



Review

Keratinous and corneous-based products towards circular bioeconomy: A research review



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ABSTRACT

Keratins and corneous proteins are key components of biomaterials used in a wide range of applications and are potential substitutes for petrochemical-based products. Horns, hooves, feathers, claws, and similar animal tissues are abundant sources of α -keratin and corneous β -proteins, which are by-products of the food industry. Their close association with the meat industry raises environmental and ethical concerns regarding their disposal. To promote an eco-friendly and circular use of these materials in novel applications, efforts have focused on recovering these residues to develop sustainable, non-animal-related, affordable, and scalable procedures. Here, we review and examine biotechnological methods for extracting and expressing α -keratins and corneous β -proteins in microorganisms. This review highlights consolidated research trends in biomaterials, medical devices, food supplements, and packaging, demonstrating the keratin industry's potential to create innovative value-added products. Additionally, it analyzes the state of the art of related intellectual property and market size to underscore the potential within a circular bioeconomic model.

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1. Introduction

The circular economy concept encompasses various approaches to encourage sustainable growth and development through the synergy between markets, societies, and the environment. Yet, at its core, this paradigm strives to generate value by extending the longevity of products and revalorising waste management from the end of the supply chain to the beginning [1–5]. Unfortunately, many of the current dominant economic systems are deep-seated in a model that thrives on the provision of goods and services but

perpetuates an environmentally inefficient throwaway culture [6–9]. In this sense, the Sustainable Development Goals set by the United Nations serve as a powerful driving force to foster economic prosperity while simultaneously protecting the environment. Among the 17 goals, “Responsible consumption and production” encompasses a range of targets that collectively address sustainable food production and appropriate waste management.

Food products' industrial processing and manufacturing inevitably generate a significant volume of waste. Approximately one-third of all food produced annually is estimated to be wasted, leading to substantial economic losses and significant environmental concerns. Yet, these wastes possess great potential as sources of valuable compounds and raw materials [10–13]. Therefore, when promoting the circular economy within food production, it is crucial not to overlook these by-products' comprehensive examination and revalorisation. The production of

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meat and its derivatives has been associated with the generation of unavoidable large quantities of side-streams, including various waste materials, in which among those, corneous and keratinous materials represent a significant component [14, 15]. These are derived from the horns, hooves, feathers, claws, and other similar tissues of animals. Those are the results of related goods manufacturing, often during the slaughtering and butchering of animals for meat production. Therefore, these materials cannot be used directly for human consumption and are typically regarded as meat industry waste or by-products [16,17]. Their disposal could be a challenge, especially for industrial quantities. Improper disposal of corneous and keratinous wastes can pollute land, water bodies, and air [18]. In this sense, ammonia and other nitrogen species are released during the disintegration of the proteins composing keratinous materials when disposed of in landfills. They can contaminate lands and water bodies easily, generating environmental issues such as uncontrolled eutrophication processes and soil acidification due to nitrogen deposition. On the other hand, when these wastes are burned, they can emit particulate matter, volatile organic compounds (VOCs), and greenhouse gases, which also compound ecological concerns. Therefore, it is crucial to comprehensively explore sustainable and environmentally friendly techniques for appropriately managing these waste materials [19,20] while possibly recovering value in a circular economy approach. Statistics report that each year, the worldwide production of keratin waste reaches approximately 10 million tons, with 8.5 million tons accounting only for feather waste [21]. Traditionally, this waste was processed into feedstuffs through thermohydrolysis despite losing some amino acids. Alternative methods include chemical-thermal hydrolysis and enzymatic-chemical hydrolysis, which improve digestibility and amino acid content. However, European Union's regulations now restrict animal by-products in feed, leading to research into other applications like cosmetics, pharmaceuticals, and agriculture. Microbial degradation of keratin waste is an eco-friendly option, with keratinolytic microorganisms providing a sustainable waste management solution [21,22]. Composting is a popular method, turning keratin waste into valuable fertilizer, improving soil properties, and supporting plant nutrition. The process involves a succession of microbial communities that transform the waste into plant-absorbable forms of nitrogen and sulfur. This approach not only recycles waste but also contributes to environmental conservation.

Historically important, the word "keratin" (from the Greek "kera", meaning horn) appeared in the literature around 1849 to describe the material that made up hard tissues such as horns and hooves. Different methods used for dissolving proteins have been ineffective with keratin, and some members of the scientific community are especially interested in dissolving hair for the further creation of manufactured products. Furthermore, the intensive commercialization of synthetic fibres like nylon and polyester was seen as a threat to the dominance in wool production, especially in Australia, and the Council for Scientific and Industrial Research established the Division of Protein Chemistry in 1940: this was done to also understand the structure of wool and keratins expanding their applications better. The fundamental methods for extracting, separating, and identifying keratins were developed. In 1965, W. Gordon Crewther and colleagues published the definitive text on the chemistry of keratins [23], containing references to more than 640 published investigations. The epidermis of scales, claws, beaks, and feathers in reptiles and birds (sauropsids) largely comprises small proteins formerly indicated as " β -keratins" but identified as corneous β -proteins to avoid confusion with true keratins as those of intermediate filament keratins (IFKs) [24–27].

Nowadays, recent research available, together with the help of industries, may have found different applications and paths to

incorporate those corneous and keratinous materials into various products. For instance, extracted keratin can be used in the production of fertilizers, cosmetics, bioplastic materials and even as a component in biomedical applications [28], such as tissue engineering and drug delivery systems [29–36]. Additionally, advancements in biotechnology approaches have opened possibilities for the management and transformation of corneous materials into biochemicals with a special interest in the agricultural field [37,38]:

Advances and development aims are underway to explore innovative processes, such as microorganism degradation and enzymatic hydrolysis [39–42] towards valuable bio-based products. The cutting-edge research and development efforts in the food production industry and the support of biotechnological procedures are triggering the revalorisation of corneous and keratinous materials. Two main approaches, (i) biotechnological (via engineered microorganisms, based on synthetic biology) and (ii) utilising collected wastes, can be exploited to obtain α -keratins and sauropsid β -keratins which could be utilized to produce bio-products (Fig. 1). In this sense, those developments will not only hit the aim of reducing waste loss but also provide disruptive profitable values through the exploitation of their promising applications, consequently matching a circular economy mindset.

Evidence from recent bibliographic research points out some review articles focused on extraction methods or waste valorisation. The present manuscript aims to fill a gap concerning state-of-the-art resource recovery, boosting sustainable heterologous protein expression and intellectual properties, and research trends related to keratinous and corneous-based products. We highlight how a new "keratinous economy" may be proposed due to the new research plus consolidated goals regarding market and patents.

