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

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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 (Akpor *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 α-keratins, β-keratins, and γ-keratins. α-keratins are fibrillar and comprised of polypeptide chains in an alpha helical structure, whereas, the polypeptide chains in β-keratins are in the form of pleated β-sheets, and those of γ-keratins are globular in shape. β-keratins are synthesized in avian feathers, claws, and beaks (Feroz *et al*., 2020; Hill *et al*., 2010).

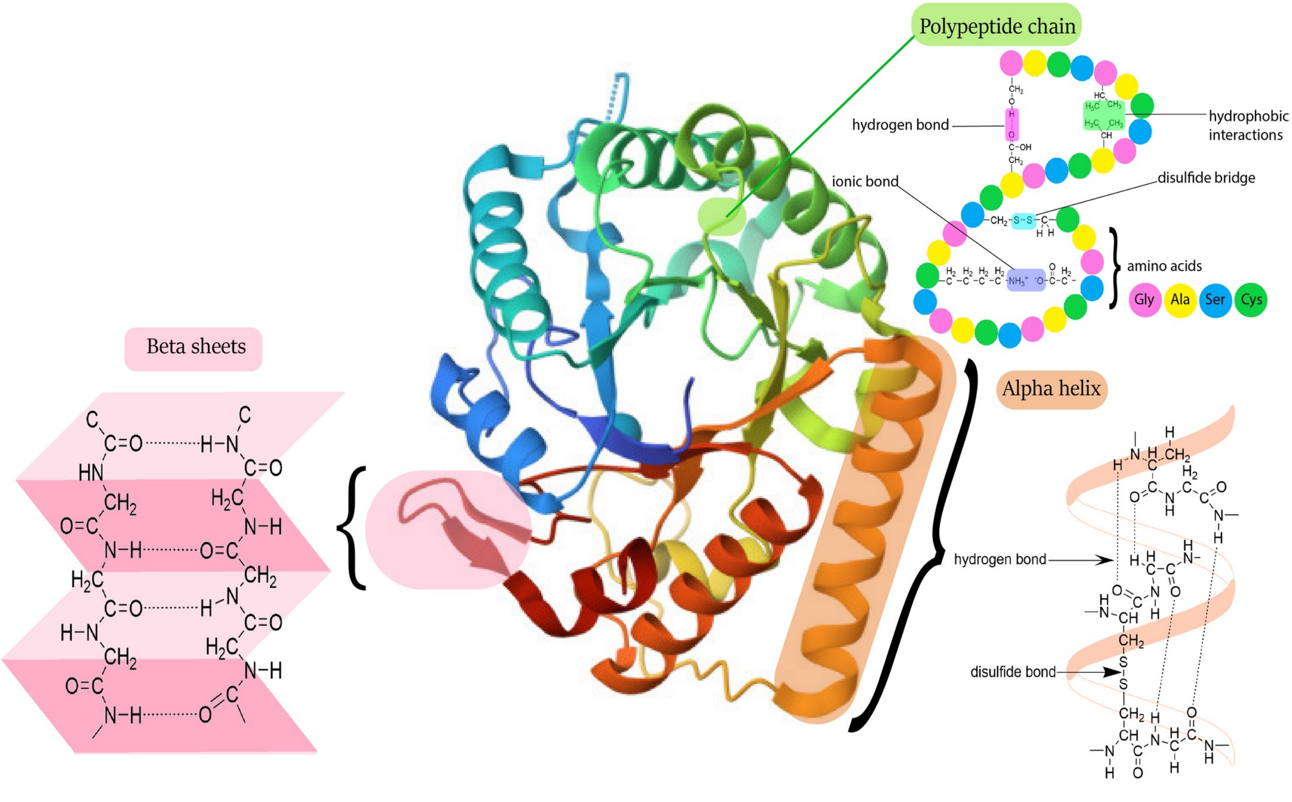


Plate 1: Bonds in keratin structures that maintain their stability. Image of 3D structure from the Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB.org) of PDB ID 6UJF (Scott *et al*., 2020).

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 ranches, 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 β-pleated sheets in the ordered regions of the polypeptide chain and other disordered structures with the feather fiber containing more α-helix structures than β-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±1.4% worth of volatile solids of which 92.0±0.48% represents the possible degradable matter which in turn 88±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.

