

DOI: 10.2478/9788367405805-006



SHORT REVIEW ON KERATIN SYNTHESIS AND ITS APPLICATION AS A BIO REINFORCEMENT AND FLAME RETARDANT AGENT IN POLYMER COMPOSITES

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Recently, attention to the environment has led to the development of a circular economy by stimulating some initiatives to obtain functional materials based on natural resources with specific characteristics. Agriculture, the food industry and the leather processing industry produce large amounts of waste, mainly in the form of fibers and fibrous materials, among them keratin fibers, which represent a good candidate for the development of material biosource. Due to their chemical composition, keratin fibers are physically resistant and most importantly, flame retardant. However, over five million tonnes of keratin-rich waste (including wool, hair, feathers, hooves and horns) are generated each year by slaughterhouses, textile industries and tanneries. The thermal degradation and flammability behavior of polymer composites from natural fibers recovered from waste is studied to obtain biocomposites, due to their wide field of application in industry, agriculture, medical field, pharmaceuticals, cosmetics. Different research studies have suggested that the thermal stability of biocomposites can be improved by adding micro/nanoparticles, additives and crosslinking compounds. This approach could be considered as an effective way of ecologically recycling keratin fiber waste and obtaining functional materials characterized by increased fire safety and low environmental impact. Keratin waste recovery and processing through different optimal methods can transform a material with low economic value (remnants of tannery hair and sheep wool) into a product with applications in various fields: leather processing, cosmetics, agriculture, pharmaceutical, medical, industry.

Keywords: Keratin, biopolymer, fire-proofing

INTRODUCTION

Wool waste from sheep farming is a source of keratin (Maurizii *et al.*, 2024; Chereji and Munteanu, 2024). The annual production of this wool waste is around 200 thousand tons in Europe alone (Chereji and Munteanu, 2024). Part of this waste is used to produce fertilizers for agriculture and animal feed. The recovery of wool waste consists of biorefining processes for the extraction of keratin and its use in compositions of added-value materials (Ossai *et al.*, 2022) with applications in the textile industry (Zhu *et al.*, 2023), air and water purification (Posati *et al.*, 2020), controlled delivery of active substances (Khorshid *et al.*, 2023), regeneration of biological tissues (Hu *et al.*, 2012; Ranjit *et al.*, 2022), composites of materials with specific mechanical properties or flame retardants (Mengistu *et al.*, 2024; Abba *et al.*, 2025). Keratin belongs to the family of fibrous structural proteins known as scleroproteins and is the most common structural protein found in animal horns, claws, nails, hair and feathers. Its unique characteristic is that it contains more cysteine than other fibrous proteins such as collagen, elastin and myofibrillar protein (Khorshid *et al.*, 2023; Hu *et al.*, 2012). The mechanical structure of keratin is due to the high concentration of hydrogen bonds, hydrophobic groups and disulfide cross-links. It contains a high percentage of cysteine

© 2024 M.D. Berechet *et al.* This is an open access article licensed under the Creative Commons Attribution 4.0 International (<u>https://creativecommons.org/licenses/by/4.0/</u>) https://doi.org/10.2478/9788367405805-006 (7-13 %) which stabilizes keratin. The recovery of keratin waste can transform a material with low economic value (remnants of tannery hair and sheep wool) into a product with applications in various fields: leather processing, cosmetics, agriculture, pharmaceutical, medical, industry (Mengistu *et al.*, 2024; Abba *et al.*, 2025; Wang *et al.*, 2016).

The purpose of this review is to highlight the main sources of keratin, extraction methods, characterization techniques and possible industrial applications. A special attention was paid to the study of those compositions with high synergy, based on keratin and other flame-retardant additives, for the development of textile coatings with multifunctional properties, and also of composite materials used in the electrical insulators industry, etc.

Material Sources of Keratin

Keratinous materials are formed by keratinized cells organized specifically and filled with proteins, mainly fibrous. They make up the hard appendages in animals, for example epidermis, wool, hair, horns, nails in mammals, as well as feathers, claws, beaks in birds and reptiles, turtle shells having a variety of functions, such as protection against external environment, armor against predators (Sarma, 2022). A thorough understanding of the relationships between the units that make up keratin materials and their functional properties provides useful knowledge in the design of new materials (Mengistu *et al.*, 2024; Wang *et al.*, 2016).

