sub> S • 9H <sub>2</sub> O)                                           | 30min,<br>50°C                                 | -                                                                    | Dou <i>et al.</i> , 2020        |
| Chicken Feather | Cysteine                                                                                                     | pH 10.5,<br>12h, 70°C                          | -                                                                    | Xu and Yang, 2014               |
| Chicken Feather | Thioglycate                                                                                                  | pH 11, 2h,<br>50-55°C                          | 76.9 ± 0.4                                                           | Fernández-d'Arlas, 2018         |
| Goose Feather   | i. Sodium Sulfide<br>ii. Ethylenediaminetetraacetic acid (EDTA)                                              | i. 1.5h,<br>25°C<br>ii. pH 4.2,<br>2h,<br>40°C | i. 86.3<br>ii. 80.9                                                  | Çakmak, 2022                    |

Table 1: Summary of extraction of keratin from feathers by reduction method

### 2.1.3 Sulfitolysis

Sulfitolysis occurs by the action of sulfite ions (sulfite (SO<sub>3</sub><sup>2-</sup>), bisulfite (HSO<sub>3</sub><sup>-</sup>), and disulfite (S<sub>2</sub>O<sub>5</sub><sup>2-</sup>)) at equilibrium in aqueous solution, which reduces the disulfide bond within keratin. The result of the reaction is the formation of Bunte salt (S-Sulfonate anion) and a thiol (Shavandi *et al.*, 2017; Vineis *et al.*, 2019). A summary of the reaction is as follows, K representing the rest of the keratin polypeptide chain:



Sulfite ions have a faster reaction rate than bisulfite ions when reacting with cystine. The concentration of sulfites increases when the pH is increased, in turn increasing

the rate at which sulfitolysis takes place. At a pH of greater than 7, sulfite is the dominant species while in acidic conditions bisulfite is dominant. At a pH of greater than 9, cystine sulfitolysis is a reversible reaction and the reaction rate decreases with this increase in pH as a result of the repulsion forces between carboxylic anions and sulfite ions (Shavandi *et al.*, 2017).

A study was performed by Isarankura Na Ayutthaya *et al.*, (2015) to extract keratin from chicken feathers with the use of sodium metabisulfite. The extraction was based on the break up of disulfide, hydrogen, hydrophobic, and ionic bonds. The cysteine disulfide bonds were cleaved with sulfite resulting in cysteine thiol and cystein-s-sulfonate. The resulting yield was 81.6% (Isarankura Na Ayutthaya *et al.*, 2015).

#### 2.1.4 Extraction of keratin using ionic liquids

An ionic liquid (IL) is a molten salt consisting entirely of cations and anions, existing as a liquid at temperatures below 100°C (Idris *et al.*, 2013). In recent years, ILs have gained a lot of interest due to their unique combination of properties in various applications including dissolution and regeneration of natural polymer material, catalyst, ion conductive media, polymer modification, polymerization, processing of polymer particles and composites, plasticization and extraction of biomass via organic synthesis (Feroz *et al.*, 2020; Shavandi *et al.*, 2017). As a solvent ILs possess superior physical and chemical properties compared to traditional volatile solvents thus allowing them to be used in a variety of applications. The anions and cations that make up an IL can be chosen and this ability allows for the creation of specifically designed functional ILs as well as numerous combinations of salts (Azmi *et al.*, 2018).

Being observed as eco-friendly and safe solvents, they have the following superior qualities: non-volatility meaning they have little to no molecular solvent discharge as compared to traditional solvents where discharge is mainly through evaporative loss; non-flammability; high thermal stability; chemical stability; low vapor pressure which contributes to their ability to be recovered by distillation after an extraction process and reused; high ion conductivity (Azmi *et al.*, 2018; Feroz *et al.*, 2020). Despite their numerous advantageous qualities, the use of ILs still has some drawbacks, including their expense, limited compatibility, difficulty in separation, and potential toxicity if improperly disposed of, which limits their use in industrial settings. An example of

the high cost is the need for an inert atmosphere for extraction due to the hygroscopic nature of ILs, which requires the use of expensive specialized equipment (Vineis *et al.*, 2019; Shavandi *et al.*, 2017). Table 2 shows a summary of ionic liquids and the keratin yields from their extraction processes.

An advantage of ILs is that they possess a high dissolution ability due to the high polarity which can break down strong inter- and intramolecular bonds in natural polymers (Ji *et al.*, 2014). The dissolution process involves immersing the keratinous materials in the ionic liquid at a specific temperature and a set period. The keratin is normally regenerated by use of a solvent(s) in a coagulation bath (Vineis *et al.*, 2019). Various studies have been carried out to investigate the mechanisms by which keratin from various source materials undergo dissolution using ILs. The dissolution of feathers takes place in two stages, the first being the permeation of the IL into the feather and the second involves the unfolding of the chains of the feather keratin due to solvation effects and reduction of disulfide bonds. The conclusion drawn from this is that the solubility of the feather is linked to the polarity of IL and the yield of keratin increases with an increase in the polarity of IL (Wang and Cao, 2012). Xu and Yang (2014) investigated the influence of different coagulation baths on the mechanical properties of regenerated feather keratin. The three baths were made up of acetic acid mixed with sodium sulfate, ethanol, and methanol respectively. The alcohol coagulation baths offered protein fibers of better mechanical performance than those from the electrolyte solution, (Xu and Yang, 2014). Ma *et al.*, (2016), used a coagulation bath composed of ethanol and acetic acid and concluded that the composition influenced the regenerated feather keratin fibers where by observing the SEM images, micropores were observed which would affect the mechanical properties of the protein fiber (Ma *et al.*, 2016)

| <b>Ionic Liquids</b>                                                                                                                                                                                                              | <b>Temp<br/>(°C)</b> | <b>Time</b> | <b>Solubility<br/>(wt%)</b>      | <b>% Yield<br/>of<br/>Keratin</b> | <b>Referen<br/>ce</b>      |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|-------------|----------------------------------|-----------------------------------|----------------------------|
| 1) Butyl-3-methylimidazolium chloride ([BMIM] <sup>+</sup> Cl <sup>-</sup> )<br>2) Allyl-3-methylimidazolium chloride [AMIM]-Cl)<br>3) Hexyl-3-methylimidazolium trifluoromethane sulfonate [HMIM]CF <sub>3</sub> SO <sub>3</sub> | 90                   | 60 min      | 1) 4.80<br>2) 4.80<br>3) 0.20    | 75.0                              | Ji, <i>et al.</i> , 2014   |
| N, N-dimethyl ethanol ammonium formate formate [DMEA][HCOO]                                                                                                                                                                       | 100                  | 7h          | 2.50                             | 63.0                              | Idris <i>et al.</i> , 2014 |
| 1) 1-Allyl-3-methylimidazolium chloride, [AMIM]Cl<br>2) 1-Butyl-3-methylimidazolium chloride ([BMIM] <sup>+</sup> Cl <sup>-</sup> )<br>3) Choline thioglycolate                                                                   | 130                  | 10h         | 1) 50.00<br>2) 50.00<br>3) 45.00 | 1) 60.0<br>2) 60.0<br>3) 55.0     | Idris <i>et al.</i> , 2013 |
| 1-hydroxyethyl-3-methylimidazolium bis(trifluoromethane sulfonyl)amide ([HOEMIm][NTf <sub>2</sub> ])                                                                                                                              | 80                   | 4h          | -                                | 21.0                              | Wang, and Cao, 2012        |

