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REVIEW

Keratin hydrolysates: a sustainable product in biotechnology sectors by microbial conversion

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Abstract

The increasing population and demand for food and feed have increased the urge to find protein sources from waste products. Due to poor management of waste valorization, it has become a pollutant to the environment. This waste can be converted into a valuable product by microbial degradation. Feather waste from poultry farms can be efficiently processed into hydrolysates, serving as an additive or in its crude form for animal feed and detergents. This approach not only reduces pollution but also boosts the economy of a country. Keratin is a hard fibrous protein, insoluble in water and organic solvents. They are accumulated in nature and are major components of feathers, nails, hairs, and wool. Microorganisms like bacteria, fungi, and actinomycetes can degrade keratin by producing the keratinase enzyme. Keratinases are thought to be promising biocatalysts for the production of animal nutrients, protein supplements, leather processing, fibre modification, detergent formulations, and pharmaceutical, cosmetic, and biomedical industries. An overview of keratin structure and composition, the mechanism of microbial hydrolysis of keratin, and their possible uses in biotechnological sectors are presented in this review.

Keywords Keratin · Microbial keratinase · Hydrolysates · Applications

Abbreviations

DTT	Dithiothreitol
IAA	Indole-3-Acetic Acid
AMF	Arbuscular Mycorrhizal Fungi
FH	Feather Hydrolysate
SEM	Scanning Electron Microscope
Aam	Acrylic acid and Acrylamide monomers

Introduction

Feathers account for about 5–7% of the body weight of birds, and about 91% of chicken feathers are made up of protein (keratin), 1% lipids, and 8% water. A typical feather comprises rachis (central shaft), barbs, barbules, and shaft with keratin protein. These feather content are discarded annually in large quantities because of no proper valorization (Verma et al. 2017). Many pathogenic organisms can survive on them that release toxic pollutants, which lead to harmful effects on human health. Keratins are the structural elements of feathers, hair, nails, horns, hooves, bones, furs, claws, hides, beaks, skin, wool, scales, and bristles. About 90% of keratins are found in birds' feathers and hairs (Gopinath et al. 2015). After chitin and cellulose, keratin is one of the most prevalent biopolymers found in nature. It is a highly insoluble, rigid fibrous material found in the tissues of mammals, amphibians, reptiles, and birds (feathers and beaks). Degradation of keratin involves two steps; the first step is keratinase adsorption to a macromolecule surface through hydrophobic and electrostatic interaction and the second step is the catalytic action. The catalysis of keratin can be of two steps; sulfitolysis or reduction of disulfide bond and the other step is proteolysis. Sulfitolysis is the major step in keratin hydrolysis before the action of keratinase, it happens in the presence of reducing compounds like disulfide reductase, sodium sulfite, dithiothreitol (DTT),

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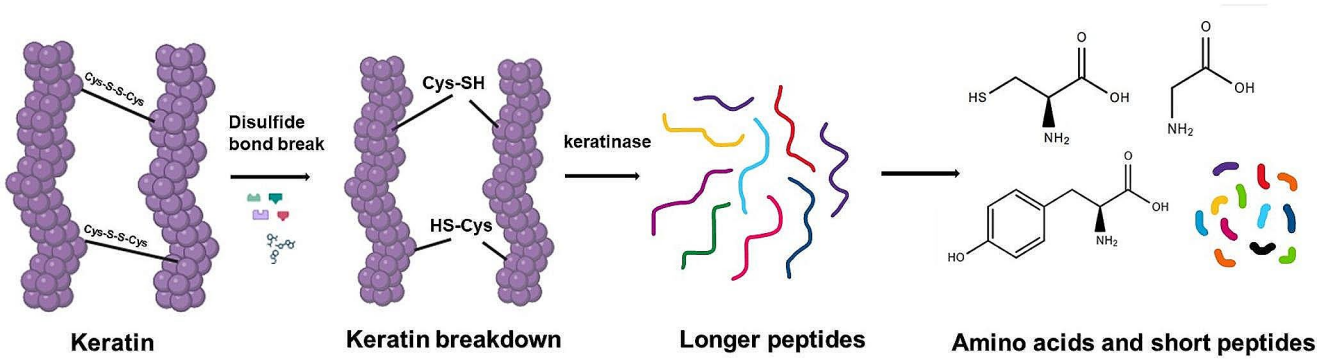

