Chapter 1
Keratin: An Introduction

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Abstract What is keratin? And why to use the keratin? Well known that protein is a part of every cell in living organism's body which plays many different roles to keep living things alive and healthy. The importance of protein for the growth and repair of muscles, bones, skin, tendons, ligaments, hair, eyes, and other tissues is proven since a very long time. Proteins also exist in the form of enzymes and hormones needed for metabolism, digestion, and other important processes. *Natural proteins* are purified from *natural* sources. Keratin is among the most copious proteins found associated with the body of reptiles, birds, and mammals. It is a structural constituent of nail, wool, feathers, and hoofs which offers strength to body and muscles. Nowadays, the keratin-rich waste biomass produced from poultry and meat industry imposes serious threat to environment and living beings. We need to explore various techniques and methods for the extractions and use of keratin from waste biomass. From the industrial point of view, keratin is a useful product in the medical, pharmaceutical, cosmetic, and biotechnological industries. Materials obtained from keratin may be converted into porous foam of different sponges, shapes, coatings, mats, microfibers, gels, and materials of high molecular weight. In this chapter, we briefly describe the various sources, properties, and structures of keratin.

Keywords Protein · Waste · Biomass · Medical · Pharmaceutical · Application
Extraction

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1 Introduction

Growing need for sustainable and safe bio-based materials due to rising environmental concern has forced the use of available natural by-products as a substitution. By-products from different animal sources are recently being used for beneficial purposes such as drug delivery, medicines, cosmetics, and bioplastic.

Keratin is among the most abundant structural proteins (Coulombe and Omary 2002), and in animals together with collagen, it is the most important biopolymer (McKittrick et al. 2012). Keratinous materials, formed by specifically organized keratinized cells filled with mainly fibrous proteins (keratins), are natural polymeric composites that exhibit polypeptide chain structure, filament–matrix structure lamellar structure, and sandwich structure ranging from nanoscale to centimeter scale. Keratin is among the most copious proteins found associated with the body of reptiles, birds, and mammals. It is a structural constituent of nail, wool, feathers, and hoofs which offers strength to body and muscles (Reichl et al. 2011). They serve several functions, such as for predation and as armor, protection, and defense. Therefore, a thorough understanding of the relationships between the units that make up a functional keratinous material would expectantly provide useful knowledge in designing new materials. Keratin is chiefly found in epithelial cells in higher vertebrates (Korniłłowicz-Kowalska and Bohacz 2011). Keratins have high strength, stiffness, and insolubility in polar as well as nonpolar solvents. The stabilization is the result of intramolecular and intermolecular disulfide crosslinks, hydrogen bonding, and its crystallinity. These properties will differentiate it from other fibrous proteins like myofibrillar and collagen protein (Schrooyen et al. 2000). With increasing urbanization, food industries particularly the wool industry, slaughterhouse, and meat market produce million tons of keratin-containing biomass. The major producers including USA, Brazil, and China, report for more than 40 million tons annually. These proteins compose keratin by-products containing 15–18% nitrogen, 2–5% sulfur, 1.27% fat, 3.20% mineral elements, and 90% of proteins (Kunert 2000; Sangali and Brandelli 2000; Gessesse et al. 2003).

From past several years, keratin has been extracted from various sources such as horn, hoof, hair, beaks, shells, toenails, claws, fingernails, and feathers (Sharma and Gupta 2016; Sharma et al. 2017c). Keratin was used in medicine for the first time by Chinese herbalist Shi-Zen in sixteenth century. Hoffmeier, in 1905, first time extracted keratin from animal hoofs with the help of lime which further was used in making gels (Rouse and Van Dyke 2010). Keratins are cystine-rich proteins associated with intermediate filaments (IFs) which are cytoskeleton element having diameter of 8–10 nm (ARAI et al. 1983; Khosa et al. 2013). It is available in two forms α- and β-keratins. α-keratins are copiously found in the soft tissues such as sheep wool, hair, and skin. These are rich in cystine. β-keratins are present in hard tissue protein of nails, fish scales, bird feathers, and others. They are rich in glycine and alanine, poor in cystine, hydroxyproline, and proline (Gupta et al. 2012). Keratins are very much stable and insoluble in most of organic solvents. The presence of cystine in ample amount has made the keratin more susceptible to hydrolytic and oxidation reactions (Schrooyen et al. 2000; Barone et al. 2006b; Endo et al. 2008).