Table 2: Summary of ionic liquids and the feather keratin yields from their extraction processes

### 2.1.5 Hydrothermal treatment

Hydrothermal treatment is normally carried out at temperatures ranging from 90°C-150°C at 10-15 psi. A base or an acid is often added to aid in the solubilization process (Martinez *et al.*, 2020). The two main methods of hydrothermal extraction include acid hydrolysis and alkaline hydrolysis. In acid hydrolysis, the acids mostly employed are Hydrochloric acid (Zhang *et al.*, 2013) or Sulfuric acid (Bouhamed and

Kechaou, 2017). During the process of acid hydrolysis, a majority of the hydrogen bonds within the keratin structure are broken leading to a more amorphous structure when compared to structures of polypeptides that underwent alkaline hydrolysis. This sometimes results in the loss of some amino acids and the conversion of others into different compounds (Martinez *et al.*, 2020).

Alkaline hydrolysis involves the use of a strong and hot alkali solution to solubilize keratin (Shavandi *et al.*, 2017). At a pH beyond 9, keratin degrades. The process involves the breaking of amide bonds on the primary chain of keratin which in turn disrupts the structure causing amino acids to be freed from the chain (Vineis *et al.*, 2019). Alkaline treatment has the following drawbacks: consumption of high amounts of alkaline chemicals alongside high amounts of acids to neutralize the products of the reaction (Vineis *et al.*, 2019), high temperatures used in the operation lead to further degrading of thermally unstable amino acids and racemization of amino acids from L-enantiomers to D-enantiomers (Martinez *et al.*, 2020). Chicken feathers were pretreated with NaOH to study the effect of alkali treatment on the morphology of chicken feathers. The study concluded that the alkali treatment improved the adhesiveness of the interface due to the removal of hydrophilic components which in turn increased surface roughness (Aranberri *et al.*, 2017).

## **2.2 Physicochemical methods of extraction**

### **2.2.1 Steam explosion**

Steam explosion (SE) is a form of biomass conversion technology mainly employed as a form of pretreatment of renewable resources. The process is based on exposing the biomass to steam at high temperatures and maintaining it for a time ranging from seconds to minutes, which will then be followed by rapid explosive decompression (Wang and Liu, 2010; Zhang *et al.*, 2015). There are variations to the steam explosion techniques developed to streamline the whole process with some examples including steam flash explosion (SFE) (Zhang *et al.*, 2014) and high-density steam flash explosion (Zhao *et al.*, 2016). The technique has the following advantages in comparison to other mechanical methods of pretreatment: it is much more energy-saving, and since water vapor is the main solvent used, it translates to a lower environmental impact as no hazardous chemicals are in use (Wang and Liu, 2010; Zhang *et al.*, 2014).

Zhang *et al.*, (2015), did a study on the feasibility of steam flash explosion (SFE)-assisted alkaline extraction from duck feathers. The alkali used was NaOH. The extraction rate was at 65.78% with a keratin yield of 42.78%. In terms of change in structure, there was a cleaving of the disulfide bonds and hydrogen bonds with the fragmentation of macromolecular chains. The method can scaled up to an industrial level despite its low keratin yield due to low operation cost, reduced energy consumption, and low environmental impact (Zhang *et al.*, 2015). Zhao *et al.*, (2012), developed a new extraction and dissolution process for feather keratin by use of a high-density steam flash explosion. The method's foundation is based on steam explosion with the one caveat being within an extremely short time, the release of high-density energy providing the force needed to disrupt the keratin structure. The resulting keratin showed the destruction of  $\beta$ -sheets crystals and intermolecular disulfide bonds with little alteration to the keratin protein chain, and an increase in accessibility of enzymes into the keratin( Zhao *et al.*, 2012).

### 2.2.2 Microwave-assisted extraction

Microwave is a form of radiation with frequencies between 300MHz-300GHz. The technology is applied in chemical reacting systems where the absorption of radiation by molecules present in the reactor is involved. The absorption causes the temperature of the solvents and reactants to increase rapidly (Zoccola *et al.*, 2012). Wietecha-Posluszny *et al.* (2010) defined microwave-assisted extraction (MAE) as "a process which involves using microwave energy to heat the extraction solvent-sample system and to transfer compounds of interest from the matrix into the extraction system" (Posluszny *et al.*, 2010, p.3234). MAE has various advantages including reduced reaction time, consistent energy saving, quick heating, thawing, selective energy dissipation, unilateral heat and mass transfer, increased efficiency, reduced solvent consumption, and improved recovery of isolate analytes (Rodríguez-Clavel, *et al.*, 2019; Zoccola, *et al.* 2012).

Lee *et al.*, (2016) conducted a study to evaluate the impact of microwave-assisted alkali treatment on the extracted chicken feather keratin product. Various microwave power levels, concentrations of sodium hydroxide, and residence times were used for feather treatment. The results showed that the optimal conditions were at 10 minutes, 800W, and 0.5 M NaOH producing 26.74mg/mL of protein. The efficiency of

microwave alkali treatment was higher than conventional treatment in the solubilization of feather keratin and the breakdown of disulfide bonds (Lee *et al.*, 2016).

### *2.2.3 Subcritical water extraction*

Subcritical or superheated water extraction, also known as the thermal hydrolysis process is an extraction and hydrolyzation method used on biomass such as keratin from keratinous waste products like hair, feathers, hooves, etc. (Tasaki, 2020). This extraction method employs the use of water under elevated pressure at temperatures between the boiling point of 100°C and the critical temperature of 374°C. Subcritical water extraction is viewed as a green approach as the subcritical water serves as a catalyst, reactant, and solvent simultaneously while absent the use of any chemicals or microbes. The operating conditions also function in the destruction of pathogens. The subcritical water breaks chemical bonds when in reaction with biomass (Faraon *et al.*, 2023; Škerget *et al.*, 2023). Faraon *et al.*, (2023) extracted keratin from ground-up feathers using subcritical water at two different temperatures and pressures, 150°C and pressure of 9 bar, and 200°C at 11 bar for two hours. The extraction at 150°C had a yield of 26.4% while 200°C had a yield of 90.5%. Though the latter sample showed a higher yield it also showed a loss of heat-sensitive amino acids (Faraon *et al.*, 2023). Škerget *et al.*, (2023), did a comparative study on the isolation of keratin from poultry feathers using a temperature range of 120-250°C and reaction time of 5-75 minutes in a batch reactor. The optimal conditions of operation were at 180°C and 60 minutes (Škerget *et al.*, 2023).