Fig. 1 Conversion of keratin protein to hydrolysates

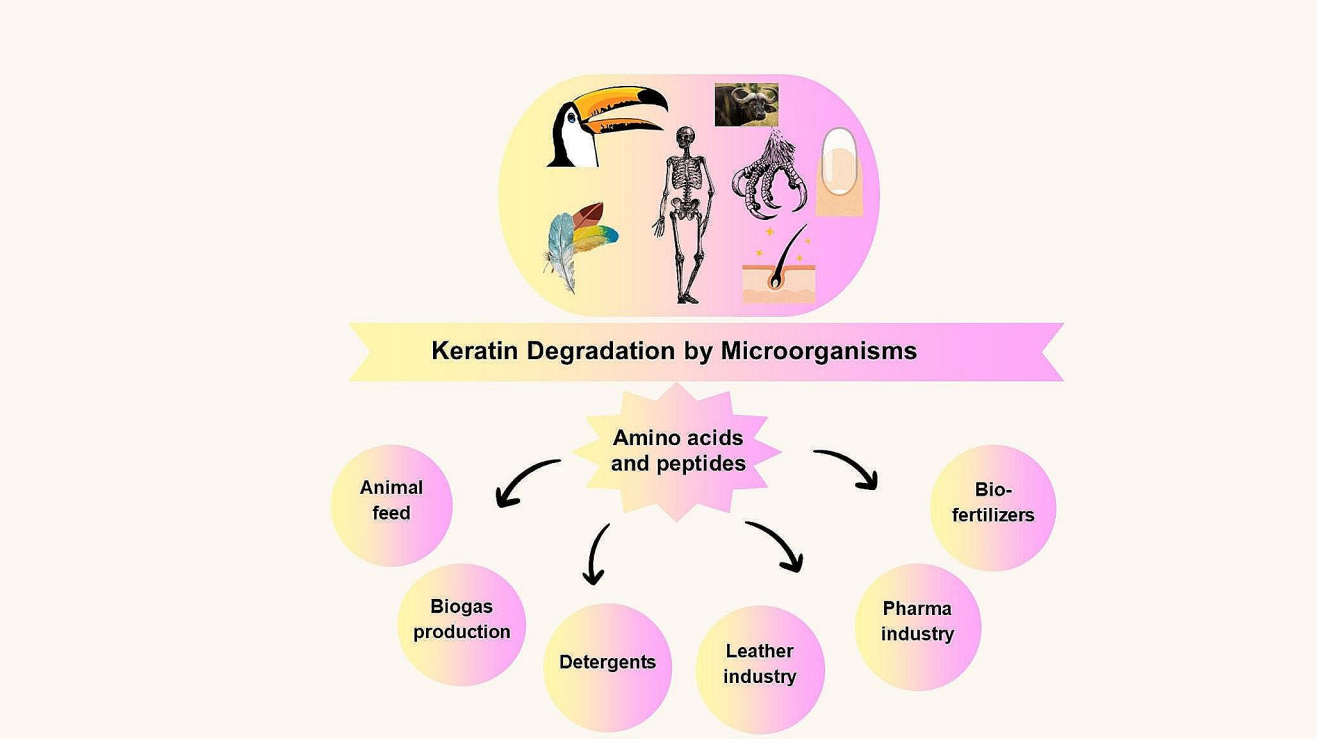

Fig. 2 Applications of keratin hydrolysates in various industrial sectors

and mercaptoethanol and acts synergistically with keratinase in degradation of keratin molecules (Yamamura et al. 2002). In proteolysis, keratinase can break the polypeptides into amino acids illustrated in Fig. 1. Keratin breakdown happens by multiple protease families since different enzymes favour different cleavage sites, and many enzymes are needed to convert keratin into amino acids completely. Additionally, disulfide reductase (Kasperova et al. 2013), lytic polysaccharide monooxygenases (Lange et al. 2016), and specific enzymes involved in lipoprotein signalling or fatty acid degradation have been related to biocatalytic keratin breakdown.