Methods of Obtaining Keratin

To solubilize keratin from various sources, numerous extraction methods have been devised. Reduction, oxidation, microwave irradiation, alkaline extraction, sulfitolysis, and ionic liquids, microbiological, enzymatic are procedures for the solubilization and isolation of keratin from keratin-rich materials (Maurizii *et al.*, 2024; Mengistu *et al.*, 2024; Sarma 2022; Olvera-Valdez *et al.*, 2024; Wang *et al.*, 2016; Abba *et al.*, 2025; Balaji *et al.*, 2012; Duverger *et al.*, 2015; Palmer *et al.*, 2008). The purpose of these keratin production processes is to break the disulfide and peptide bonds in the protein chains and their fragmentation (Table 1).

Keratin production process	Action	Applications
Alkaline hydrolysis	Obtaining an advanced fragmentation of protein chains.	Pharmaceuticals, cosmetics
Acid hydrolysis	It creates extremely severe conditions that can be harmful certain amino acids.	Composites with industrial applications
Reduction processes	Preserves keratin microstructure by reducing (with mercaptoethanol) disulfide bridges in protein fibers without appreciably cleaving peptide bonds.	Composites with industrial applications
Enzymatic hydrolysis	A very slow approach unsuitable for industrial applications, produces the lowest degree of species splitting.	Composites with miscellaneous applications, agriculture, animal feed
Sulphytolysis	Breaking disulfide bonds by treating with sulfite, bisulfite.	Fertilizers in agriculture
Ionic liquids	It accelerates the dissolution of natural polymers. The most used ionic liquids are soluble salts of imidazole derivatives.	Industrial applications in different polymer compositions

Keratin production process	Action	Applications		
Microbiological degradation	The action of some microorganisms such as Actinomycete (Vasileva-Tonkova et al., 2009; Syed et al., 2009), Bacillus sp. (Suntornsuk & Suntornsuk 2003; Gessesse et al., 2003), Seratia sp. (Khardenavis et al., 2009), Kocuria rosea, led to the obtaining of keratin hydrolysates by ecological methods.	Soil improvement products and protein supplements in animal feed		
Microwave	Microwave at 60°C, 400 W, 40 kHz for 10 min. Reduces extraction time from hours to 10 min. Mw	In water treatment processes		

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Keratin Characterization Techniques

Various procedures are used to evaluate and characterize extracted keratin to confirm protein and amino acid content. Investigation on crosslinking mechanism can be performed using attenuated total FTIR (ATR-FTIR) and XRD but also the presence of specific amino acids. The infrared absorption spectrum of keratin shows the characteristic absorption bands attributed mainly to the amide bond (-CONH-): the broad band centered at 3200 cm⁻¹ is associated with stretching vibrations for O-H, the band from approximately 1600-1700 cm⁻¹ is mainly associated with stretching vibrations for C=O (amide I), while the amide II band, which falls at about 1550 cm⁻¹, is associated with bending vibrations for N-H and stretching vibrations for C-N. Around 1200-1300 cm⁻¹ is the band corresponding to amide III, which results from the combination of C-N stretching and N-H bending vibrations, with the contribution of C-C stretching vibrations and C-O bending vibrations. All these peaks are visible in the keratin spectrum, suggesting the presence of a α -helix and a β -sheet structure. Following hydrolysis, the amide bands move to longer wavelengths, which indicates conformational changes in the protein chains (Fraser & Parry 2011; Zhao et al., 2015; Khosa et al., 2011). Circular dichroism (CD) spectroscopy can be utilized to analyze the secondary protein structure of keratins (Lin et al., 2024). Rheological characterization with a rotational rheometer can investigate crosslinking properties by storage modulus, G' and loss modulus, G", the morphology can be analyzed with Scanning Electron Microscopy (SEM), also texture analyzer (Maurizii et al., 2024; Lin et al., 2024). The sodium dodecyl sulfate and polyacrylamide gel electrophoresis analysis (SDS-PAGE) shows the molecular masses of the obtained hydrolysates and highlights the electrophoretic model of keratin specific to the presence of the primary group of proteins associated with keratin, the proteins from the intermediate filaments but also bands corresponding to proteins with high molecular weight at the level of 50 kDa, attributed to the proteins of the intermediate filaments with low sulfur content, which are mainly characteristic of the α -helical keratin chain (Lv *et al.*, 2016; Olvera-Valdez et al., 2024).

Mechanical testing can be done by determining Young's modulus, stress and tensile strength. Micro-computed tomography (Micro-CT) evaluates the 3-D structures of keratin as films. After scanning, pore size, porosity and connectivity are analyzed. Investigation by confocal laser scanning microscopy is done at different excitation wavelengths. The obtained images highlight the network structure of keratin through red fluorescence (Lv *et al.*, 2016). Field emission scanning electron microscopy (FE-SEM), with energy dispersive spectroscopy (FESEM-EDS) is performed on network structures to investigate the surface morphology. The demineralization of the samples is evaluated by an energy dispersive spectroscopy unit attached to the SEM. In the SEM results of keratin, the characteristic three-dimensional network structure is highlighted, which is consistent with the confocal laser scanning image

(Pon-On *et al.*, 2016). Thermogravimetric analysis (TG) can be used to confirm the thermal stability and composition of the synthesized materials (Olvera-Valdez *et al.*, 2024). The antimicrobial and antifungal activity of wet keratin films can be investigated by the diffusion method in the presence of microorganisms of interest.