## **2.3 Microbial and enzymatic hydrolysis**

Feathers can be found in different environments and keratin-degrading organisms have adapted to efficiently break down feather keratin (Qiu *et al.*, 2020). Strategies such as evolving specialized enzymes, resisting competition, forming symbiotic relationships, and utilizing different keratin sources help these organisms outcompete others (Lai *et al.*, 2023; Rios *et al.*, 2022; Sypka *et al.*, 2021). Keratinase enzymes, used in industries like textiles (Srivastava *et al.*, 2020) and cosmetics (Herzog *et al.*, 2014), break down keratin waste into valuable products. Microbial keratinase production has environmental and industrial applications, including dehairing in

leather (Devi and Lakshmi, 2015), and converting keratin waste into feed supplements (Kumawat *et al.*, 2018).

Microorganisms are used for the extraction of keratin by utilizing keratinolytic and proteolytic enzymes known as keratinases which catalyze the bioconversion of keratinous waste. Microorganisms exhibiting keratinolytic activity are termed keratin degraders and sometimes work in synergy with other enzymes that increase the effectiveness of the degradation process (Babbar *et al.*, 2022; Chilakamarri *et al.*, 2021). The degradation of keratin occurs in three different ways, namely; deamination, proteolysis, and sulfitolysis. Deamination involves the microbial enzymes breaking down keratinous protein substrate to release molecules containing free amino groups. Sulfitolysis and proteolysis have sulfite released by microorganisms to cleave disulfide bonds (Adelere and Lateef, 2019; Chilakamarri *et al.*, 2021).

The majority of bacteria that produce keratinolytic enzymes are classified as Gram-positive bacteria, with a focus on the *Bacillus* genus. Species within this genus known for their potential to produce keratinase include *Bacillus cereus* (Arokiyaraj *et al.*, 2019), *Bacillus licheniformis* (Abdel-Fattah *et al.*, 2018; Alahyaribeik *et al.*, 2020), and *Bacillus tequilensis* (Noor-E-Hira *et al.*, 2024). Other species of bacteria have been isolated and studied for their potential use in the degradation of keratin namely, *Arthrobacter oryzae* (Smirnova *et al.*, 2023), *Brevibacterium luteolum* (Thankaswamy *et al.*, 2018), *Paenibacillus woosongensis* (Paul *et al.*, 2014b), *Brevibacillus brevis* (Jaouadi *et al.*, 2013), *Rhodococcus erythropolis*, and *Geobacillus stearothermophilus* (Alahyaribeik *et al.*, 2020). Actinomycetes, particularly species within the *Streptomyces*, and *Actinomyces* genera, have also been noted for their ability to produce keratinases (Espersen *et al.*, 2021; Falco *et al.*, 2019; Li *et al.*, 2020; Malek *et al.*, 2013). The production of keratinase by keratinolytic fungi is often induced by the presence of keratin substrate in their environment. When the fungi come into contact with keratin-rich material, such as feather waste, they will start to produce and secrete keratinase to degrade the keratin and utilize it as a nutrient source for growth and reproduction. The predominant active keratinolytic fungi are found in the genera *Aspergillus* (Mazotto *et al.*, 2013; Paul *et al.*, 2014a), *Aphanoascus* (Bohacz *et al.*, 2020), *Chrysosporium* (Bohacz *et al.*, 2020; Moorthy *et al.*, 2011), *Fusarium*

(Moorthy *et al.*, 2011), *Penicillium* (Nwadiaro *et al.*, 2015) and *Trichoderma* (Bagewadi *et al.*, 2018).

Khalilipourroodi *et al.*, (2022) carried out a study to investigate the optimal parameters for enzymatic hydrolysis of feathers working with the variables concerning extraction time ranging from 1.5 to 6 hours, enzyme concentration, and reductant concentration. The extraction was carried out utilizing sodium sulfite as a reductant, sodium dodecyl sulfate as a surfactant, and Savinase enzyme as an alkaline protease. The conclusion to the study found that the optimal conditions were 1.5h extraction time, an enzyme concentration of 2%, and 15g/L of the reducing agent (Khalilipourroodi *et al.*, 2022).

Biological degradation of keratin waste by microbial enzymes offers a cost-effective and eco-friendly alternative to traditional methods. Keratinase enzymes hydrolyze keratin substrates, providing a more efficient and toxin-free way to convert waste into useful products (Gong *et al.*, 2020; Kanoksilapatham and Intagun, 2017). Overall, utilizing keratinophilic microorganisms on a larger scale can reduce the environmental impact of traditional waste treatments (Gong *et al.*, 2020; Kumawat *et al.*, 2018).

### **3. Applications of keratin extracted from feathers**

Feather keratin possesses a plethora of exclusive properties such as good processing characteristics, biocompatibility, mechanical strength, good cell adhesion, non-toxicity, and biodegradability. The extracted keratin can take the form of gels, membranes, scaffolds, nanofibers, beads, micro/nanoparticles, and sponges. The versatility in form allows feather keratin to find applications in agricultural, biomedical, cosmetic, food, pharmaceutical, and other sectors (Khumalo *et al.*, 2019; Škerget *et al.*, 2023).

#### **3.1 Packaging**

The packaging industry is heavily reliant on petroleum-based materials, especially in food packaging where oftentimes the plastic used is single-use and promptly discarded after use with low to no possibility of being recycled. To solve this, bio-based plastic packaging has begun to become more popularized as a biodegradable

and eco-friendly alternative to conventional plastics. Bio-based plastics or bioplastics often utilize a biopolymer as a backbone in the development of the plastic. Feather keratin is a likely candidate for the creation of bioplastics and its versatile nature allows various additives to be added to it to improve its functionality for example, mixing antimicrobial particles into the polymer matrix to exterminate pathogens and increase shelf-life (Motelica *et al.*, 2020; Pourjavaheri *et al.*, 2014). Oluba *et al.*, (2021) fabricated a bio-composite film from waste feather keratin and ginger starch. The film exhibited good mechanical properties, stability in water, and thermal stability while also retaining biodegradability (Oluba *et al.*, 2021).