Conventional methods like acid hydrolysis are quite effective, but they also lose some essential amino acids, such as tryptophan, thus the residue that is left over is not very nutritious (Goda et al. 2021). Alkali hydrolysis at 80 °C with 2% NaOH for three hours produced a 25% yield which also lost some essential amino acids like cysteine, tryptophan, methionine, and tyrosine (Chilakamarry et al. 2021). Feather degradation using microorganisms is an easy and less expensive process for converting raw feathers to hydrolysates as a useful end product. Microbial degradation of keratin waste can yield high-quality protein hydrolysates (Nnolim et al. 2020a). Figure 2 represents the use of feather hydrolysates for animal feed, as a bio-fertilizer, producing coatings, films, and glues (Ozdal and Kurbanoglu 2018, 2019a, b), in cosmetics production, and pharmaceutical industries. They can also be used in peptone-substituted

Table 1 Summary of some studies on keratin-degrading organisms, their substrate and applications

Application	Microorganisms	Substrate	References
Animal feed	*Bacillus licheniformis* LMUB05, *Bacillus licheniformis* ER-15, *Bacillus amyloliquefaciens* 3–2, *Kocuria rosea, Flavobacterium sp.*	Feather meal, feather	(Adetunji and Adejumo 2018), (Tiwary and Gupta 2012), (Zhou et al. 2022), (Bertsch et al. Bertsch and Coello 2005), (Riffel and Brandelli 2002)
Biogas production	Recombinant *Bacillus megaterium, Bacillus licheniformis* KK1, *Bacillus* sp.C4, *Bacillus* sp.CL18, *Thermococcus litoralis*	Chicken feathers, feather meal, feather hydrolysate	(Forgacs et al. 2011), (Balint et al. 2005), (Patinvoh et al. 2016), (Schommer et al. 2020), (Balint et al. 2005)
Butanol production	*Clostridium sp.*	Wheat staw hydrolysates and Chicken feather waste	(Liberato et al. 2019)
Promotes plant growth activity	*Stenotrophomonas maltophilia, Bacillus sp*. MBRL 575	Chicken feather	(Jeong et al. 2010), (Kshetri and Ningthoujam 2016)
Dehairing	*Bacillus safensis, Bacillus subtilis* S14	Feathers, hair, wool	(Lateef et al. 2015), (Cai et al. 2008)
De-staining	*Paenibacillus woosongensis* TKB2, *Bacillus tequilensis* hsTKB2	Chicken feather	(Paul et al. 2016), (Nnolim et al. 2020b)
Detergent additive	*Coriolopsis byrsina, Citrobacter diversus, Actinoallteichus sp*. MA-32, *Bacillus safensis* LAU 13, *Arthrobacter sp*. KFS-1, *Streptomyces aureofaciens* K13	Chicken feathers, wool	(Duffeck et al. 2020a), (Duffeck et al. 2020b), (Manivasagan et al. 2014), (Lateef et al. 2015), (Nnolim et al. 2020a), (Gong et al. 2015)
Detergent additive, leather dehairing	*Bacillus aerius* NSMk2	Chicken feathers	(Bhari et al. 2019)
Leather industry	*Streptomyces gulbargensis*	Feather waste	(Schrooyen et al. 2001)
Degrade prion proteins	*Nocardiopsis* strain TOA-1, *Bacillus licheniformis* strain PWD-1, *Bacillus licheniformis* N22	Feathers	(Adelere and Lateef 2019), (Langeveld et al. 2003), (Okoroma et al. 2013)

microbial growth media (Ozdal et al. 2017a, b; Kurbanoglu et al. 2015), plant growth hormones (Ozdal et al. 2017a, b), citric acid production (Ozdal and Kurbanoglu 2019a, b) and animal feedstocks (Nnolim and Nwodo 2021). Many studies have reported the use of single strain identification for degrading keratins, which has potential, but the use of microbial consortium showed better results which have less evidence (Kang et al. 2020). More research on microbial consortium-based degradation methods should be studied for efficient hydrolysis, and reduced loss of amino acids can be obtained. Different microorganisms have different capacities to degrade based on their biochemical factors (Callegaro et al. 2018). Some of the studies on keratin-degrading microorganisms and their applications are described in Table 1. Employment of microbial consortium, keratin can be degraded effectively and contribute to industries in an eco-friendly manner, such as pharmaceuticals, cosmetics, animal feed, and bio-fertilizers (Kang et al. 2021).

Structure of keratin

Keratin, a fibrous and structural polypeptide, belongs to a heterogeneous family and is highly complex to degradation by proteolytic enzymes. Disulfide bonds and hydrogen bonds that interconnect keratin make them insoluble in water, weak acids, and organic solvents. It has been classified according to its secondary structure: α keratins and β keratins. α-keratins are found in the hair, horns, nails, claws, wool, and hooves of mammals. It has an average molecular weight between 40 and 60 kDa, has a reduced content of sulfur, is partly crystalline, and also self-assembles into filamentous fibres (Lee et al. 2014). Feathers, claws, and beaks of birds contain β-keratins, which are insoluble sulfur compounds with disulfide bridges. The interchain hydrogen bonds that exist between the amino and carbonyl groups define the β-sheet structure. These sheets are formed by hydrophobic interactions between four β-strands and later form dimers by various types of interactions like disulfide bonds, salt bridges, and hydrophobic interactions which in turn assemble into β-keratin filaments. Based on the sulfur content that comes from cysteine residues, feathers are further divided into soft and hard keratins. Hard keratins are present in feathers, hairs, and nails, while soft keratins are found in the skin and callus (Gopinath et al. 2015).