This review aims to compile research developments and utilise corneous and keratinous materials to produce bio-commodities, which have been debated. It also focuses on related biotechnological approaches. The review has been developed through a narrative literature methodology in which a critical analysis and summary of current empirical results have been executed together with drawing conclusions and identifying the gaps plus inconsistencies regarding this field of knowledge. The authors independently extracted articles from the different areas of the paper, focusing on recent scientific literature and older references relevant to the article's drafting. The study supervisors then harmonised the text at the final revision stage. Intellectual propriety and the market size are discussed to give an insight into the situation of this scientific topic in the industry.

2. Chemical-physical properties of α -keratins and corneous β -proteins

A-Keratins are fibrous proteins of the intermediate filament (IF)-family, specifically with a variable content of cysteine and glycine, having poor solubility in water and most common solvents [43–48]. These represent a wide family of biomacromolecules characterised by high-sulfurated proteins with filament-forming structures assembled in a hierarchical structure. The polypeptide units are arranged into structures of higher complexity by folding into α conformation. These structures are further assembled at the microscale in the bigger organisation of keratinized cells, forming (i) fibre, (ii) lamellar, (iii) tubular, or (iv) sheet structures, arranged in various forms of macroscale compact shields, resulting in highly resistant non-reactive materials able to withstand mechanical stress. The amino acid composition of keratin is variable, being coded by specific genes in every keratinous material, but sequences of certain polypeptides have similarities in different species. Based on gene substructure and protein sequence homology, types 1 and 2 of keratin can generally be distinguished. Furthermore, through

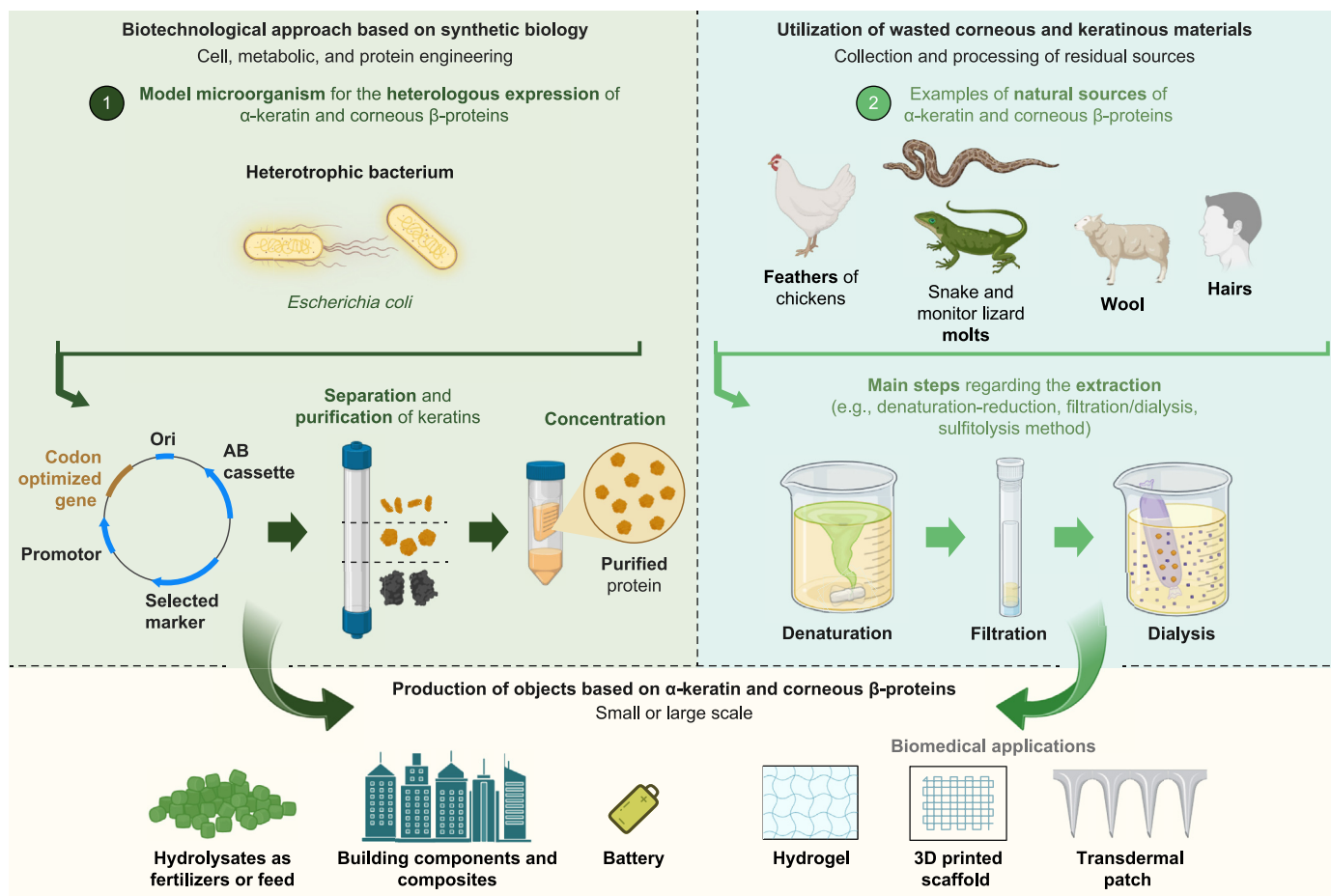


Fig. 1. Two main strategies for α -keratin and corneous β -proteins production: biotechnological and waste-based. Schematic representation of two approaches for α -keratin and corneous β -proteins production, (i) biotechnological approach and (ii) utilisation of wasted corneous and keratinous materials, and subsequent downstream steps to generate products. Ori: origin of replication. AB: antibiotic.

two-dimensional gel electrophoresis, type I keratins, acidic (pI 4.5–6) with smaller molecular weight (MW) of 40–55 kDa, can be separated from type II keratins that are basic-neutral (isoelectric point (pI) 6.5–8.5), and larger (50–70 kDa) [49]. The well-known keratin genes comprise 8–9 exons corresponding to various types I and II [25,44,47]. Keratin can also be classified in terms of sulfur cross-links, distinguishing from soft keratins (e.g., stratum corneum) and hard keratins (e.g., hair, nails, claws, beaks, and quills) [45,46]. Besides, cysteine and glutamic acid are common residues for whole wool keratin, while glycine, proline and serine are frequent amino acids in feather keratin [27,43,50].

The different amino acids are organised in secondary structures known as α -helix and corneous β -sheet (Fig. 2), the two major secondary protein structures. α -Keratins are IF keratins of type I and II that in amniotes are also acidic (type I) or basic (type II): their genes are localised in specific loci, there is one locus of type I keratin genes and another locus of type II genes [25,44,46,47,51]. These hydrogen bonds stabilise a twisted structure and MW of 40–80 kDa. The pairing of two α -helix bonded by disulfide cross-links forms a left-handed coiled coil, mentioned as a dimer. The aggregation of dimers in an end-to-end and side-by-side manner by disulfide bonds determines the formation of the so-called “protofilament”, a building block of the intermediate filament, which is a further arrangement of protofilaments organised in groups of four elements [52].