Nowadays, a large amount of keratin by-products are unused which is probable hazard to the environment (Cavello et al. 2012; Park et al. 2013). Keratin waste is classified into three categories in regulation (EC) 1774/2002 of the European Parliament and Council of 3rd October 2002 laying down health rules concerning animal by-products which are not intended for human consumption but does not spread diseases to humans or animals (Korniłłowicz-Kowalska and Bohacz 2011). In the form of solid biomass, keratin is less prone to enzymatic hydrolysis due to high cross-linking by hydrogen bonding, disulfide bonds, and hydrophobic interactions (Korniłłowicz-Kowalska and Bohacz 2011).

Thus, use of keratin as a biopolymer requires its extraction from the biomass. In recent years, several attempts have been made for the extraction of keratin using chemical, mechanical, and enzymatic methods (Korol 2012; Jeong et al. 2010; Chaudhari et al. 2013; Fang et al. 2013). There are number of ways for extraction of keratin from the waste biomass including acidic hydrolysis (Breinl and Baudisch 1907; Earland and Knight 1956), alkaline hydrolysis (Tsuda and Nomura 2014; Song et al. 2013; Poole et al. 2008), enzymatic hydrolysis (Eslahi et al. 2013), ionic liquid hydrolysis (Idris et al. 2014; Wang and Cao 2012), and alkaline–enzymatic hydrolysis (Yin et al. 2007). Acidic hydrolysis provides very severe conditions which can destroy some useful amino acids during hydrolysis. Conversely, enzymatic hydrolysis provides less species alteration but with a slower process and is more expensive (Staroń et al. 2014), which makes its commercial use more difficult. On the other hand, ionic liquids are too costly and protein recovery is very low (Cevasco and Chiappe 2014) to be used for industrial purpose. Hence, research into simple, cheap, environmentally sustainable, and industrial applicable method to extract keratin seems justifiable.

Keratin is a useful product in the medical, pharmaceutical, cosmetic, and biotechnological industry. Materials obtained from keratin may be converted into porous foam of different sponges, shapes, coatings, mats, microfibers, gels, and materials of high molecular weight. Keratin is attracting the attention of the researchers due to its abundance. Keratin biomaterial is applied in the development of wound healing gels, tissue engineering, drug delivery, trauma and medical devices, biomedical, and cosmetic applications. One of the impending applications of purified keratin is to produce biomaterials in regeneration and tissue repair (Alsarra 2009; Ramshaw et al. 2009; Natarajan et al. 2012; Ramadass et al. 2013; Kumaran et al. 2016).

These are the polymers formed by various amino acids capable of promoting intra- and intermolecular bonds, allowing the resultant materials to have a large variation in their functional properties (Gupta and Perumal 2013). Feather keratin is a potential source of abundant, inexpensive, eco-friendly, and commercial biomaterial (Poole et al. 2009; Shi et al. 2014). Keratin word first emerged around 1850 and it illustrates the material which constitutes hard tissues such as animal horns and hoofs. The name was taken from the word “kera” which means horn. Keratin is an insoluble, highly stable structure, small proteins, and uniform in size. Feather keratin has a molecular weight of about 10 kDa (Fraser et al. 1972; ARAI et al. 1983; Ullah et al. 2011; Kamarudin et al. 2017). It is composed of α-helix, β-sheet structures as discussed in further chapters. Also, the internal structure of every keratin has α-helices and β-sheets that support the protein. The elastic nature of keratin fiber is due to the interplay between α-helices and β-sheet configuration of the protein. Feathers consist of 50% of each fiber and quill by weight (Reddy and Yang 2007a). In a feather, fiber has a larger percentage of α-helix (41%) as compared to β-sheet (38%) and quill fraction consists of more β-sheet (50%) structure than α-helix (21%) (Barone et al. 2006a; Schmidt and Jayasundera 2004; Wallenberger and Weston 2003; Fraser et al. 1972). According to a previous study (Sun et al. 2009a), feather has 9.38% α-helix, 47.19% β-sheet, 32.25% β-turn, and 11.18% in random.