Keratinase

Keratinase [EC 3.4.21/24/99.11] is a group of hydrolytic enzymes that can catalyze the degradation of keratins. Keratinases are serine and metalloprotease or serine-metalloprotease enzymes capable of degrading keratinous protein (Hassan et al. 2020). Though, to date, they cannot completely

solubilize keratins, their nature of catalysis is still a puzzle (Gupta and Ramnani 2006). A detailed description of the diversity and classification of keratinase has been provided in the MEROPS database (Rawlings et al. 2018), based on the amino acid sequence and conserved domains. So far, at least fourteen distinct protease families represent the known keratinolytic enzymes such as S1, S8, S9, S10, S16, M3, M4, M14, M16, M28, M32, M36, M38, and M55 (Qiu et al. 2020). Based on so far recognized enzymes and the sequences filed in databases it is estimated that the molecular weight of keratinase ranges from 20 to 200 kDa. The ability to classify keratinases according to their amino acid sequence offers a distinct and understandable perspective on the mechanism and function of keratinases, indicating that figuring out the amino acid sequence of newly discovered keratinases is a crucial task (Li 2021; Gopinath et al. 2015).

Microbial degradation of keratin

Bacterial strains like *Bacillus licheniformis* (Lin et al. 1997), B. *pumilus*, *B. cereus*, and *B. Subtilis* (Nagal and Jain 2010) as well as non-sporogenic bacteria *Stenotrophomonas sp*. (Yamamura et al. 2002), *Fervidobacterium pennavorans* (Friedrich and Antranikian 1996), *Lysobacter sp*. (Pereira et al. 2014) and *Kocuria sp.* (Bernal et al. 2006) have been reported to be capable of degrading keratin. According to a study, the anaerobic bacterium *Serratia marcescens* EGD-HP20 is responsible for the breakdown of feathers through the production of proteolytic enzymes that hydrolyze keratin (Fuke et al. 2018). Two fungi were isolated from feathers: *Fusarium oxysporum* and *Aspergillus sp.* exhibited effective keratinase production of which *Fusarium oxysporum* showed high enzyme activity on the 6th day with a value of 243.25 U mL^{-1} and *Aspergillus sp.* 113.50 U mL^{-1} on 9th day (Preczeski et al. 2020). Recent research revealed actinomycetes strain *Streptomyces werraensis* KN23 showed high keratinase production reporting 51.60 U/mL and enhanced its effect by chemical mutagenesis recording its value of 106.92 U/mL (Abd El-Aziz et al. 2023). Factors like pH, temperature, carbon, nitrogen source, and agitation rate influence keratin hydrolysis (Revankar et al. 2023).

Applications

Feather lysate as animal feed

Feather meals as an additive for livestock have been practised for many years. Still, there has been concern about its nutritional value due to the inadequacy of essential amino acids in the feed. This is due to the conventional chemical-based treatment of feathers which involves higher energy and denatures some heat-labile amino acids. Chicken fed with feather lysate prepared by anaerobic fermentation of feather with *Bacillus licheniformis* PWD-1 showed an increase in growth response of 19.3% with that of corn-soybean meal used as control. The chickens fed with feather hydrolysate supplemented with lysine, methionine, and histidine as additives produced a similar growth curve as soybean meal (Williams et al. 1991). In a study, the *Kocuria rosea* strain was cultured under aerobic conditions with feathers as the substrate by submerged fermentation to obtain feather meal. Pepsin digestibility of the feather meal obtained by this method was about 88%, which is similar to the value seen in commercially available meals. Apart from that the feather meal fermented by *Kocuria rosea* was observed to have more amino acid contents when compared to commercial feather meal. Additionally, the bacterial biomass increased the amount of amino acids, notably lysine by 3.46%, histidine by 0.94%, and methionine by 0.69% (Bertsch and Coello 2005). The growth of broiler chicken fed with *f*eather meal prepared by *Bacillus licheniformis* LMUB05 fermentation was comparable with that of the growth of broiler chicken fed standard meal (Adetunji and Adejumo 2018). *Bacillus licheniformis* ER-15 produced dimeric keratinases that enhanced the decaying activity of feathers at 50◦C pH 8 within 8 h. Also, 14% nitrogen, 44% carbon, and a few necessary amino acids were present in the feather meal (Tiwary and Gupta 2012). This research gives a way to replace commercial meals with feather lysate as a protein source to reduce the overhead cost of production.