Several IF-keratins show an extended and conserved region

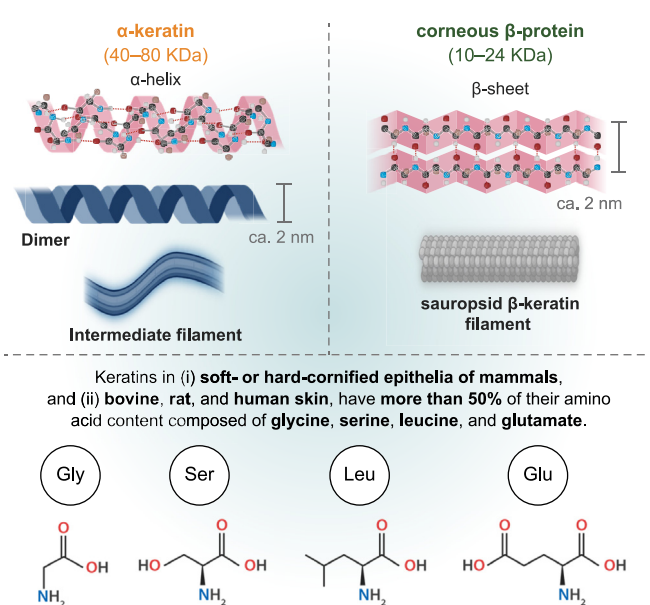


Fig. 2. Schematic representation of α -keratin and corneous β -protein structure levels. The secondary structure assembles in α -helix or a β -sheet, and the structures for the amino acid glycine (gly), serine (ser), leucine (leu), and glutamate (glu) are shown. Ca.: circa.

mainly conformed as α -helix, called a central rod, separated by short non- α linker regions [44,46]. The organisation of protofilaments along the periphery — epidermal keratins, or peripheral protofilaments and in the central region — hair keratins, gives rise to the IF-keratins of 8–10 nm in diameter. The assemblage of these proteins, analysing materials containing these in large amounts, shows a prevalent α -pattern under X-ray analysis since most IF-proteins possess few regions confirmed in β -sheets contrasting the central rod region [43,44]. Under mechanical tension and high temperature and/or humidity, the α -regions are turned into β -sheet and random coiled regions [29,43,53], inducing a β -X ray pattern [43]. In corneous β -proteins, the amino acid chains are folded in four β -strands localized in a central region termed β -region or core-box [25,27]. This β -region initially pairs with a β -region of another corneous β -protein through polar and apolar bonds forming a dimer [24]. Then, each dimer rotates 45° and polymerizes into a linear macromolecule that forms a β -filament. When extracted from corneous material and analysed by electrophoresis, corneous β -protein (the monomers derived from the β -filament) show a MW of 10–24 kDa [25,26,54]. X-ray diffraction analysis (started in 1931 on human hairs [55]) allows us to associate the specific diffraction pattern of α -keratin to wool, hair, quills, fingernails, horns, hooves and stratum corneum, and corneous β -proteins to feathers, avian beaks and claws, reptilian claws and scales [54].

Especially due to the presence of disulphide bonds for the high cysteine content, α -keratins are generally insoluble in most organic and water-based solvents. Because of its large composition variability, the solubility changes based on the amount of ionic, hydrogen and hydrophobic bonds. Wool keratin, for example, is reported to have lower solubility and higher stability thanks to its high amount of cysteine disulphide bonds. The pH highly influences the presence of ionic bonds, mainly due to carboxylic anions and ammonium cations. Acidic pH determines the protonation of the carboxylic group, while basic pH is the deprotonation of amine [50], lowering the solubility. α -Keratin and corneous β -proteins exhibit exceptional mechanical properties such as high stiffness and elasticity (Fig. 3) and confer interesting structural features when combined with the organizations of keratinized cells at the micro and macro scale. Corneous β -proteins usually show higher stiffness compared to α -keratin, although, under tensile load, it is possible to twist α -helices into β -sheets [27,53,56]. The main parameters that influence α -keratin and corneous β -proteins mechanical properties are the alignment of the filaments, the presence of ions, i.e., calcium, and the water content. The increase in humidity tends to decrease strength and Young's modulus, acting as a swelling agent because of the reduction of interchain

bonds and increased free volume [57].

3. Biotechnological approaches to extract and produce raw materials based on α -keratin and corneous β -protein

The extraction of α -keratins from biomasses obtained from by-products of the food industry poses a significant challenge due to disulphide bonds, which confer strong resistance to chemical, enzymatic, and thermal treatments. As previously discussed, their solubility proves to be an arduous task. Hence, it is crucial to prioritise discovering an effective, environmentally friendly, and cost-efficient method. Various approaches can be employed for keratin extraction (Fig. 3), including chemical hydrolysis, enzymatic and microbial treatments, dissolution in ionic liquids, and microwaving techniques [58–60].

Hydrolysis and sulfitolysis are commonly accepted methods with variable experimental conditions for dissolution [59]. Processes based on mercaptoethanol have been generally replaced with sodium sulphite, and other supporting chemicals are being substituted with ionic liquids. The digestion product is usually made by heating wool or feathers with a selected solvent from glycols, alkanol amines, and polyamines: the resulting digested α -keratin and the corneous β -proteins-based product is a derived polyol, useful for making polymeric materials [59].

One approach to maintaining protein's structure during extraction is through enzymatic treatment. Enzymes (e.g., keratinases from mesophilic fungi, actinomycetes, and some species of *Bacillus*) specifically designed to target and break down the disulphide bonds can selectively hydrolyse the IFKs [61,62]. It has been proven that the effects of different factors, such as the use of surfactant, enzyme loading, substrate type, and hydrolysis time, should be considered to optimise the enzymatic treatment [63]. Other techniques like steam explosion procedures and superheated processes have demonstrated effectiveness in recovering α -keratins and corneous β -proteins from biomasses [64–67]. However, it is important to note that these techniques may have the disadvantage of not maintaining the original structure of recovered proteins well. Steam explosion procedures involve subjecting the corneous biomasses to high-pressure steam, followed by rapid decompression, while superheated processes involve subjecting the corneous and keratinous biomasses to elevated temperatures above their boiling points. These processes break down the α -keratin and corneous β -protein structures, facilitating extraction. Even though these techniques can successfully recover high yields, they may cause important structural alteration, which might impact their functional properties and potential revalorising applications [68,69].