### **3.2 Agriculture**

The use of organic waste in the creation of fertilizers is becoming a more common practice as a way of reducing reliance on chemicals as well as the effectiveness of the fertilizers is nearly comparable to their commercial alternatives. Using subcritical water extraction, Zul *et al.*, (2020) were able to obtain a liquid fertilizer for spinach plants. They used chicken feathers as the source of keratin and created a liquid fertilizer containing 34,200 mg/L of nitrogen and 1,380 mg/L of phosphorus. A comparison was done using agronomic parameters to measure plant growth between control plants and the subcritical water liquid fertilizer and the results showed that there was significant growth on the plants with the fertilizer (Zul *et al.*, 2020).

### **3.3 Biomedical Application**

The biocompatible and non-toxic nature of feather keratin makes it a prime candidate for the development of biomedical applications. Esparza (2017) was able to develop scaffolds for potential use in tissue engineering from feather keratin. The keratin was solubilized by reduction and formed into a hydrogel (Esparza, 2017). To improve the functionality of cotton fabric used in wound dressing Khalilipourroodi *et al.*, (2022) added extracted keratin to carboxymethylated cotton nonwoven fabrics. The result of the addition resulted in an increase in tensile strength to 200%, a doubling of air permeability, and enhanced properties suitable for wound dressing applications (Khalilipourroodi *et al.*, 2022).

## 4. Conclusion

Further research is needed to improve and enhance current keratin extraction techniques in order to minimize their drawbacks. Emerging technologies have the potential to create more value-added products in this sector, but will require dedicated research and development. The key to unlocking the full potential of feather keratin lies in finding a balance between a cost-effective, scalable, eco-friendly, and energy-efficient extraction method and creating high-value products from the extracted keratin. If these conditions are met, feathers could become a valuable resource for sustainable production. Moving forward, it is essential to explore and develop more efficient and sustainable extraction methods for keratin. Alternative techniques and technologies should be considered to make the process more environmentally friendly and cost-effective. Additionally, raising awareness about the potential uses of feathers in industries such as cosmetics, agriculture, and medical can help reduce their environmental impact and open up new economic opportunities. By promoting the concept of up-cycling feathers, we can shift the mindset towards viewing feathers as a valuable resource. Through innovation in extraction methods and a change in perspective, feathers can be maximized for their potential and contribute to a more sustainable future.

## References

- Adelere, I. A., and Lateef, A. (2019). Degradation of Keratin Biomass by Different Microorganisms. In S. Sharma and A. Kumar (Eds.), *Keratin as a Protein Biopolymer: Extraction from Waste Biomass and Applications* (pp. 123–162). Springer International Publishing. [https://doi.org/10.1007/978-3-030-02901-2\\_5](https://doi.org/10.1007/978-3-030-02901-2_5)
- Akpor, O. B., Odesola, D. E., Thomas, R. E., and Oluba, O. M. (2019). Chicken feather hydrolysate as alternative peptone source for microbial cultivation. *F1000Research*, 7(1918), 1–25. <https://doi.org/10.12688/f1000research.17134.3>
- Alahyaribeik, S., Sharifi, S. D., Tabandeh, F., Honarbakhsh, S., and Ghazanfari, S. (2020). Bioconversion of chicken feather wastes by keratinolytic bacteria. *Process Safety and Environmental Protection*, 135, 171–178. <https://doi.org/10.1016/j.psep.2020.01.014>
- Alashwal, B., Saad Bala, M., Gupta, A., Sharma, S., and Mishra, P. (2020). Improved properties of keratin-based bioplastic film blended with microcrystalline cellulose: A comparative analysis. *Journal Of King Saud University - Science*, 32(1), 853-857. <https://doi.org/10.1016/j.jksus.2019.03.006>

Aranberri, I., Montes, S., Azcune, I., Rekondo, A., and Grande, H.-J. (2017). Fully Biodegradable Biocomposites with High Chicken Feather Content. *Polymers*, 9(593), 1–15. <https://doi.org/10.3390/polym9110593>

Arokiyaraj, S., Varghese, R., Ali Ahmed, B., Duraipandiyar, V., and Al-Dhabi, N. A. (2019). Optimizing the fermentation conditions and enhanced production of keratinase from *Bacillus cereus* isolated from halophilic environment. *Saudi Journal of Biological Sciences*, 26(2), 378–381. <https://doi.org/10.1016/j.sjbs.2018.10.011>

Azmi, N. A., Idris, A., and Yusof, N. S. M. (2018). Ultrasonic technology for value added products from feather keratin. *Ultrasonics - Sonochemistry*, 47, 99–107. <https://doi.org/10.1016/j.ultsonch.2018.04.016>

Babbar, N., Sharma, G., and Arya, S. K. (2022). Effective degradation of chicken feather waste by keratinase enzyme with triton X-100 additive. *Biocatalysis and Agricultural Biotechnology*, 44, 102447. <https://doi.org/10.1016/j.bcab.2022.102447>

Bagewadi, Z. K., Mulla, S. I., and Ninnekar, H. Z. (2018). Response surface methodology based optimization of keratinase production from *Trichoderma harzianum* isolate HZN12 using chicken feather waste and its application in dehairing of hide. *Journal of Environmental Chemical Engineering*, 6(4), 4828–4839. <https://doi.org/10.1016/j.jece.2018.07.007>

Benesova, P., Kucera, D., Marova, I., and Obruca, S. (2017). Chicken feather hydrolysate as an inexpensive complex nitrogen source for PHA production By *Cupriavidus necator* on waste frying oils. *Letters In Applied Microbiology*, 65(2), 182–188. <https://doi.org/10.1111/lam.12762>

Bohacz, J., Kornilowicz-Kowalska, T., Kitowski, I., and Ciesielska, A. (2020). Degradation of chicken feathers by *Aphanoascus keratinophilus* and *Chrysosporium tropicum* strains from pellets of predatory birds and its practical aspect. *International Biodeterioration and Biodegradation*, 151, 104968. <https://doi.org/10.1016/j.ibiod.2020.104968>

Bouhamed, S., and Kechaou, N. (2017). Kinetic study of sulphuric acid hydrolysis of protein feathers. *Bioprocess And Biosystems Engineering*, 40(5), 715–721. <https://doi.org/10.1007/s00449-017-1737-7>

Çakmak, E. (2022). Keratin Isolation Methods From Waste Goose Feather: An Effective Comparison. *Turkish Journal of Nature and Science*, 11(2), 113–117. <https://doi.org/10.46810/tdfd.1113394>

Chilakamarry, C. R., Mahmood, S., Saffe, S. N. B. M., Arifin, M. A. B., Gupta, A., Sikkandar, M. Y., Begum, S. S., and Narasaiah, B. (2021). Extraction and application of keratin from natural resources: A review. *3 Biotech*, *11*(220), 1–12. <https://doi.org/10.1007/s13205-021-02734-7>