Keratin has about 7% cystine, which forms S–S bonds with other cystine molecules (ARAI et al. 1983) and forms cysteine by disulfide bridges. The presence of disulfide, hydrophobic, and hydrogen bond (Onifade et al. 1998; ARAI et al. 1983; Ullah et al. 2011; Cardamone 2010; Bulaj 2005) in keratin provides it strength, mechanical stability, rigidity, and resistance to degradation by proteolytic enzymes such as trypsin, pepsin, and papain (Yamamura et al. 2002; Agrahari and Wadhwa 2010; Paul et al. 2013) to keratin in the solid state. However, these cross-links are a hindrance to processing in the melt state (Barone et al. 2006b). The presence of reactive functional groups, especially peptide backbone, disulfide (–S–S) bridges, amino ($-NH_2$), and carboxylic acid (–COOH), makes it chemically reactive under favorable reaction conditions. During controlled reduction, protonation of keratin occurs. Thus, the keratin protein attains positive surface charge and becomes pseudo-cationic biopolymer.

Keratin is insoluble in polar and nonpolar solvents and has very low chemical reactivity. At low pH, high temperature and in presence of reducing agents the solubility of the keratin are increased. The biodegradability and nontoxic nature of keratin make it versatile biopolymer which can be modified and extended in various forms such as films, gel, beads, and nano/microparticles. The modified keratin has plenty of applications in food sciences, green chemistry, cosmetic industries, and pharmaceuticals.

2 Chemical Composition and Occurrence of Keratin

Keratin is the most important component of hair, wool, nails, hooves, claws, scales, horn, beaks, and feathers as shown in Table 1. These are least affected by chemical and physical environmental factors (Teresa and Justyna 2011). The keratin extracted is with 90–100 amino acids and 10.2–10.4 kDa molecular weight (Kamarudin et al. 2017; Barone and Schmidt 2005). Chemical structure of keratin showed α-helix, β-helix, or β-pleated sheet (Fraser et al. 1972; Lee and Baden 1975). Keratin has a high amount of cystine residues (7–15 mol% of amino acids) as compared to other which help to make intermolecular cross-links (Rouse and Van Dyke 2010). The amount of cysteine residues depends on the keratin source, which varies from 7% in feather to 15% in wool keratin (Fraser et al. 1972; ARAI et al. 1983).

Table 1 Distribution of α- and β-keratins

Types of keratin	Source organ
α-Keratin	Wool, quills, hair, horns, fingernails, hooves; stratum corneum
β-Keratin	Feathers, avian beaks and claws, reptilian claws and scales
	α- and β-keratin reptilia epidermis, pangolin scales

The high cystine content is the unusual characteristic of keratin which differentiates it from other structural proteins like elastin and collagen. Main amino acids present in keratin are cystine, proline, serine, and glycine (Fraser et al. 1972; Fraser and Parry 2003). In another study, γ-keratin was extracted which is nonstructural and associated with α- and β-sheets (Fraser et al. 1972; Hill et al. 2010). The sulfur content ratio plays an important role in keratin physical properties. Some researchers based upon sulfur content classified keratin as soft and hard forms (Rizvi and Khan 2008; Zoccola et al. 2009). Soft keratin has lower cystine content, week cross-linking, and smaller resistant to other chemicals found in the hair core and outer layer of epidermis (Fraser et al. 1972).

Hard keratin found in mammalian epidermal appendages, such as hairs, nails, horns, and in avian or reptilian tissues. In recent years, equine hoof (Douglas et al. 1996), bovine hoof, wool (Feughelman and Robinson 1971), and especially the sheep horn (Tombolato et al. 2010) are the attractive sources for keratin extraction.