In addition to the methods mentioned above, using several solvents has been deeply investigated in this field [70]. Those are the ones considered for performing mild chemical hydrolysis (involving the controlled degradation, using gentle reagents such as mild acids or alkaline solutions), and those known as ionic liquids (organic and inorganic anions or bulky cations, with a high solvation capacity plus exceptional characteristic like melting and boiling points). Notably, while their use for α -keratin and corneous β -protein extraction offers significant advantages, not all could be considered eco-friendly. Therefore, the environmental impact of these is a crucial aspect to consider when evaluating their suitability for revalorisation applications. Certain solvents have relevant environmental risks due to their potential corrosiveness or toxicity, presenting detrimental environmental effects if not handled and disposed of properly. Moreover, producing and synthesising some ionic liquids can involve energy-intensive processes and potentially hazardous chemical reactions, contributing to their ecological footprint. In this context, ongoing efforts are being made

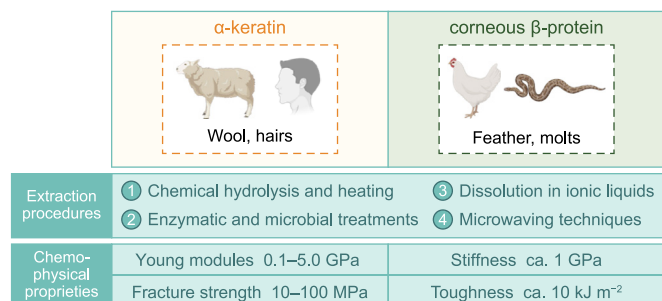


Fig. 3. Different procedures to obtain α -keratin and corneous β -protein and typical values of their main properties. Extraction procedures from natural sources (chemical hydrolysis and heating, enzymatic and microbial treatments, dissolution in ionic liquids, and microwaving techniques) and chemical-physical properties (young modulus, fracture strength, and toughness) are shown. Gpa: gigapascal. Mpa: megapascal. Ca.: circa.

to develop and identify more environmentally friendly solutions or techniques that can effectively extract α -keratin and corneous β -protein while minimizing ecological risks [71]. Furthermore, Cassoni et al. showed that the costs to obtain them with their method, based on one biodegradable reagent constituted by non-ionic surfactants (<5%) and NaOH (5–15%) combined with physical ultrafiltration, were approximately ten times lower than precipitation method with different reagents [71]. This might also be the case with techniques such as microwave-assisted extractions [72–74] and deep ionic liquids [75–78]. These methods offer the advantage of shorter extraction times and reduced exposure to harsh conditions, which can help preserve the structural integrity of α -keratin and corneous β -protein for revalorisation applications.

Another biotechnological tool for the valorisation of wasted keratinous material is the use of keratinases. Keratinases are unique enzymes that show a strong efficiency in feather transformation. A key factor of keratinase activity is the reductive cleavage of disulphide bonds in keratin, enhancing the solubility and the synergistic action of proteases and hydrolysis [79]. Moreover, novel searches performed by Cowan et al. [80] developed a colourimetric method using an anthraquinone-dyed substrate named α -keratin azure, which may better investigate the keratinase activity. This screening tool was then applied to investigate the reduction of the keratin disulfide bond on keratinase-catalysed degradation. These enzymes have been classified as proteases that break peptide and disulfide bonds, promoting the degradation of α -keratin and the production of α -keratin-based by-products. Different microorganisms can produce extracellular keratinases, which can degrade the α -keratin containing substrates, opening a wide range of industrial applications in waste treatment [81]. The above-mentioned approach may be considered an eco-friendly valorisation of keratinous materials, which is impossible using common proteases like trypsin pepsin or papain [82]. Qiu et al. studied the mechanism of action, proposing three strategies: endo-attack, exo-attack, and peptide-oligomers enzymatic action [40]. In the former case, the catalytic activity occurs on peptide bonds placed inside the polypeptide, while the end of the aminoacidic chain is involved in the latter. The oligopeptidase families can attack both the C-terminal and the N-terminal of the involved oligopeptides. Due to the multiple disulfide bond structure of α -keratin, enzymatically degradation is particularly hard. It seems to proceed by a two-step mechanism: the disulfide bond cleavage and the resulting hydrolysis of proteins into peptides and amino acids. According to a green technology approach, keratinases have a great value for natural wastes having α -keratin at high content and offer potential industrial application in animal feed, pharmaceuticals, detergent formulation, cosmetics, organic fertilisers, biogas, agriculture, and leather industry [82,83]. Some keratinase-based formulations have been made available for commercial use. These products were formulated with keratinases predominantly from *Bacillus licheniformis* strains. PROTEOS Biotech has four branded formulations in the market: Keratoclean® sensitive PB, Keratoclean® HYDRA PB, Keratoclean® BP, and PURE100 KERATINASE [84]. They are used as topical agents for treating skin problems and related conditions. Valkerase® and Versazyme® are products of BioResource International, Inc. (BRI). Differently, CIBENZA® DP100 and FEED-0001 were introduced to the feed market by Novus® International and Creative Enzymes®, respectively. These formulations can potentially be utilized to improve the nutritional values of animal feeds [84,85]. Keratin proteins, found in feathers and other keratin-based materials, are largely indigestible by ruminants and other livestock. This is primarily due to their structural orientation, determined by various chemical groups. Unless these structures are significantly broken down, the keratin proteins remain mostly inaccessible [85].

Sharma et al. [86] have been studying keratinase-based

nanocomplexes to immobilise enzymes and their potential use in industrial applications. The isolation of keratinase-producing microorganisms has been performed from very different contexts, thus showing various urban or natural environments that are potential keratinase sources. Recent examples are the isolation of *Pseudomonas geniculata* H10 from hair residual of bathroom drains in South Korea, *Bacillus licheniformis* KRLr1 from poultry litter in Iran, *Bacillus velezensis* ZBE1 forest soil of Western Ghats of Karnataka in India [22,87,88]. Within the process of extracting α -keratin and corneous β -protein from natural sources, an important challenge emerges in association with the rigorous processing conditions. Unfortunately, as mentioned before, these procedures may lead to the formation of undesired molecules or modified and biologically damaged structures, which may affect the targeted final applications. This issue underscores the necessity for alternatives to overcome these drawbacks while upholding the physicochemical and potentially bioactive attributes. In this sense, recombinant proteins might be a promising avenue that holds the potential for α -keratin production and utilisation, especially when the targeted applications are biomedical, in which the legislation and requirements are considerably tighter [89,90].