Colunga-Sánchez, L. M., Salazar-Cruz, B. A., Rivera-Armenta, J. L., Morales-Cepeda, A. B., Ramos-Gálvan, C. E., and Chávez-Cinco, M. Y. (2019). Evaluation of Chicken Feather and Styrene-Butadiene/Chicken Feather Composites as Modifier for Asphalts Binder. *Applied Sciences*, *9*(5188), 1–11. <https://doi.org/10.3390/app9235188>

Costa, J. C., Barbosa, S. G., and Sousa, D. Z. (2012). Effects of pre-treatment and bioaugmentation strategies on the anaerobic digestion of chicken feathers. *Bioresource Technology*, *120*, 114–119. <https://doi.org/10.1016/j.biortech.2012.06.047>

Devi, D. A., and Lakshmi, V.V. (2015). Enhancing the Utilisation of Keratinases by using Immobilised Chitosan Beads. *IOSR Journal of Pharmacy and Biological Sciences*, *10*(4), 1–4. <https://doi.org/10.9790/3008-10440104>

Dwivedi, S., and Kaneko, T. (2018). Aromatic Bioplastics with Heterocycles. In *Green Polymer Chemistry: New Products, Processes, and Applications* (Vol. 1310, pp. 201–218). American Chemical Society. <https://doi.org/10.1021/bk-2018-1310.ch014>

Esparza, Y. (2017). *Fabrication of feather keratin bio-based materials: Thermoplastics and tissue engineered scaffolds* [Doctoral thesis, University of Alberta]. <https://doi.org/10.7939/R39W09C65>

Espersen, R., Huang, Y., Falco, F. C., Häggglund, P., Gernaey, K. V., Lange, L., and Svensson, B. (2021). Exceptionally rich keratinolytic enzyme profile found in the rare actinomycetes *Amycolatopsis keratiniphila* D2T. *Applied Microbiology and Biotechnology*, *105*(21), 8129–8138. <https://doi.org/10.1007/s00253-021-11579-2>

Falco, F. C., Espersen, R., Svensson, B., Gernaey, K. V., and Eliasson Lantz, A. (2019). An integrated strategy for the effective production of bristle protein hydrolysate by the keratinolytic filamentous bacterium *Amycolatopsis keratiniphila* D2. *Waste management*, *89*, 94–102. <https://doi.org/10.1016/j.wasman.2019.03.067>

Faraon, V., Mihaila, E., Tritean, N., Trică, B., Capra, L., Roman, B., Constantinescu Aruxandei, D., and Oancea, F. (2023). Keratin extraction from chicken feathers in aqueous solutions. *Scientific Bulletin*, *27*(1), 105–112. <https://www.researchgate.net/publication/373652800>

- Fernández-d'Arlas, B. (2018). Improved aqueous solubility and stability of wool and feather proteins by reactive-extraction with H<sub>2</sub>O<sub>2</sub> as bisulfide ( -S-S- ) splitting agent. *European Polymer Journal*, 103, 187-197. <https://doi.org/10.1016/j.eurpolymj.2018.04.010>
- Fernández-d'Arlas, B. (2019). Tough and Functional Cross-linked Bioplastics from *Sheep Wool Keratin*. *Scientific Reports*, 9(1), 1-12. <https://doi.org/10.1038/s41598-019-51393-5>
- Feroz, S., Muhammad, N., Ranayake, J., and Dias, G. (2020). Keratin - Based materials for biomedical applications. *Bioactive Materials*, 5(3), 496-509. <https://doi.org/10.1016/j.bioactmat.2020.04.007>
- Foresman, P. S. (2019). Feather 2 (PSF) [Digital]. Pearson Scott Foresman. [https://commons.wikimedia.org/wiki/File:Feather\\_2\\_\(PSF\).png](https://commons.wikimedia.org/wiki/File:Feather_2_(PSF).png)
- Ge, N., Zhang, Y., Zhang, H., Cheng, L., and Shi, X. (2021). Extraction and Characterization of Keratin and Keratin Hydrogels from Wasted Rabbit Hair. *Journal of Physics: Conference Series*, 1790(1), 1–8. <https://doi.org/10.1088/1742-6596/1790/1/012008>
- Gong, J.-S., Ye, J.-P., Tao, L.-Y., Su, C., Qin, J., Zhang, Y.-Y., Li, H., Li, H., Xu, Z.-H., and Shi, J.-S. (2020). Efficient keratinase expression via promoter engineering strategies for degradation of feather wastes. *Enzyme and Microbial Technology*, 137(109550), 1–8. <https://doi.org/10.1016/j.enzmictec.2020.109550>
- Gupta, A., Kamarudin, N., Yeo, C., Chua, G. K., Bin, R., and Yunus, R. (2012). Extraction of Keratin Protein from Chicken Feather. *Journal of Chemistry and Chemical Engineering*, 6, 732–737.
- Herzog, B., Overy, D., Haltli, B., and Kerr, R. (2014). Keratinases – A viable approach for hair removal and younger looking skin. *Maritime Natural Products*, 6, 1. <https://doi.org/10.13140/2.1.4891.8403>
- Hill, P., Brantley, H., and Van Dyke, M. (2010). Some properties of keratin biomaterials: Kerateines. *Biomaterials*, 31(4), 585–593. <https://doi.org/10.1016/j.biomaterials.2009.09.076>
- Idris, A., Vijayaraghavan, R., Rana, U. A., Fredericks, D., Patti, A. F., and MacFarlane, D. R. (2013). Dissolution of feather keratin in ionic liquids. *Green Chemistry*, 15(2), 525–534. <https://doi.org/10.1039/C2GC36556A>

Isarankura Na Ayutthaya, S., Tanpichai, S., and Wootthikanokkhan, J. (2015). Keratin Extracted from Chicken Feather Waste: Extraction, Preparation, and Structural Characterization of the Keratin and Keratin/Biopolymer Films and Electrospuns. *Journal of Polymers and the Environment*, 23(4), 506–516. <https://doi.org/10.1007/s10924-015-0725-8>

Jaouadi, N. Z., Rekik, H., Badis, A., Trabelsi, S., Belhoul, M., Yahiaoui, A. B., Ben Aicha, H., Toumi, A., Bejar, S., and Jaouadi, B. (2013). Biochemical and molecular characterization of a serine keratinase from *Brevibacillus brevis* US575 with promising keratin-biodegradation and hide-dehairing activities. *PloS one*, 8(10), e76722. <https://doi.org/10.1371/journal.pone.0076722>

Ji, Y., Chen, J., Lv, J., Li, Z., Xing, L., and Ding, S. (2014). Extraction of keratin with ionic liquids from poultry feather. *Separation And Purification Technology*, 132, 577-583. <https://doi.org/10.1016/j.seppur.2014.05.049>

Kamaraj, S., and Vuppu, S. (2024). In-silico study of bacterial keratinase and optimization of extraction procedure for keratin from Country chicken and Indian blue rock pigeon feathers. *Kuwait Journal of Science*, 51(1), 1–10. <https://doi.org/10.1016/j.kjs.2023.10.016>