3 Keratin Sources

Keratin biomass is derived from living organisms or from their body parts after death. The major livestock's of keratin includes sheepskins, goatskins, cattle hides, feathers, hairs, and buffalo hides as shown in Fig. 1. Skin and its appendages such as feathers, wool, nails, hooves, hair, scales, and stratum corneum are the richest sources of keratin (Kim 2007). It can be extracted from animal horns and hooves, wool, feathers, and human hairs (Fig. 1). Food industry produces million tons of keratin biomass. About 80% of human hair is formed of keratin only (Kaplin 1982; Wagner and Joekes 2005). It provides flexibility, strength, durability, and functionality to the hair in the form of different conformations (Velasco et al. 2009). Keratinous materials based on α- and β-keratins are discussed in Table 2.

Fig. 1 Main sources of keratin biomass

Table 2 Keratinous material based on the presence of α- and β-keratins

Keratinous materials based on α-keratin	Keratinous materials based on β-keratin	Keratinous materials based on α- and β-keratins
Stratum corneum	Feathers	Reptilian epidermis
Wool and hair	Beaks	Hard and soft epidermis of Testudines
Quills	Claws	Pangolin scales
Horns		
Hooves		
Nails		
Whale baleen		
Hagfish slime threads		
Whelk egg capsules		

4 Applications of the Keratin Protein

Keratin biomass is hydrolyzed by alkali, acid, or enzyme to extract keratin. The extracted keratin has various applications in various industries such as cosmetic, biomedical, and pharmaceutical industries. Furthermore, it does not have harmful effects and thus can be used for variety of cosmetics such as creams, shampoos, hair conditioners, and biomedical products. They have been used as a treatment of skin and human hair as reported before (Weigmann et al. 1990; Innoe 1992). Its existence in the hair cuticle and stratum corneum helps in preserving skin moisture while interacting with cosmetics. Its combination with other natural polymers such as chitosan, collagen, and silk fibroin was used as a component for cosmetic products (Sionkowska 2015). Keratin with high molecular weight is mostly used for skin care applications due to its individuality like film forming and hydrophilic. Keratin film or coating on skin provides smooth and soft sensation. Keratin-associated proteins from different sources were developed and applied as microscaffolds in medicine and cosmetics (Lipkowski et al. 2009). Proteins are useful ingredients for healthy skin and hair. There are studies which reported the role and efficacy of using protein in cosmetics; proteins can be obtained from simple and conjugated proteins (Secchi 2008). There are also studies which described the keratin derivatives and cationizing agent in the various cosmetics having specific functional groups (Matsunaga et al. 1983). Figure 2 shows the hair treatment cream formed using keratin extracted from chicken feathers. Other applications from different biomasses are discussed in Table 3.

Because the presence of high cross-linking by cystine formulation of micro- and nanoparticle from feathers is difficult, some researchers have prepared micro- and nanoparticles successfully from feather keratin. Keratin was converted into useful microparticles by treatment with ionic liquid, 1-butyl-3-methylimidazoliumchloride (Sun et al. 2009b). Treated feathers have low surface area but have higher ion sorption capacity than untreated feathers due to their hydrophilic nature. (Xu et al. 2014b) developed nanoparticles (50–130 nm) from feather keratin which showed good biocompatibility and stability essential for controlled drug release. Here, the chicken feather keratin nanoparticles were developed and used as a haemostatic agent which resulted in decrease in the bleeding time and blood loss in tail amputation and liver scratch rat models (Wang et al. 2016). In another study, quail feathers keratin incorporated into silver nanoparticles and formed the nanofibrous scaffold which gave 99.9% and 98% of the antibacterial activity against Gram-negative (*Escherichia coli*) and Gram-positive (*Staphylococcus aureus*) bacteria, respectively. Thus, it can be used for biomedical applications (Khajavi et al. 2016). As chitosan has good properties of biodegradation and biocompatibility, when they are mixed with keratin nanoparticles, they form scaffold. The biodegradation and protein adsorption of the scaffold had increased, and it was noncytotoxic to human osteoblastic cells. Thus, this scaffold can work as biomimetic substrate for bone tissue engineering applications (Saravanan et al. 2013). In one study, keratin nanopowder from chicken feather was produced by electrospraying which has a small particle size and has less crystallinity