Unlike the conventional extraction approach, recombinant techniques involve the utilisation of genetically engineered microorganisms, which act as bio-factories to produce keratin proteins. Genetic accessibility is necessary to chassis cells with potential industrial applications, allowing them to engineer strains towards improved expression of proteins and robustness. On the one hand, numerous methods for genome editing with direct synthetic biology applications have been established for model microorganisms (e.g., *Saccharomyces cerevisiae* [*S. cerevisiae*] and *Escherichia coli* [*E. coli*]). Conversely, the available toolkit for non-model cells (e.g., cyanobacteria and microalgae) often prevents specific strain engineering. Expanding this scientific area is challenging but vital to accelerate bioprocesses [91]. Still, such transformative strategy offers a multifaceted solution that addresses the limitations of conventional extraction methods, diminishing the formation of unwanted by-products by sidestepping harsh extraction conditions: this can result in a purer final product, as the three-dimensional (3D) structure and composition of natural α -keratin and corneous β -protein are more faithfully preserved through recombinant techniques [92–94]. Industries dependent on specific properties can transit seamlessly to these recombinant versions without compromising product performance and ensuring the robustness of the designed applications [95].

The scientific community has recently tested it towards miscellaneous applications, including biotechnological applications like wound healing, tissue engineering, and cosmetic products [50]. However, the expression level, protein structure, and functional properties may vary depending on the specific applied microorganism and the methods employed for genetic modification, protein production, and recovery [96]. In this sense, Guo et al. used a certain strain of *E. coli* as a host strain for expressing recombinant human hair α -keratins (K37 and K81) that were utilized for rat halting bleeding, thanks to their capacity to promote the fibrin clot formation at different sites of injury [97]. A similar strategy was performed by Gao et al., in 2019, in which recombinant human hair α -keratins nanoparticles were recognized as promising candidates in dermal wound healing. Regarding the improvement of human hair repairing [98], Basit et al. demonstrated how recombinant K31 α -keratin showed marked thickness, strength and smoothness improvements when applied to human-damaged hair [99]. Despite the promising results, further studies must be carried out since some biological mechanisms (e.g., the solubilisation or the breakage of disulfide bonds) are not fully understood, whilst they play a key role in the healing capabilities [100]. The rational design

and synthesis of water-soluble keratins was recently performed using the Glutamine (Q) Threonine (T) Tyrosine (Y) (QTY) code [94]. For the first time in recombinant α -keratins, through heterologous expression in *E. coli*, the homotypic self-assembly was observed in an aqueous environment minus urea. Without apparent structural changes compared to the native proteins, a 200-fold increase in water solubility of the QTY variant keratins was shown via *In vitro* biophysical analyses and molecular dynamic simulation [94].

The research is not yet focussed on microorganisms different than *E. coli*, like model methylotrophic yeast (e.g., *Komagatella phaffii* [*K. phaffii*]) and photosynthetic bacteria (e.g., *Synechocystis* sp. PCC 6803 [*Syn.* PCC 6803]), regarding heterologous α -keratin and corneous β -protein production, even if these are hosting model microorganisms for different recombinant proteins production [91,101]. The heterologous expression of keratin and corneous proteins is still in its infancy, given that most efforts have been made to recover wasted keratinous and corneous material directly.

4. Emerging trends in corneous and keratin-derived materials in biomedical and other industrial applications

Corneous and keratin-derived materials represent an innovative frontier in the biomedical and other industrial fields, showing an interesting approach concerning circular economy and sustainability [34,79,102–105]. Several studies focused on developing eco-friendly proteins-based materials for wound healing applications, skin cancer, post-surgery treatment, drug delivery systems, tissue engineering and homeostasis [106,107]. Hydrogel dressings, nanocomposite hydrogel biomaterials and nanofibrous/keratin hydrogel represent an important biomedical research trend increasingly supported by scientific evidence due to their synergistic ability to promote regeneration and closure of skin wounds, reducing reverse effects such as inflammation [108–110]. Keratin-based transdermal patches and hydrogels have been investigated for diabetic and radiated wounds, showing promising preliminary results to decrease the healing time [103]. IFKs-based nanofibers can be obtained by electrospinning and find application in the production of sponges, films, and scaffolds, in combination with biodegradable and non-biodegradable biocompatible polymers [111]. Keratinous biomaterials have proven effective in accelerated coagulation, enhancing the proteolytic coagulation cascade and decreasing the inflammatory phase, common in classic foreign body reactions and graft rejection. These also improve epithelialization and stimulate the formation of myofibroblasts and, consequently, collagen. In addition to intrinsic wound healing properties, some studies have focused on functionalizing wound dressings with insulin, antibiotics, silver nanoparticles, and stimuli-responsive compounds. These efforts pave the way for new lines of research, including clinical studies, that should improve skin tolerance of wound dressings and enable customized treatments according to the different needs of patients [112]. Developing protein-based materials with higher antibacterial functionality and sporicidal activity is also desirable for more demanding applications in the medical sectors [113,114]. Moreover, *in vitro* and *in vivo* findings reported that keratin-based hydrogel showed a promising potential to promote the regeneration of injured skeletal muscle, nerve regeneration and repair of peripheral neuropathy [115]. 3D printing of scaffolds is another important application of natural polymers, including keratinous materials, in tissue engineering and regenerative medicine. Several natural compounds are biodegradable and biocompatible, but the high cost and lack of availability decrease the sustainability of large-scale biomedical applications. In contrast, α -keratins (and corneous β -proteins) can be purchased from many low-cost sources and allow the production of non-toxic inks for 3D printing applications [116].

In addition to biomedical applications, α -keratins and corneous β -proteins derivatives have found their way into research related to various fields, such as animal feed, food, innovative materials, and agriculture. These have been the subject of studies in human and animal nutrition and in developing supplements. Due to its high cysteine content, α -keratins may be considered the best natural source for high-quality nutraceutical supplements enriched in cysteine. Vitamin C and E are the most common antioxidant supplements for convalescent patients or healthy athletes wishing to improve their performance. However, the central role of cysteine (and thus of dietary thiols) as a promoter of the endogenous antioxidant activity of glutathione must be considered [117–119]. Systemic inflammatory response syndrome brings about a dangerous and persistent inflammatory response, which involves multiple organ damage. Cysteine has a booster effect on the glutathione pathways, increasing the natural antioxidant defences. As reported by Plaza et al. the number of publications concerning the potential application of cysteine in nutraceutical and drug industries has grown significantly during the last two decades [120]. Preliminary results, also in clinical trials, concerning antioxidant activity, immune system regulation and hair and digestive system protection have been reviewed, and some promising data are available. α -keratin supplementation also seems to increase lean body mass in athletes [121]. Additionally, as reported by Gasmi et al., these proteins have been mentioned as a potential active compound in hair loss treatment [122]. However, the scientific community agrees that further studies need to support the use of α -keratins and corneous β -proteins as a potential supplement/active ingredient. Concerning food applications, Giteru et al. and Dias et al. reported several chemical and enzymatic methods useful for obtaining edible α -keratins from wool waste. The above-mentioned data open a new trend for developing functional ingredients for amino acid supply [123,124]. Regarding the food packaging industry, α -keratins and corneous β -proteins have also been presented as a potential protein source to develop innovative edible films: the preliminary results seem to allow better thermal resistance and mechanical properties when mixed with collagen for collagen-based films [125]. Furthermore, keratinous hydrolysate from several wastes has been studied as a potential animal-feed additive for ruminants and fishes [95,126].