Kanoksilapatham, W., and Intagun, W. (2017). A Review: Biodegradation and Applications of Keratin Degrading Microorganisms and Keratinolytic Enzymes, Focusing on Thermophiles and Thermostable Serine Proteases. *American Journal of Applied Sciences*, 14(11), 1016–1023. <https://doi.org/10.3844/ajassp.2017.1016.1023>

Khalel, A., Alshehri, W., and Aly, M. (2020). Enhancing Plant Growth by Chicken Feather Compost Obtained from Feather Degradation by *Streptomyces enissocaesili*. *Bioscience Biotechnology Research Communications*, 13(4), 1847-1853. <https://doi.org/10.21786/bbrc/13.4/32>

Khumalo, M. B. (2021). *Valorisation of waste chicken feathers: Regeneration of keratin fibre into tubular nanofibres via electrospinning* [Doctoral thesis, University of Kwazulu Natal]. Doctoral Degrees (Chemical Engineering) University of KwaZulu Natal. <https://researchspace.ukzn.ac.za/handle/10413/20300>

Khumalo, M., Tesfaye, T., Sithole, B., and Ramjugernath, D. (2019). Possible beneficiation of waste chicken feathers via conversion into biomedical applications. *International Journal of Chemical Sciences*, 17(1), 1–21. <https://doi.org/10.21767/0972-768X.1000298>

Khalilipourroodi, K., Dadashian, F., and Solouk, A. (2022). Effect of extraction method on properties of feather keratin grafted modified cotton nonwoven fabric for biomedical applications. *Journal of Industrial Textiles*, 51(2S), 2558S-2575S. <https://doi.org/10.1177/15280837211006208>

Kumawat, T., Sharma, A., Sharma, V., and Chandra, S. (2018). Keratin Waste: The Biodegradable Polymers. In *Keratin* (pp. 149–169). IntechOpen. <https://doi.org/10.5772/intechopen.79502>

Łaba, W., and Szczekała, K. B. (2013). Keratinolytic Proteases in Biodegradation of Pretreated Feathers. *Polish Journal of Environmental Studies*, 22(4), 1101–1109.

Lai, Y., Wu, X., Zheng, X., Li, W., and Wang, L. (2023). Insights into the keratin efficient degradation mechanism mediated by *Bacillus* sp. CN2 based on integrating functional degradomics. *Biotechnology for biofuels and bioproducts*, 16(1), 59. <https://doi.org/10.1186/s13068-023-02308-0>

Lee, Y., Phang, L., Ahmad, S., and Ooi, P. (2016). Microwave-Alkali Treatment of Chicken Feathers for Protein Hydrolysate Production. *Waste And Biomass Valorization*, 7(5), 1147-1157. <https://doi.org/10.1007/s12649-016-9483-7>

Li Q. (2021). Structure, Application, and Biochemistry of Microbial Keratinases. *Frontiers in microbiology*, 12, 674345. <https://doi.org/10.3389/fmicb.2021.674345>

Li, Z. W., Liang, S., Ke, Y., Deng, J. J., Zhang, M. S., Lu, D. L., Li, J. Z., and Luo, X. C. (2020). The feather degradation mechanisms of a new *Streptomyces* sp. isolate SCUT-3. *Communications biology*, 3(1), 191. <https://doi.org/10.1038/s42003-020-0918-0>

Ma, B., Qiao, X., Hou, X., and Yang, Y. (2016). Pure keratin membrane and fibers from chicken feather. *International Journal Of Biological Macromolecules*, 89, 614-621. <https://doi.org/10.1016/j.ijbiomac.2016.04.039>

Malek, E., Moosazadeh, M., Hanafi, P., Abbasi Nejat, Z., Amini, A., Mohammadi, R., Kohsar, F., and Niknejad, F. (2013). Isolation of Keratinophilic Fungi and Aerobic Actinomycetes From Park Soils in Gorgan, North of Iran. *Jundishapur Journal of Microbiology*, 6(10), 1–5. <https://doi.org/10.5812/jjm.11250>

Martinez, J. P. D. O., Cai, G., Nachtschatt, M., Navone, L., Zhang, Z., Robins, K., and Speight, R. (2020). Challenges and Opportunities in Identifying and Characterising Keratinases for Value-Added Peptide Production. *Catalysts*, 10(2), 1–23. <https://doi.org/10.3390/catal10020184>

Maurya, S. D., and Singh, A. (2023). Extraction and Characterization of Keratin (Natural Protein) from Waste Broiler Chicken Feathers. *Environmental Protection Research*, 3(2), 372–381. <https://doi.org/10.21203/rs.3.rs-2794301/v1>

Mazotto, A. M., Couri, S., Damaso, M. C. T., and Vermelho, A. B. (2013). Degradation of feather waste by *Aspergillus niger* keratinases: Comparison of submerged and solid-state fermentation. *International Biodeterioration and Biodegradation*, 85, 189–195. <https://doi.org/10.1016/j.ibiod.2013.07.003>

Mishra, S., Kumar, A., Singh, O., Upadhyay, A., and Antony, R. (2015). Assessment of a Bio-inspired Artificial Wing for Micro Aerial Vehicle Based on Structural Bio-mimetics. *Materials Today: Proceedings*, 2(4-5), 2407-2413. <https://doi.org/10.1016/j.matpr.2015.07.179>

Moorthy, K., Prasanna, I., Vimalan, S., Lavanya, V., Selvi, A. T., Mekal, T., and Thajuddin, N. (2011). Study on Keratinophilic and Keratinolytic Fungi Isolated from Birds' Feathers and Animal Hairs. *Biosciences Biotechnology Research Asia*, 8(2), 633–640.

Motelica, L., Ficai, D., Ficai, A., Oprea, O. C., Kaya, D. A., and Andronescu, E. (2020). Biodegradable Antimicrobial Food Packaging: Trends and Perspectives. *Foods*, 9(1438), 1–36. <https://doi.org/doi:10.3390/foods9101438>

Nnolim, N. E., Okoh, A. I., and Nwodo, U. U. (2020). *Bacillus* sp. FPF-1 Produced Keratinase with High Potential for Chicken Feather Degradation. *Molecules (Basel, Switzerland)*, 25(7), 1505-1521. <https://doi.org/10.3390/molecules25071505>

Noor-E-Hira, Haq, I., Arshad, F., Aftab, M. N., Rehman, A., and Akram, F. (2024). Isolation and screening of keratinase-producing indigenous bacteria from keratin rich waste sites and slaughter-houses. *Journal of Xi'an Shiyu University, Natural Science Edition*, 20(3), 20–27.