Fig. 2 Hair treatment cream made by using keratin extracted from chicken feathers

than raw keratin (Rad et al. 2012). Water stable nanoparticles were developed from feather keratin. Figure 3 shows the keratin nanoparticles extracted from chicken feathers. Nanoparticles can be a good veterinary diagnostic, and it can penetrate

Table 3 Applications from different keratin biomasses

Sr. no.	Sources	Industrial application(s)	Reference(s)
1	Human hairs	Medicinal use	Zheng et al. (2005)
2	Hoof and horn’s	Preparation of firefighting composition	Datta (1993)
3	Human hair and wool	To explore structural and biological properties of self-assembled keratins	Xu et al. (2014b)
4	Feathers	Development of protein fibers and 2D and 3D scaffolds for tissue engineering	Xu et al. (2014a), Rouse and Van Dyke (2010)
5	Chicken feathers	Keratin film for drug delivery system	Poole et al. (2009), Yin et al. (2013)
		Regenerated fibers	Xu et al. (2014a)
		Micro- and nanoparticles	Sun et al. (2009b)
		Graphene oxide and its derivative in biomaterials	Amieva et al. (2014)
		As a diet supplement for feeding ruminants	Coward-Kelly et al. (2006), Dalev (1994), Dalev et al. (1996), Dalev et al. (1997)
		Microporous material used as electrode material	Zhan and Wool (2011)
		Thermoplastic films	Reddy et al. (2013), Jin et al. (2011)
		Waste management using microorganisms for degradation	Vasileva-Tonkova et al. (2009), Syed et al. (2009), Grazziotin et al. (2006)
		Leather processing	Sastry et al. (1986), Sehgal et al. (1987), Karthikeyan et al. (2007)
		Handspun yarn	Reddy and Yang (2007b)
		Textile yarns	Reddy et al. (2014a, b), Yang and Reddy (2013)
		Keratinases in detergents formulation	Balakumar et al. (2013), Manivasagan et al. (2014), Rai et al. (2009)
		Flame retardant	Wang et al. (2014)
		Bio-composites or composite fabrication	Flores-Hernández et al. (2014), Spiridon et al. (2012), Huda and Yang (2008, 2009)
		Biofertilizer	Ichida et al. (2001), Kornillowicz-Kowalska and Bohacz (2010), Gurav and Jadhav (2013), Hadas and Kautsky (1994), Gousterova et al. (2012)

(continued)

Table 3 (continued)

Sr. no.	Sources	Industrial application(s)	Reference(s)
		Nanoparticle and microparticles for pharmaceutical application	Xu et al. (2014b), Sundaram et al. (2015), Yu et al. (2014), Sharma et al. (2017a, b)
		Keratin and graphene oxides within biomaterials	Amieva et al. (2014)
		Cement-bonded composites	Acda (2010)
		Keratin hydrogel	Wang et al. (2017), Barati et al. (2017), Priyaah et al. (2017)
		Tissue regenerative applications	Li et al. (2013), Saravanan et al. (2013), Kumar et al. (2017)
		Paper production	Tesfaye et al. (2017)
		Bioplastic	Sharma et al. (2018), Ramakrishnan et al. (2018)

easily into cells and organs due to their nano size. They are good for the drug delivery as compared to synthetic polymer and carbohydrates. Xu et al. (2014b) found that keratin nanoparticles have supportive function for the cell growth and stable in physiological environment for up to 7 days. The keratin nanoparticle shows anticancer properties. When keratin nanoparticles combine with chlorin e6, it resulted in greater cell death percentage (90%) as compared to free chlorin e6 on osteosarcoma (U2OS) and glioblastoma (U87) cells lines, and thus keratin nanoparticles are effective and promising delivery vehicles for photodynamic therapy applications (Aluigi et al. 2016). In one study, keratin-based drug-loaded nanoparticles were made and it was showing pH and glutathione dual-responsive behavior. Thus, from the results of the study conducted, it was concluded that keratin-based drug carriers had potential for drug delivery and cancer therapy in clinical medicine (Li et al. 2017).