A recent study showcases the utilisation of chicken feathers for pioneering applications in thermal insulation within the field of building construction [127–130]. Thanks to their lower skeletal density (0.9 g cm^{-3}) compared to cellulose fibres (1.5 g cm^{-3}) and wool fibres (1.3 g cm^{-3}), they possess attractive advantages as lightweight materials [130–132]. Composites reinforced with chicken feather fibres were fabricated via hand layup technique in the laboratory: the composites were manufactured using epoxy as the matrix [127]. Feathers are a plentiful by-product of the poultry industry, and there is an opportunity to establish a circular economy that harnesses their unique natural characteristics. Generally, α -keratins and corneous β -proteins are a source of bioinspired designs engineering materials and ecological fibre-reinforced composites. The “bio-composites” concept involves using natural fibres to reinforce synthetic polymer structures, with a promising potential in the automotive and building sectors [133].

Importantly, several protein-based compounds can be obtained from biotechnological approaches due to proteolytic enzymes such as keratinases or through chemical and thermal treatments: α -keratin hydrolysates obtained by keratinases can be applied as pre-treatment in biogas and biofertilizers production [83,95]. In the filtration material segment, eco-friendly materials based on α -keratins and corneous β -proteins are potential alternatives to conventional synthetic fibres. The oxidation process of wool keratin disrupts the sulfur-sulfur bonds, which in turn activates the ion-

binding cysteic acid groups. This transformation makes the oxidised wool a bio-based filter material for heavy metal ions [114]. Moreover, the advanced oxidation processes have been highlighted as an eco-friendly alternative for treating wastewater from organic dye removal. Keratin-carbon dots nanocomposites show a strong photodegradation efficiency and represent a new research frontier towards a sustainable approach [134]. Finally, biopolymers are an emerging class of novel materials in energy applications. α -keratins and corneous β -proteins-based materials enhance lithium battery function as they can restrict dendrite formation that may cause undesired cathode and electrode contacts. Moreover, keratin is a promising biopolymer for capacitor optimisation, offering decreased charging rates due to the inherent porous separator structures [135]. All these findings prove the potential of the proteins from corneous and keratinous sources to constitute biomaterials for biomedical and other industrial applications.

5. Intellectual properties for corneous and keratinous polymers

The initial phase of the intellectual property related to keratinous polymer from raw natural material could be dated around 1900. John Hoffmeier described a process for extracting α -keratin and corneous β -protein from animal horns using lime in a United States (U.S.) patent issued in 1905 (German Pat No. 184,915, 1905 [136]). The extracted proteins were then used to make gels that could be strengthened by adding formaldehyde. Between 1905 and 1935, several methods were developed to extract α -keratin and corneous β -protein using oxidative and reductive chemistries [137–141]. Different techniques had been developed for breaking down the structures of hair, horns, and hooves by the late 1920s. The number of derivative materials that could be produced with keratins grew exponentially once the knowledge about “how to” extract keratins from hair fibres, purify and characterize them, has been achieved. Since 1970, methods to form related films, powders, gels, fibres, coatings, and foams have been developed and published by several international researchers [142–144].

In 1982, the worldwide application for “Keratin derivative, the process for its preparation and treatment composition containing it” (EP Pat. No. 0 099,780 A1, 1983) was presented [145]. This invention relates to keratinous derivatives, particularly for treating human skin and/or hair, and to a process for their preparation. One of the first patent applications regarding macromolecular products based on keratin from hair is dated 1997. This has been focused on keratin-based hydrogel (U.S. Pat. No. 5,932,552 A, 1999 — “Keratin-based hydrogel for biomedical applications and method of production”, expired for a lifetime [146]) and classified under C08H1/06 “Macromolecular products derived from proteins derived from horn, hoofs, hair, skin or leather”. Within this classification, the worldwide application of the “Method for extracting keratin” was more recently performed in 2018 (WO Pat. No. 2019/116357 A1, 2019 [147]). This has been related to a method for extracting keratin from pigs’ hair, comprising several steps (e.g., degreasing the animal hair and filtering to remove residues and obtain a solution of keratins). Another patent converging on method was entitled “Digestion of keratin” and granted in 2018 (U. S. Pat. No. 10,030,099 B2, 2018 [148]; expired for fee-related reasons). This mentions the chemical digestion of wool and avian feathers, probably both α -keratins and corneous β -proteins. The digested product is made by heating the feathers or wool with a solvent selected from glycols, alkanolamines, polyamines, and their combinations. The resulting digested keratin product corresponds to a derived polyol, useful for producing polymeric materials such as polyurethanes. For patents focused on keratinase, among the countries where the inventors are domiciled, China recorded the highest number, which

invariably indicated the participation of prolific researchers from this nation [84]. Different active patents (US11173233B2, bio-ceramic bone graft compositions and methods of using the same [149]; US10821211B2, keratin biomaterials and the use in biomedical applications [150]; US9968706B2, hydrogel matrix as an acellular scaffold for axonal regeneration [151]; JP4707154B2, solubilized keratin from keratin-containing raw materials [152]) and a pending one (US20230173799A1, biodegradable and/or home compostable sachet from naturally keratin sources [153]) are mentioned in Table 1. US9498419B2 [154] is an example of an active patent for treating and repairing hairs, cited by more than 55 exclusive applications on keratin caring for hair and skin.

6. Market niche of corneous and keratin-based products

Adopting new materials in various industries follows a prolonged gestation period, typically over 20 years, from initial technical innovation to widespread acceptance and commercial utilisation of the material [157]. This delay can be attributed to market readiness, technological limitations, regulatory challenges, and societal acceptance. For instance, the introduction of early bioplastics like polylactic acid (PLA), discovered around 1890, faced significant delays before gaining prominence in the packaging industry, finally taking off in the 1960s [158]. Guaranteeing commercial success, material should exceed functional suitability and include social and cultural acceptance. Thus, besides its inherent properties, a material must resonate with users, evoke meaningful experiences, and elicit desired responses and behaviours [159,160]. Past research has aimed to assist designers in defining and designing for such meaningful experiences with innovative materials [159]. The methods went beyond evaluating a material solely based on its inherent characteristics and emphasize its actions, expressions it elicits from users, and the behaviours it inspires. This approach called for divergent thinking and explored multiple possibilities during early concept design, particularly in dynamic, collaborative settings like workshops [161]. Design tools and methods have proven effective in supporting interdisciplinary short-term ideation workshops for new product development and innovation activities [162]. More specifically, stimulating cross-disciplinary ideation between the field of product design and biotechnology has been investigated, showcasing how going beyond external stimuli and integrating internal triggers can lead to a new model of innovation [163–165].