Nur-E-Alam, M., Akter, N., Chakma, S., Fatema, K., Azad, A., Jaman Chowdhury, M., and Abu Sayid Mia, M. (2019). Alkali Enzymatic Extraction of Keratin Protein from Chicken Feather Waste in Bangladesh. *Iranian (Iranica) Journal Of Energy And Environment*, 10(4), 235-241. <https://doi.org/10.5829/ijee.2019.10.04.02>

Nuutinen, E.-M., Virtanen, T., Lantto, R., Vähä-Nissi, M., and Jääskeläinen, A.-S. (2021). Ductile keratin films from deep eutectic solvent fractionated feathers. *RSC Advances*, 11, 27512–27522. <https://doi.org/10.1039/d1ra05123g>

Nwadiaro, P. O., Chuku, A., Onyimba, I. A., Ogbonna, A. I., Nwaukwu, I. A., and Adekojo, D. A. (2015). Keratin Degradation by *Penicillium purpurogenum* Isolated from Tannery Soils in Jos, Nigeria. *British Microbiology Research Journal*, 8(1), 358–366. <https://doi.org/10.9734/BMRJ/2015/16339>

Okoya, A., Ochor, N., Akinyele, A., and Olaiya, O. (2020). The Use of Chicken Feather Waste as an Adsorbent for Crude Oil Clean Up from Polluted Water. *Journal Of Agriculture And Ecology Research International*, 21(3), 43-53. <https://doi.org/10.9734/jaeri/2020/v21i330136>

Oluba, O. M., Obokare, O., Bayo-Olorunmeke, O. A., Ojeaburu, S. I., Ogunlowo, O. M., Irokanulo, E. O., and Akpor, O. B. (2022). Fabrication, characterization and antifungal evaluation of polyphenolic extract activated keratin starch coating on infected tomato fruits. *Scientific Reports*, 12(4340), 1–12. <https://doi.org/10.1038/s41598-022-07972-0>

Ozdal, M., Gurkok, S., and Ozdal, O. (2017). Optimization of rhamnolipid production by *Pseudomonas aeruginosa* OG1 using waste frying oil and chicken feather peptone. 3 *Biotech*, 7(2), 1-8. <https://doi.org/10.1007/s13205-017-0774-x>

Ozdal, M., and Kurbanoglu, E. (2018). Citric Acid Production by *Aspergillus niger* from Agro-Industrial By-Products: Molasses and Chicken Feather Peptone. *Waste And Biomass Valorization*, 10(3), 631-640. <https://doi.org/10.1007/s12649-018-0240-y>

Ozdal, M., and Başaran Kurbanoglu, E. (2019). Use of Chicken Feather Peptone and Sugar Beet Molasses as Low Cost Substrates for Xanthan Production by *Xanthomonas campestris* MO-03. *Fermentation*, 5(1), 9. <https://doi.org/10.3390/fermentation5010009>

Pahua-Ramos, M. E., Hernández-Melchor, D. J., Camacho-Pérez, B., and Quezada-Cruz, M. (20017). Degradation of Chicken Feathers: A Review. *BioTechnology: An Indian Journal*, 13(6), 153–177.

Pandey, S. C., Pande, V., Sati, D., Gangola, S., Kumar, S., Pandey, A., and Samant, M. (2019). Microbial keratinase: A tool for bioremediation of feather waste. In P. Bhatt (Ed.), *Smart Bioremediation Technologies* (pp. 217–253). Academic Press. <https://doi.org/10.1016/B978-0-12-818307-6.00013-5>

Paul, T., Das, A., Mandal, A., Halder, S. K., DasMohapatra, P. K., Pati, B. R., and Mondal, K. C. (2014a). Production and purification of keratinase using chicken feather bioconversion by a newly isolated *Aspergillus fumigatus* TKF1: Detection of

valuable metabolites. *Biomass Conversion and Biorefinery*, 4(2), 137–148.  
<https://doi.org/10.1007/s13399-013-0090-6>

Paul, T., Das, A., Mandal, A., Halder, S. K., DasMohapatra, P. K., Pati, B. R., and Mondal, K. C. (2014b). Valorization of Chicken Feather Waste for Concomitant Production of Keratinase, Oligopeptides and Essential Amino Acids Under Submerged Fermentation by *Paenibacillus woosongensis* TKB2. *Waste and Biomass Valorization*, 5(4), 575–584. <https://doi.org/10.1007/s12649-013-9267-2>

Pourjavaheri, F., Mohaddes, F., Shanks, R. A., Czajka, M., and Gupta, A. (2014). Effects of Different Purification Methods on Chicken Feather Keratin. *Advanced Materials Research*, 941, 1184–1187. <https://doi.org/10.4028/www.scientific.net/AMR.941-944.1184>

Qin, X., Yang, C., Guo, Y., Liu, J., Bitter, J. H., Scott, E. L., and Zhang, C. (2023). Effect of ultrasound on keratin valorization from chicken feather waste: Process optimization and keratin characterization. *Ultrasonics Sonochemistry*, 93(106297), 1–11.  
<https://doi.org/10.1016/j.ultsonch.2023.106297>

Qiu, J., Wilkens, C., Barrett, K., and Meyer, A. S. (2020). Microbial enzymes catalyzing keratin degradation: Classification, structure, function. *Biotechnology Advances*, 44(107607), 1–23. <https://doi.org/10.1016/j.biotechadv.2020.107607>

Rios, P., Bezus, B., Cavalitto, S., and Cavello, I. (2022). Production and characterization of a new detergent-stable keratinase expressed by *Pedobacter* sp. 3.14.7, a novel Antarctic psychrotolerant keratin-degrading bacterium. *Journal of Genetic Engineering and Biotechnology*, 20(81), 1–14.  
<https://doi.org/10.1186/s43141-022-00356-x>

Rodríguez-Clavel, I., Paredes-Carrera, S., Flores-Valle, S., Paz-García, E., Sánchez-Ochoa, J., and Pérez-Gutiérrez, R. (2019). Effect of Microwave or Ultrasound Irradiation in the Extraction from Feather Keratin. *Journal Of Chemistry*, 2019, 1-9.  
<https://doi.org/10.1155/2019/1326063>

Scott, W., Lowrance, B., Anderson, A. C., and Weadge, J. T. (2020). Identification of the Clostridial cellulose synthase and characterization of the cognate glycosyl hydrolase, CcsZ. *PloS One*, 15(12), e0242686. <https://doi.org/10.2210/pdb6UJF/pdb>

Shavandi, A., Silva, T., Bekhit, A., and Bekhit, A. (2017). Keratin: dissolution, extraction and biomedical application. *Biomaterials Science*, 5(9), 1699-1735.  
<https://doi.org/10.1039/c7bm00411g>