Feather lysate as biofertilizer

Feather hydrolysate as a biofertilizer is currently needed as it is an environment-friendly, cost-effective product for agricultural production, as chemical fertilizers are harmful to the soil. Feather lysate with amino acids and peptides can be used as a plant growth promoter and slow-release nitrogenous fertilizer (Bhari et al. 2021). A feather degrading bacteria *Bacillus tropics* LS 27 isolated from a poultry dumping site degraded chicken feathers and the feather hydrolysate showed antioxidant properties and obtained 1.5 mg/mL by DPPH scavenging assay. It also showed that plants supplemented with 20% feather hydrolysate showed increased plant growth-promoting properties when utilized as a liquid biofertilizer (Stanly and Umesh 2023). By composting pig bristles in a mixture containing sawdust and lignite dust, the keratinolytic *Bacillus cereus* PCM 2849 strain shows improvement in the decomposition of organic waste. The carbon and nitrogen ratio, carbon solubility, oxidation index of mineral forms of nitrogen, and humification ratio were all favourably impacted by the bacterial inoculum, which also improved the transformation of mineral compounds

(Choinska-Pulit et al. 2019). *Amycolatopsis sp*. MBRL 40 was found in another study to have antifungal efficacy against four significant fungal pathogens *Pyricularia oryzae, Rhizoctonia solani, Fusarium oxysporum, Curvularia oryzae*, and plant growth regulators like indole-3-acetic acid (IAA) production and phosphate solubilization. Rice seeds treated with *Amycolatopsis sp.* MBRL 40 showed increased germination, vigor indices, and seedling growth. Protein hydrolysate obtained from *Trichoderma asperellum* showed improved activity on crop health (Calin et al. 2019). The growth rate of plants was observed higher when the feather hydrolysate pellet was supplemented with bioinoculant compared to plants provided only with feather hydrolysate pellet (Tamreihao et al. 2017). When chicken feathers are used as foliar feeding to banana plants, *Chryseobacterium sp.* RBT degraded them and banana fruit's protein content rose from 15 mg/g to 16 mg/g and its amino acid content from 2 mg/g to 2.96 mg/g. Furthermore, the fertilizing action of banana fruit significantly increased its anti-oxidant capacity through the presence of flavonoids and phenolics. Two native Arbuscular Mycorrhizal Fungi (AMF), *Glomus caesaris* and *Acaulospora bireticulata* obtained from sandy soil and *Bacillus licheniformis* ASU from chicken feathers. Hydrolysate from the feathers and AMF or a combination of both was inoculated in pots where faba bean seeds were grown. When compared to non-inoculated plants, the total dry biomass of plants co-inoculated with AMF and feather hydrolysate (FH) increased substantially. Therefore, the combination of FH and AMF may develop into a useful biofertilizer in the future (Nafady et al. 2018).

Partially hydrolyzed keratin for producing films, coatings, and glues

A study used microcrystalline cellulose (0.2%) and glycerol (3.5%) in NaOH to create the bioplastic film and keratin hydrolysate were obtained after chemical treatment of feathers for 48 h at 60 °C where the thickness of the bioplastic film was found to be 1.12×10^{-4} mm (Sharma et al. 2018). Using non-soluble keratin for biodegradable and eco-friendly composites has developed a new process to combat sustainable economic needs. Keratin powder was added to polylactic acid pellets to obtain composites using hot-melt extrusion technology. The samples exhibited the same thermal stability compared to Poly lactic acid alone with decreased toughness (Pulidori et al. 2022). A study developed an efficient biomaterial that converted keratin waste to bio-composite film in combination with ginger starch (Oluba et al. 2021). Feather protein-based resins were created by utilizing three pH values, two formaldehyde-phenol (F-P) ratios, and two hydrolysis techniques. Feather hydrolysate was prepared with an F-P ratio of 2.0 at pH 10.5 and 30% phenol substitution with feather protein. It was made from one part feather meal hydrolyzed in an alkaline solution with two parts phenol, and it performed better than a commercial phenol-formaldehyde resin. These results suggest that feather hydrolysate functions as an efficient co-polymer in these types of resin formulations and could represent a reasonably priced additional raw material for the preparation of phenol-formaldehyde-type wood adhesive resins (Jiang et al. 2008).