The keratin market worldwide is diversified into sectors such as pharmaceuticals, textiles, and fertilizers, among others, each offering its unique value. The demand for sustainable and biodegradable materials is rising in various industries, including fashion, automotive, and packaging. Polymers derived from α -keratins and corneous β -proteins, known for their strength and thermal stability, cater to these needs, indicating potential commercial expansion. Presently, the market showcases numerous keratin-based products, largely due to the European Union’s Bioeconomy strategy and funding organizations like EIT Raw Materials that finance projects to transition from research institutions and expedite their technology’s commercialization. Start-ups like Kerline Aeropowder [166] and projects like UNLOCK exemplify this shift by offering products in sustainable packaging, non-woven geotextiles, and insulation materials. However, for these budding markets to flourish, it’s essential to tackle entry barriers. Dieckmann’s study in 2020 underscores considerable regulatory and financial hurdles impeding the incorporation of keratin-based innovations into conventional commerce [167]. The introduction of higher-value products could potentially facilitate this transition. The global keratin market had a value of approximately United States Dollars (USD) 1.4 billion in 2021 and is projected to expand at a compound

Table 1
Exemplary patents for inventions related to products based on α -keratins or corneous β -proteins. Active and pending patents are listed, excluding those expired [155,156].

Patent No.	Title	Short description	Status
US11173233B2 (Granted in 2021)	Keratin bioceramic compositions	Bioceramic bone graft compositions (keratose, particulate filler, antibiotic, and water) and methods of using the same	Active
US10821211B2 (Granted in 2020)	Coatings and biomedical implants formed from keratin biomaterials	Generally related to keratin biomaterials and the use thereof in biomedical applications	Active
US9968706B2 (Granted in 2018)	Nerve regeneration employing keratin biomaterials	Keratin hydrogel matrix as an effective acellular scaffold for axonal regeneration, facilitating functional nerve recovery	Active
US9498419B2 (Granted in 2016)	Keratin treatment formulations and methods	Generally related to formulations and methods for treating keratin in hair, skin, or nails (particularly, for strengthening and/or repairing hair during or after a colouring or permanent wave treatment)	Active
JP4707154B2 (Granted in 2011)	Method for producing solubilized keratin	Method for producing solubilized keratin from keratin-containing raw materials like animal hairs	Active
US20230173799A1	Biodegradable and/or home compostable sachet containing a solid article	Biodegradable and/or home compostable sachet from naturally sourced polymers, including those of plant origin, seed extracts, fruit extracts, and those of animal origin, including keratin, keratin hydrolysates, and sulfonic keratins	Pending

annual growth rate (CAGR) of 6.2% from 2022 to 2030 (Grand View Research: “Keratin Market Size, Share & Trends Analysis Report By Product (Hydrolysed, Others), By Application By Source (Food & Beverages, Pharmaceutical & Healthcare), By Region, And Segment Forecasts, 2022–2030” [168]). The market size in the U.S. between 2020 and 2030 of “keratin” (it has not specified exactly which, like α -keratins, corneous β -proteins, or both), hydrolysed and other types (Fig. 4). The increasing demand for the personal care and cosmetic industry primarily drives its growth. A-keratins are especially used in skincare and hair care products, such as shampoos and conditioners, to address various concerns like removing dead skin cells, reducing skin oil levels, and repairing hair and skin damage [169] caused by heat and/or chemicals. Peptide treatments, conducted at a neutral pH, create intra and intermolecular disulphide bonds. These bonds form between cysteine-based peptides and hair proteins, demonstrating significant potential for cosmetic applications, particularly as regulators of hair shape [170]. The suggested methods, which rely on peptide formulations, are eco-friendly. They present a viable alternative to traditional chemical treatments, heralding a new era for the environmentally conscious haircare cosmetic industry. One of the main factors contributing to

the rising demand for IFKs in the personal care and cosmetics industry is its suitability as a sustainable, safe, and multifunctional alternative to other acid-based ingredients in cosmetic formulations (Grand View Research: “Keratin Market Size, Share & Trends Analysis Report By Product (Hydrolysed, Others), By Application By Source (Food & Beverages, Pharmaceutical & Healthcare), By Region, And Segment Forecasts, 2022–2030” [168]).

Additionally, the revitalising effects on hair and skin further enhance its appeal. The preference for bio-based products and increased disposable income among consumers also drive the growth of the related market. Manufacturers are continually expanding their product portfolios to meet the evolving needs of end-users, leading to increased consumption of keratin. Furthermore, its market's growth is influenced by the growing global population, urbanisation, industrialisation, and increased manufacturing expenditure in emerging economies. The cosmetics industry is experiencing a shift towards innovative ingredients that are more efficient and have fewer adverse effects, which has boosted the use of IFKs, especially in hair care products. The demand for these solutions and the increasing awareness among individuals are also contributing factors. This finds applications in various industries beyond personal care and cosmetics, including health and pharmaceuticals, as well as food and beverages. This widespread utilisation is expected to create lucrative opportunities for market players, particularly in the Asia Pacific region. The hair care segment is projected to witness significant market penetration globally, driving the overall demand. The increasing global consumption of green chemicals is also anticipated to further propel the utilisation of “vegan-based keratins”. It should also be considered that the anticipation of “vegan-based keratin” or “plant-based keratin” (consisting of hydrolysed corn, wheat, and soy proteins) in the market of the hair and skin care sector, could perhaps harm the utilisation of keratins from animal sources. Finally, consumer research has been carried out, reflecting a product's social and cultural acceptance, revealing that the market is ready for bio-waste products and that investing in supply chain reconfiguration to adopt closed loops can be viable.

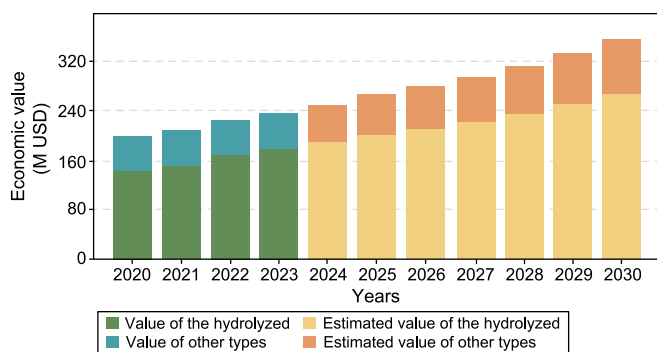


Fig. 4. Market size in the United States between 2020 and 2030 of α -keratins and corneous β -proteins. The economic values for the hydrolysed (dark green and light orange) and other types (light cyan and dark orange) are shown. The millions (M) of United States Dollars (USD) for 2020, 2021, and 2022 correspond to \$212 M, \$219 M, and \$232 M. The estimated values between 2024 and 2030 are illustrated in light (hydrolysed) and dark (other types) orange. The values are reported according to the content shown in Grand View Research: “Keratin Market Size, Share & Trends Analysis Report By Product (Hydrolysed, Others), By Application By Source (Food & Beverages, Pharmaceutical & Healthcare), By Region, And Segment Forecasts, 2022–2030” [168].