Sinkiewicz, I., Śliwińska, A., Staroszczyk, H., and Kołodziejska, I. (2017). Alternative Methods of Preparation of Soluble Keratin from Chicken Feathers. *Waste And Biomass Valorization*, 8(4), 1043-1048. <https://doi.org/10.1007/s12649-016-9678-y>

Škerget, M., Čolnik, M., Zemljič, L. F., Gradišnik, L., Semren, T. Ž., Lovaković, B. T., and Maver, U. (2023). Efficient and Green Isolation of Keratin from Poultry Feathers by Subcritical Water. *Polymers*, 15(2658), 1–16. <https://doi.org/10.3390/polym15122658>

Srivastava, B., Singh, H., Khatri, M., Singh, G., and Arya, S. K. (2020). Immobilization of keratinase on chitosan grafted- $\beta$ -cyclodextrin for the improvement of the enzyme properties and application of free keratinase in the textile industry. *International Journal of Biological Macromolecules*, 165, 1099–1110. <https://doi.org/10.1016/j.ijbiomac.2020.10.009>

Stiborova, H., Branska, B., Vesela, T., Lovecka, P., Stranska, M., Hajslova, J., Jiru, M., Patakova, P., and Demnerova, K. (2016). Transformation of raw feather waste into digestible peptides and amino acids. *Journal of Chemical Technology and Biotechnology*, 91(6), 1629–1637. <https://doi.org/10.1002/jctb.4912>

Subash, H. A., Santhosh, K., Kannan, K., and Pitchiah, S. (2024). Extraction of Keratin degrading enzyme from marine Actinobacteria of *Nocardia sp* and their antibacterial potential against oral pathogens. *Oral Oncology Reports*, 9, 100184. <https://doi.org/10.1016/j.oor.2024.100184>

Sypka, M., Jodłowska, I., and Białkowska, A. M. (2021). Keratinases as Versatile Enzymatic Tools for Sustainable Development. *Biomolecules*, 11(12). <https://doi.org/10.3390/biom11121900>

Tasaki, K. (2020). A novel thermal hydrolysis process for extraction of keratin from hog hair for commercial applications. *Waste Management*, 104, 33-41. <https://doi.org/10.1016/j.wasman.2019.12.042>

Taskin, M., Esim, N., and Ortucu, S. (2012). Efficient production of l-lactic acid from chicken feather protein hydrolysate and sugar beet molasses by the newly isolated *Rhizopus oryzae* TS-61. *Food And Bioproducts Processing*, 90(4), 773-779. <https://doi.org/10.1016/j.fbp.2012.05.003>

Thankaswamy, S. R., Sundaramoorthy, S., Palanivel, S., and Ramudu, K. N. (2018). Improved microbial degradation of animal hair waste from leather industry using

*Brevibacterium luteolum* (MTCC 5982). *Journal of Cleaner Production*, 189, 701–708. <https://doi.org/10.1016/j.jclepro.2018.04.095>

Vineis, C., Varesano, A., Varchi, G., and Aluigi, A. (2019). Extraction and Characterization of Keratin from Different Biomasses. In S. Sharma and A. Kumar (Eds.), *Keratin as a Protein Biopolymer: Extraction from Waste Biomass and Applications* (pp. 35–76). Springer International Publishing. [https://doi.org/10.1007/978-3-030-02901-2\\_3](https://doi.org/10.1007/978-3-030-02901-2_3)

Wang, X., and Liu, L. (2010). Steam Explosion Pretreatment Technique and Application in Biomass Conversion. *Advanced Materials Research*, 113(116), 525-528. <https://doi.org/10.4028/www.scientific.net/amr.113-116.525>

Wang, Y., and Cao, X. (2012). Extracting keratin from chicken feathers by using a hydrophobic ionic liquid. *Process Biochemistry*, 47(5), 896-899. <https://doi.org/10.1016/j.procbio.2012.02.013>

Wietecha-Posłuszny, R., Garbacik, A., Woźniakiewicz, M., and Kościelniak, P. (2010). Microwave-assisted hydrolysis and extraction of tricyclic antidepressants from human hair. *Analytical And Bioanalytical Chemistry*, 399(9), 3233-3240. <https://doi.org/10.1007/s00216-010-4440-y>

Wool, R. P., and Sun, X. S. (2005). *Bio-Based Polymers and Composites* (pp. 411-412,424). Academic Press. <https://doi.org/10.1016/B978-0-12-763952-9.X5000-X>

Xia, Y., Massé, D. I., McAllister, T. A., Beaulieu, C., and Ungerfeld, E. (2012). Anaerobic digestion of chicken feather with swine manure or slaughterhouse sludge for biogas production. *Waste Management*, 32(3), 404–409. <https://doi.org/10.1016/j.wasman.2011.10.024>

Xu, H., and Yang, Y. (2014). Controlled De-Cross-Linking and Disentanglement of Feather Keratin for Fiber Preparation via a Novel Process. *ACS Sustainable Chemistry and Engineering*, 2(6), 1404-1410. <https://doi.org/10.1021/sc400461d>

Zhang, J., Li, Y., Li, J., Zhao, Z., Liu, X., and Li, Z. *et al.* (2013). Isolation and characterization of biofunctional keratin particles extracted from wool wastes. *Powder Technology*, 246, 356-362. <https://doi.org/10.1016/j.powtec.2013.05.037>

Zhang, Y., Yang, R., and Zhao, W. (2014). Improving Digestibility of Feather Meal by Steam Flash Explosion. *Journal Of Agricultural And Food Chemistry*, 62(13), 2745-2751. <https://doi.org/10.1021/jf405498k>

Zhang, Y., Zhao, W., and Yang, R. (2015). Steam Flash Explosion Assisted Dissolution of Keratin from Feathers. *ACS Sustainable Chemistry and Engineering*, 3(9), 2036-2042. <https://doi.org/10.1021/acssuschemeng.5b00310>

Zhao, W., Yang, R., Zhang, Y., and Wu, L. (2012). Sustainable and practical utilization of feather keratin by an innovative physicochemical pretreatment: high density steam flash-explosion. *Green Chemistry*, 14(12), 3352. <https://doi.org/10.1039/c2gc36243k>

Zoccola, M., Aluigi, A., Patrucco, A., Vineis, C., Forlini, F., and Locatelli, P. *et al.* (2012). Microwave-assisted chemical-free hydrolysis of wool keratin. *Textile Research Journal*, 82(19), 2006-2018. <https://doi.org/10.1177/0040517512452948>

Zul, S. M., Iwamoto, K., Rahim, M. A. M., Abdullah, N., Mohamad, S. E., Shimizu, K., and Hara, H. (2020). Production of Liquid Fertilizer from Chicken Feather Waste by Using Subcritical Water Treatment for Plant and Algal Growth. *IOP Conference Series: Earth and Environmental Science*, 479, 1–9. <https://doi.org/10.1088/1755-1315/479/1/012033>