Biogas production from keratin waste

The conversion of keratin waste to biogas is an eco-friendly and cost-effective method. A two-process system was developed that combines keratin degradation and biogas production. Chicken feather degradation by recombinant *Bacillus megaterium* strain showed a yield of 0.51 mg/mL soluble proteins after 8 days of cultivation. During the anaerobic batch digestion process methane gas produced about 0.35 Nm^3/kg of dry feathers (Forgacs et al. 2011). A similar study was carried out where keratin was first degraded by *Bacillus licheniformis* KK1 and then the hydrolysate obtained was optimized and metabolized by *Thermococcus litoralis* generating hydrogen gas as a fermentation byproduct (Balint et al. 2005). These methods can be effective and environment friendly where waste is converted into a byproduct along with biogas production that can reduce the manpower and cost for their production.

Feather hydrolysate in cosmetics

Hydrolyzed keratin peptides obtained from wool were applied to the skin, improving hydration and elasticity. It can also be applied with wool internal lipids, enhancing absorption and desorption profile (Barba et al. 2008). Keratin hydrolysates from chicken feather waste showed potential antioxidant and anti-tyrosinase activity (Kshetri et al. 2020). Keratin peptides obtained from enzymatic hydrolysis of chicken feathers by *Bacillus subtilis* AMR tend to penetrate hair or nail cuticles and hydrate the hair follicles. The keratin peptides made the hair fibres more hydrated, and the sealed cuticles in the fibres treated with the hydrolysates also showed a significant improvement in brightness and softness by Scanning Electron Microscope (SEM) analysis (Villa et al. 2013). Hydrolysates of keratin by alkaline-enzymatic hydrolysis strengthen the skin barrier by reducing transepidermal water loss and hydrating the skin. Moisturizing properties were examined in both men and women at different time intervals and concentrations. According to the results of the hydration measurement, adding 2% keratin hydrolysate to the ointment base during

the monitored measurement interval (1–48 h) results in an increase in stratum corneum hydration of 11–19% for male volunteers and 12–22% for female volunteers and it also showed a reduction in transepidermal water loss (Mokrejs et al. 2017a).

Pharmaceutical applications

2% of keratin hydrolysate combined with an ointment base can act as a good humectant when tested over 48 h and has increased skin hydration. The results interpreted a 14-23% increase in hydration of stratum corneum and for trans-epidermal water loss about 4% of keratin hydrolysate was preferred for the reduced trans-epidermal water loss of about 26-46%. Such formulations are stable in their structure and do not cause phase separation (Mokrejs et al. 2017b). Acrylic acid and acrylamide monomers (AAm) were added with keratin hydrolysate obtained from bovine hair using the alkali technique to create superabsorbent hydrogels through free radical graft copolymerization. After 48 h, the highest swelling ratio of 1791% was observed at pH 9. With a swelling capacity of 1430.7%, the ideal formulation of the synthesized hydrogel contains 3 g of keratin hydrolysate and 4 g of AAm copolymer. This suggested technique effectively transformed low-biodegradable keratin waste into superabsorbent hydrogel that can be used in cosmetics and biomedical applications (Arican et al. 2021).

Conclusion

Poultry processing industries and farms produce enormous amounts of feather-keratin waste which creates a problem of solid waste in the environment. Microbial conversion of feather waste is an eco-friendly and sustainable approach. Chicken feather waste can be processed into a useful by-product for various industries mainly in the cosmetics and bio-fertilizer domain. Feather hydrolysates contain rich amino acids which could be potent for poultry feed development. In the future, feathers can be converted into enriched products that can support industries at a large scale without looking for any other source of raw material that can cause damage to the environment. This review highlights the versatile uses of keratin hydrolysates in various biotechnology sectors which paved a new way to combat environmental pollution by transforming community waste into a valuable product.

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Author contributions KM - Study conception and design, literature search, data collection, analysis and interpretation of results, and writing the original draft. PK– Review and Editing. LMS– Conceptualization, Supervision, Review, and Editing.

Data availability All the data used for the study has been cited and included in the references.

Declarations

Conflict of interest The authors declare that there is no conflict of interest for the submission of this manuscript.

Declaration statement The work reported in this manuscript has not been published elsewhere and is not currently under any other considerations.

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