Biocompatible composites were developed which can be used for high-performance dressing, to treat chronic ulcerous infected wounds by the combination of cellulose and keratin with the silver nanoparticles (Tran et al. 2016). Hair keratin nanoparticle had faster clotting time, and it significantly reduces blood loss and coagulation time which forms the viscosity gel on wound; hence, it has a great potential for haemostatic application (Luo et al. 2016). The soluble keratin would have applications in tissue regeneration, cell seeding, wound healing, and drug delivery (Yamini Satyawali 2013). Soluble keratin can be used to make 2D and 3D scaffolds, and protein fibers which further utilized for tissue engineering (Xu et al. 2014b). Due to self-congregation and polymerization property of keratin proteins has led to work as scaffolds for tissue engineering (Rouse and Van Dyke 2010).

When the partially oxidized keratin is added into water, it forms the hydrogel which can be used as an absorbent material, as a therapeutic for skin. The hydrogel can be used for the implantation. The keratin can be incorporated into films which are

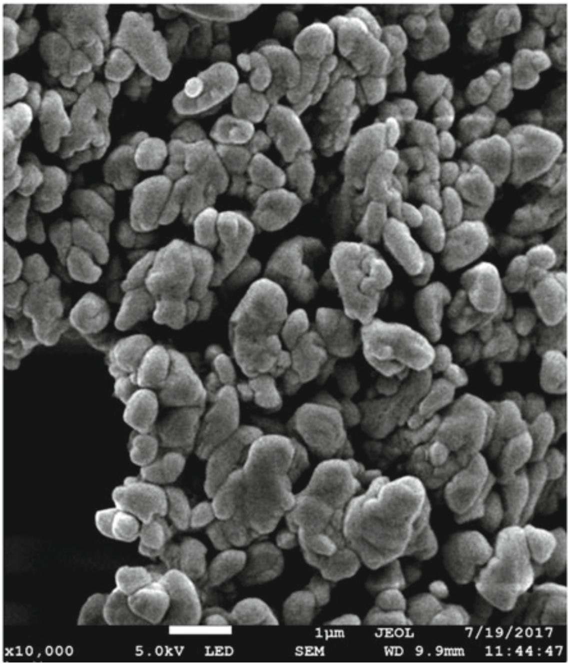

Fig. 3 Keratin nanoparticles extracted from chicken feathers

nonwoven, and these two materials are suitable for use in tissue engineering scaffolds (Blanchard et al. 2002). Keratin biomaterials have the potential to interact with cells and tissues but the composition, structure, and cell-instructive characteristics are not clear. Burnett et al. (2013) made keratin-based biomaterial, demonstrate self-assembly of cross-linked hydrogels, investigate a cell-specific interaction, and find the utilization in drug and cell delivery, tissue engineering, regenerative medicine, and trauma. The huge amount of waste biomass generated by animals as well as food industries can be used as raw material for the production of keratin at industrial level. The keratin waste biomass management by reconversion into industrially used products will save the ecosystem from large amount of sludge and boost up industries such as pharmaceutical and cosmetic industry economically.

5 Conclusion

To develop the strategies for efficient extraction of keratin from poultry biomass will prove to be very beneficial for sustainable management of huge waste. Researchers are working to develop various chemical, biological, and physical methods individually as well as in combined form for keratin extraction. The insoluble protein has many advantages in biomedical industry to develop products of pharmaceutical use, in tissue engineering as well as in agriculture industry too. Recent advances in the sustainable management of poultry waste biomass, extraction techniques used by various researchers, and numerous applications of the extractions have been discussed in the subsequent chapters of this book.

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