7. Conclusions

Within this framework, we aimed to highlight biotechnological approaches using corneous and keratinous materials to produce bio-commodities of relevant technological interest. Corneous and keratinous materials present distinctive functions and hierarchal

structures valuable for crafting everyday products that the population benefits from, such as cosmetics or bioplastic materials. The importance of these applications is underscored by both the ongoing and, thus, the anticipated growth in demand for such products, highlighting the need for the purposeful and efficient use of α -keratin and corneous β -protein-based resources to meet these rising demands. The creation of materials derived from these proteins underscores the potential for repurposing waste into beneficial products in a sustainable manner. From an economic perspective, the sector grapples with issues concerning processing costs and gaining market share. Nonetheless, the increasing consumer preference for eco-friendly materials and progress in bioprocessing techniques paints an optimistic future for the keratin industry. Regarding environmental impact, materials produced from α -keratin and corneous β -protein offer substantial advantages, including waste minimisation, lower energy use, and reduced greenhouse gas emissions, thereby contributing to achieving worldwide sustainability objectives.

Ongoing scientific research is being directed towards advancing biomaterials, especially for practice in applying biomedical fields and food additives. The different types of these proteins are an abundant resource in nature, as protective integument of several animals, and the number of wastes rich in them has increased in the last decades, especially due to the expansion of intensive meat production. However, the societal effort towards a circular economy has provided several methods and protocols at the academic and industrial levels, some of which are covered by intellectual properties for utilising these industrial by-products. Although some studies claimed successful extraction, the results of comprehensive analyses did not completely support these claims.

8. Future perspectives

Scalability issues are crucial concerns. Meeting industry quality standards is another important aspect. Ensuring consistent production is also a significant concern. These are particularly important when considering their intended use in the biomedical field (e.g., drug release in hydrogels, patches, or 3D-printed scaffolds). Furthermore, some extraction protocols present high energy requirements, and using environmentally challenging solvents is crucial to tailor methods for the final application of the extracted α -keratins and corneous β -proteins. To establish a stable source for its production, ensuring a consistent and reliable supply is crucial, as relying solely on by-product wastes may not always be suitable or sufficient in meeting industry demands. To address this challenge, one promising approach would be the utilisation of genetically modified microorganisms: this will ensure a dependable, high-quality supply while enabling customisation to suit specific industry requirements. In essence, recombinant IFK proteins stand as a promising tool, effectively tackling the drawbacks raised by traditional extraction methods. This approach ushers in a cleaner production process. It also conserves the intrinsic qualities of natural proteins. As a result, it ensures a wide range of resource-stable applications. These applications span across diverse sectors. The sectors include cosmetics, biomaterials, medical devices, food supplements, food packaging, and textiles. Through the convergence of biotechnology and material science, recombinant α -keratins and corneous β -protein chart a transformative course that elevates the potential of their related innovations. Methylophilic yeasts and photosynthetic microorganisms, which are already studied and utilized to express heterologous proteins, have the potential to be investigated as “one-cell factories” towards α -keratins and corneous β -proteins production in addition to the extensively engineered *E. coli*. In this sense, while a significant amount of information is available for their structural

characteristics, part of the knowledge is still lacking, including their available gene sequences, biosynthesis, molecular assembly, and structural and mechanical nature. Furthermore, research must also be carried out in synthetic biology to obtain their heterologous expression.

Three main approaches are available, each at a different status of development. (i) *In vitro* biocatalytic reactions and application of keratinolytic enzymes for the sustainable treatment of keratinous wastes. (ii) *In vivo* application of wild-type or engineered microorganisms expressing keratinolytic enzymes to upgrade the commercial value of keratinous wastes for different applications. (iii) Chemical transformation of animal residues and food company bio-wastes into valuable products. The use of genetically modified organisms (GMOs) in biotechnological applications has been debated for decades. While GMOs can potentially provide significant benefits, regulatory and ethical concerns must also be considered. Public opinion has generally supported the worries about the risks of releasing GMOs into the environment, which may change the natural equilibrium within an ecosystem. Among the proteases expressed by microorganisms, keratinases have been identified as a promising tool for deleting keratin-rich wastes. These enzymes have been used in various industries, such as agriculture, animal feed, cosmetics, pharmaceuticals, detergent additives, and leather. Additionally, keratin-based products, such as “keragel” and “keramatrix”, have been proposed as potential treatments for skin damage. The engineering of keratinase to improve its catalytic activity towards different keratin or corneous proteins-rich substrates, has high relevance for application purposes in resource recovery.

All these aspects are of paramount significance, and further research and insights in these areas hold the potential to drive substantial advancements in biomaterial development and application. Furthermore, it is noteworthy that most ongoing research efforts primarily centre on IFK extraction processes, but it has been detected that there is currently no widely recognized approach that delves into the generation of valuable peptides or amino acids from residual biomass. This could represent a significant opportunity to optimise resource utilisation and expand the horizons of potential applications in a field where such an approach has yet to be fully developed. One aspect to explore is the composition of the proteins extracted from corneous and keratinous material. Besides α -keratins and corneous β -proteins, other proteins are present: how do they influence the properties of corneous or artificial keratin-based material? Research into this topic is probably necessary. Molecular biology, structural biology, microbiology, cells and metabolic engineering, biochemistry, and computation will play important roles in maximising the advantages offered by keratin, ultimately contributing positively to both global population well-being and environmental prosperity.

CRedit authorship contribution statement

Giovanni Davide Barone: Writing - Review & Editing, Writing - Original Draft, Visualization, Validation, Supervision, Resources, Project Administration, Methodology, Investigation, Funding Acquisition, Formal Analysis, Data Curation, Conceptualization. **Irene Tagliaro:** Writing - Review & Editing, Writing - Original Draft. **Rodrigo Oliver-Simancas:** Writing - Review & Editing, Writing - Original Draft. **Matteo Radice:** Writing - Review & Editing, Writing - Original Draft. **Livia M. Kalossaka:** Writing - Original Draft. **Michele Mattei:** Writing - Original Draft. **Antonino Biundo:** Writing - Original Draft. **Isabella Pisano:** Writing - Original Draft. **Amparo Jiménez-Quero:** Writing - Review & Editing, Writing - Original Draft, Supervision, Funding Acquisition, Data Curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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