This method is performed in petri dishes on solid agar culture medium. Wet keratin films are placed in Petri dishes with agar cultured with the microorganism to be tested. The test samples are then incubated at 37°C for 24 hours, and the antimicrobial activity of the wet keratin films is determined by measuring the diameter of the zone of inhibition (Pon-On *et al.*, 2016).

Composition and Structure of Keratin

Sheep wool contains up to 95% by weight of pure keratin with molecular mass ranging from 45 to 60 kDa and 11–28 kDa. Keratin consists of intermediate filaments, structured in different models: the α model, the β model and the amorphous model (Table 2). The α -keratin proteins are organized as spirals with an α -helix conformation of the polypeptide chains stabilized by hydrogen bonds. β -keratin is like a folded sheet, made up of laterally assembled strands, which can be parallel or antiparallel (more stable), and the chains are held together by intermolecular hydrogen bonds. Keratinized materials present a complex hierarchical structure consisting of polypeptide chains and filamentary matrix structures on a nanometric scale. The primary structure of keratin is a chain of amino acids, with various different sequences. Keratin is rich in cysteine (17.5%), serine (11.7%), glutamic acid (11.1%), threonine (6.9%), glycine (6.5%), arginine (5.6%) (Gupta *et al.*, 2012; Mengistu *et al.*, 2024).

Keratin type	Keratin Resources
α-keratin	Wool, hair, nails, hooves, horns, stratum corneum (Mengistu et al.,
	2024; Olvera-Valdez et al., 2024).
β-keratin	Bird feathers, beaks and claws, reptilian claws and scales (Wang et
	al., 2016; Olvera-Valdez et al., 2024).
α and β -keratin	Reptile epidermis, Pangolin scales (Wang et al., 2016).

Table 2. Keratin sources and distribution of α - and β - keratin

Properties of Keratin

Keratin is one of the hardest biological materials, with a high modulus of elasticity, although it contains only polymeric compounds and rarely minerals, it is a complex mixture of proteins and enzymes taken from epithelia. They are insoluble in dilute acids, alkaline solutions, water and organic solvents and are resistant to degradation by the pepsin and trypsin proteases. Keratins are insoluble in aqueous salt solutions, but are soluble in solutions with denaturing agents such as urea (Sarma, 2022). Keratin has specific mechanical properties, biodegradation, biocompatibility, bioactivity through the ability to promote cell growth, the ability to bind toxic substances (heavy metals, formaldehyde) and transport hydrophilic and lipophilic active ingredients (Wang *et al.*, 2016).

Processing Keratin Extracts – Additivation and Crosslinking Methods

Keratin-based products can be processed by various chemical and physical cross-linking methods with the potential to increase mechanical resistance. The formation of new interchain disulfide bonds can be favored by oxidative methods or by acid treatment, by thermal or dehydration methods. The inclusion of some solvents in the solubilized keratin can favor increased chain mobility. Also, various chemical agents can be used, such as aldehydes ICAMS 2024 - 10th International Conference on Advanced Materials and Systems

(formaldehyde, glutaraldehyde), or nanoparticles can be incorporated as reinforcing agents (montmorillonite) in keratin-based materials (Table 3) (Mengistu *et al.*, 2024; Wang *et al.*, 2016).

Crosslinking compounds	Remarks
Carbonyl compounds such as formaldehyde and glutaraldehyde	It reacts through condensation reactions with the amide groups in the structure of glutamine and asparagine. Different cross-linking densities of the formed networks are obtained with the improvement of the superior mechanical properties (Young's modulus, resistance to elongation) of keratin cross-linked with formaldehyde and glutaraldehyde, compared to non-cross-linked keratin
Montmorillonite, comes from the class of clay materials	Nanostructured material is obtained, used as a filling agent
Acrylic monomers of the 1,3-diglycerolate diacrylate type	"Click-chemistry"-type chemical treatment that gives keratin fibers a superior resistance to contraction

Table 3.	Compounds	and	methods	of	keratin	crosslin	king
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Industrial Applications of Keratin