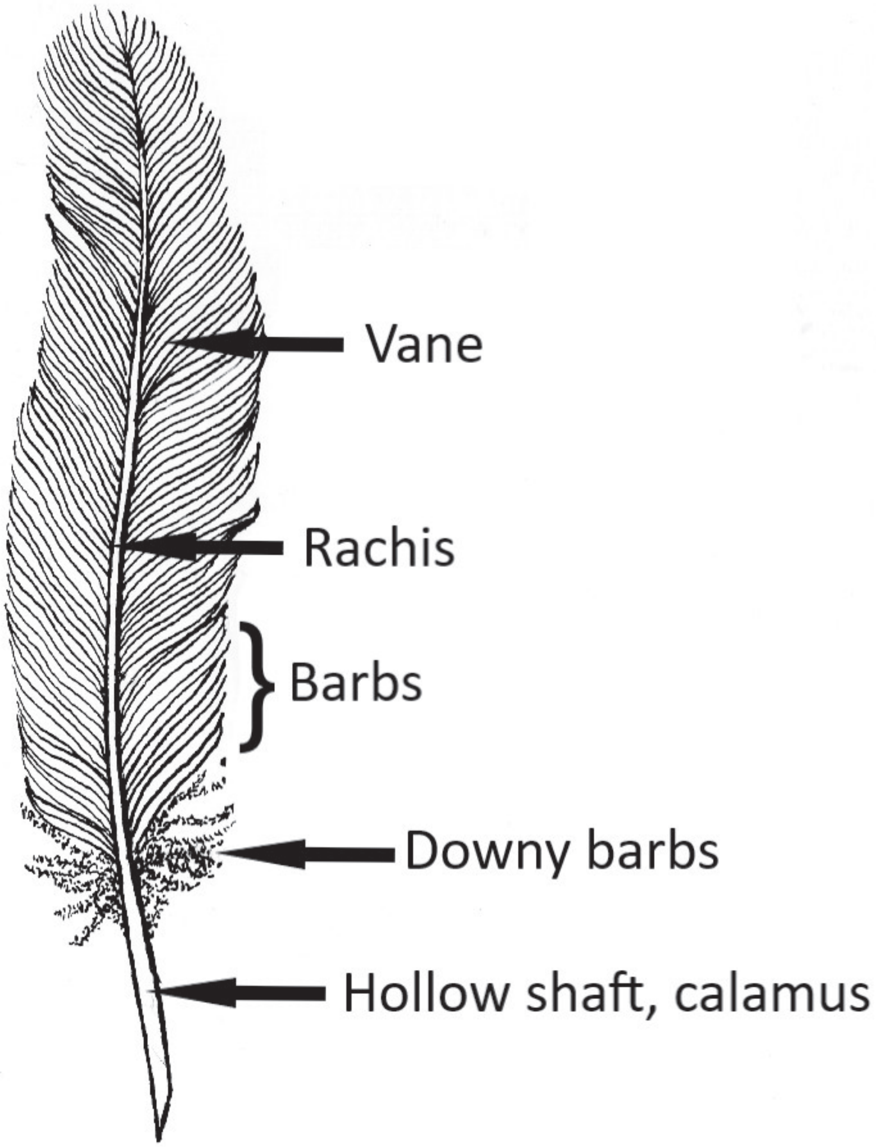


Plate 2: General anatomy of a bird feather (Foresman, 2019).

2. Extraction techniques for the valorization of feather keratin

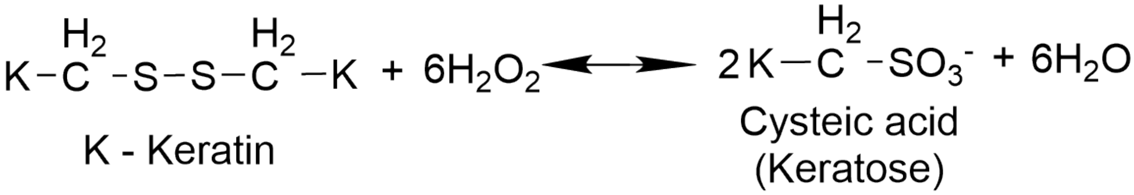
Several factors come into play when dissolving and extracting keratin from various biomasses. Its strong physical and mechanical properties render it difficult to process without the aid of external intervention be it chemical, physical, or even microbial. Techniques have been developed to extract and/or 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:



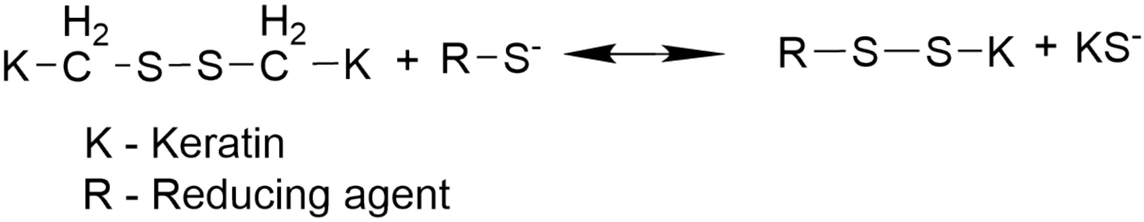
$$K-\overset{H_2}{C}-S-S-\overset{H_2}{C}-K + 6H_2O_2 \longleftrightarrow 2K-\overset{H_2}{C}-SO_3^- + 6H_2O$$

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:



$$K-\overset{H_2}{C}-S-S-\overset{H_2}{C}-K + R-S^- \longleftrightarrow R-S-S-K + KS^-$$

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 30ºC, 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.

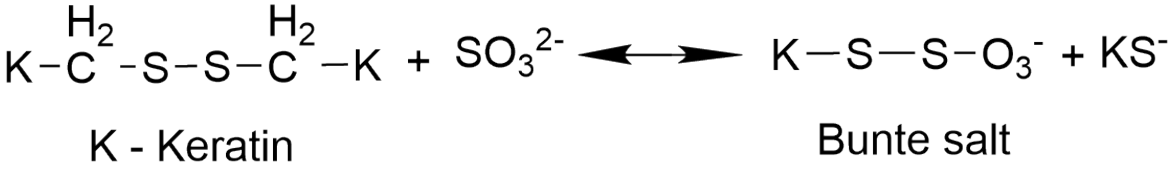
Keratin source	**Reducing agent**	**Reaction Conditions**	**Yield %**	**Reference**
Chicken Feather	Sodium Sulfide	pH 10-13, 6h, 30°C	63.3	Nur-E-Alam *et al*., 2019

Chicken Feather	i. 2-mercaptoethanol ii. Dithiothreitol (DTT) iii. Sodium-*m*-bisulfite iv. Sodium bisulfite	2h, 50°C	i. 83.8 ± 0.3 ii. 77.6 ± 1.4 iii. 62.9 ± 1.0 iv. 82.4 ± 0.1	Sinkiewicz *et al.*, 2017
Chicken Feather	L-Cysteine	pH 10.5, 12h, 70°C	60.0	Ma *et al.*, 2016
Chicken Feather	Sodium Sulfide Nonahydrate ($Na_2S \bullet 9H_2O$)	30min, 50°C	-	Dou *et al.*, 2020
Chicken Feather	Cysteine	pH 10.5, 12h, 70°C	-	Xu and Yang, 2014
Chicken Feather	Thioglycate	pH11, 2h, 50-55°C	76.9 ± 0.4	Fernández-d'Arlas, 2018
Goose Feather	i. Sodium Sulfide ii. Ethylenediaminetetraacetic acid (EDTA)	i. 1.5h, 25°C ii. pH 4.2, 2h, 40°C	i. 86.3 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_3^{2-}), bisulfite (HSO_3^{-}), and disulfite ($S_2O_5^{2-}$)) 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:



$$K-\overset{H_2}{C}-S-S-\overset{H_2}{C}-K + SO_3^{2-} \longleftrightarrow K-S-S-O_3^{-} + KS^{-}$$

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 100ºC (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)

Ionic Liquids	**Temp (°C)**	**Time**	**Solubility (wt%)**	**% Yield of Keratin**	**Reference**
1) Butyl-3-methylimidazolium chloride ([BMIM]$^+$Cl$^-$) 2) Allyl-3-methylimidazolium chloride [AMIM]-Cl) 3) Hexyl-3-methylimidazolium trifluoromethane sulfonate [HMIM]CF$_3$SO$_3$	90	60 min	1) 4.80 2) 4.80 3) 0.20	75.0	Ji, *et al.*, 2014
N, N-dimethyl ethanol ammonium formate formate [DMEA][HCOO]	100	7h	2.50	63.0	Idris *et al.*, 2014
1) 1-Allyl-3-methylimidazolium chloride, [AMIM]Cl 2) 1-Butyl-3-methylimidazolium chloride ([BMIM]$^+$Cl$^-$) 3) Choline thioglycolate	130	10h	1) 50.00 2) 50.00 3) 45.00	1) 60.0 2) 60.0 3) 55.0	Idris *et al.*, 2013
1-hydroxyethyl-3-methylimidazolium bis(trifluoromethane sulfonyl)amide ([HOEMIm][NTf$_2$])	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 90ºC-150ºC 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 β-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-Posłuszny *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" (Posłuszny *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; Chilakamarry *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; Chilakamarry *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 alliterative 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.

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