Keratin is a protein with specific characteristics that contains amino acids with active amino (-NH₂) and carboxylic (-COOH) functional groups. In addition to these groups, it also has a high content of cysteine in the amino acid sequence, with thiol (-SH) groups, which are responsible for intra- and intermolecular sulfur-sulfur (disulfide) bonds. Keratin is a biopolymer with numerous functional properties, applicable for the creation of biomaterials with controlled characteristics used in various fields. For the textile industry, it is important to develop filaments with a high keratin content for the renewal of raw wool (Cao *et al.*, 2020) and for the pharmaceutical and cosmetic industry for the creation of protein scaffolds that mimic the cellular matrix capable of promoting the biological reconstruction of tissues and skin regeneration (Mengistu *et al.*, 2024; Ye *et al.*, 2020; Liu *et al.*, 2023). Potential uses of keratin compounds may be in agriculture as organic fertilizers, and animal feed or in the production of adhesives, processing of leather products, polymer composites for household or industrial products, eliminating common chemical compounds used in cosmetics, pharmaceuticals or biomedical materials (Mengistu *et al.*, 2023).

Keratin Flame Retardant

Proteins in keratin fibers contain 50% carbon, 21%–24% oxygen, 12%–21% nitrogen, 6%–7% hydrogen, 2%–5% sulfur, and other elements. Due to their chemical composition, keratin fibers are physically resistant and have flame retardant properties (Alaffi *et al.*, 2020). Only a few articles can be found on the use of keratin fibers as a raw material for the preparation of composites with flame retardant properties. Perez-Chavez *et al.* (2022) used keratin fiber in a biodegradable cornstarch copolyester material to make a composite with flame retardant properties avoiding the use of toxic flame retardants. Keratin fibers have also been used as a filler in polyurethane foams to reduce flammability and improve mechanical and thermal properties (Wrzesniewska-Tosik *et al.*, 2020). The use of deoxyribonucleic acid (DNA) in combination with keratin as green intumescent additives has been studied in polymer blends based on polyethylene (PE) / ethylene vinyl acetate (EVA) with applications as an insulator in the electrical wire and cable industry. The flame retardancy mechanism of keratin is based on the formation of a carbonaceous intumescent layer with the role of

protecting the polymer matrix during combustion (Albite-Ortega et al., 2019). Another approach focused on the extraction of keratin from bird feathers and wool by the hydrothermal method, without additional pretreatments, in order to use it as an innovative multifunctional layer with new properties (UV protection, flame retardancy and antioxidant activity) for polyester fabrics (Zemljič et al., 2024). Another research study describes a chemical method of modifying keratin with melamine (a nitrogen-rich compound) and sodium pyrophosphate (a phosphorus-rich compound) for use as a flame-retardant additive for cotton fabrics. The limiting oxygen index (LOI) of cotton after the application of modified keratin increased by ~ 66.7% compared to that obtained on untreated cotton (Patankar et al., 2021). The use of keratin and tannin derived from renewable biomass (rich in polyphenols) as a reinforcing agent in the polyamide 66 (PA66) matrix was studied, in order to improve the fire-retardant and anti-drip performance. The obtained results showed that the peak of heat release rate (PHRR) and total heat release (THR) of the fireproof PA66 composite decreased by 75.3% and 38.4%, compared to PA66. Following the determination of the LOI, the fireretardant PA66 composite reached 29.65%, which places it in the V0 combustibility class (Jiang et al., 2023).

Future Prospects

Keratin is one of the most durable biological materials. The specific characteristics can be the basis for the development of new bio-inspired models. Biomaterials based on keratin, such as sponges, hydrogels, patches, films, fibers, various biocomposites can be created for biomedical, pharmaceutical and cosmetic applications. Due to its resistance to acids, bases and solvents as well as its flame-retardant nature, keratin can be recommended for polymer compositions for use in anti-shock, fire-resistant materials or with specific mechanical properties for different industrial sectors or for household use. The main methods of disposal of waste from poultry farms, sheep farms, the leather processing industry are waste storage, burning or dumping in the open air at the landfill, all of which pollute the environment. Recycling and efficient use of these resources is important from an ecological and financial point of view (Mengistu *et al.*, 2024; Abba *et al.*, 2025).

CONCLUSION

The application of knowledge from the area of keratinous materials to the design of new structures is a vast field of research, in which carbon nanotubes, graphene and other synthetic fibers can be combined with biopolymer matrices, offering many possibilities for the development of biomaterials with applicability in different industrial sectors, in medicine or in agriculture. Keratin waste recycling and processing through optimal methods can generate functional materials characterized by excellent fire safety and low environmental impact.

Acknowledgements

The research was carried out under ERANET project contract no. 15/2024, funded by The Executive Unit for the Financing of Higher Education, Research, Development and Innovation (UEFISCDI).

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