




Review

Bioplastics from waste biomass of marine and poultry industries

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Plastics are indispensable and typically derived from non-renewable sources. The extensive production and indiscriminate use of synthetic plastics pose a serious threat to the environment and lead to problems due to their non-biodegradability. Various forms of plastics that are used in daily life should be limited and replaced by biodegradable materials. To deal with the challenges of sustainability or environmental issues that occur due to the production and disposal of synthetic plastics, biodegradable and environment-friendly plastics are crucial. Utilizing renewable sources such as keratin derived from chicken feathers and chitosan from shrimp cell wastes as an alternative to obtain safe bio-based polymers has gained much attention because of rising environmental issues. Approximately, 2–5 billion tons of waste are produced by the poultry and marine industries each year, adversely impacting the environment. These polymers are more acceptable and eco-friendly compared with conventional plastics due to their biostability, biodegradability, and excellent mechanical properties. The replacement of synthetic plastic packaging with biodegradable polymers from animal by-products significantly reduces the volume of waste generated. This review highlights important aspects such as the classification of bioplastics, properties and use of waste biomass for bioplastics production, their structure, mechanical properties, and demand in industrial sectors such as agriculture, biomedicine, and food packaging.

Keywords. Biomaterial; bioplastics; biopolymers; chitosan; keratin; PHA; plastic

1. Introduction to bioplastics

Global environmental changes and depleting natural resources are probably the most recognized challenges the world is facing today (Vijayavenkataraman *et al.* 2012). We are facing severe changes in climate due to the emission of greenhouse gases such as carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), and chlorofluorocarbons (CFCs) produced by the combustion of fossil fuels (Piemonte 2011). Complete reliance on synthetic plastics and their overuse contribute to environmental pollution because of their non-degradability in the environment (Abbasi 2020). Conventional plastics have a wide range of properties and physicochemical

characteristics that make them a daily life product. Synthetic plastics belong to a large family of polymers and are derived from petroleum-based products. Various types of plastics such as polyethylene (PE), polyurethane (PUR), polystyrene (PS), polyethylene terephthalate (PET), polypropylene (PP), polyvinyl chloride (PVC), polybutylene terephthalate (PBT), and nylons are commonly used in our daily life because they are easy to manipulate, have low molecular weight, low cost, and excellent thermal properties (Muneer *et al.* 2021a).

Serious climate and health hazards are caused due to the non-biodegradable nature of synthetic plastics and their overuse in our daily lives (Rhodes 2018). About 34 million tons of plastics are produced every year and only

7% is recycled while the remaining 93% is dumped into oceans and landfills (Pathak *et al.* 2014). In Europe 25.8 million tons of plastic waste are produced annually, of which 30% is recycled and 40% is incinerated, giving rise to further environmental challenges (Narancic *et al.* 2020). Measurements and concepts for plastic waste management are relatively unknown in many developing countries. Municipalities lack a proper system for the management of solid/plastic waste. For example, more than 3.3 million tons of plastic waste are produced every year in Pakistan, and most of it ends up in landfills, mismanaged dumps, or on land and in water bodies across the country (Urbanek *et al.* 2018). Global concerns about plastic waste are increasing. The pollution rate has escalated due to limited and insufficient plastic waste management systems. Waste treatments like incineration lead to the emission of toxic gases and cause air pollution, while landfills are limited (Sushmitha *et al.* 2016). Drastic climate change, and economic and global health issues, have increased the demand and research interest to replace synthetic or fossil fuel-based polymers with biodegradable polymers (Hatti-Kaul *et al.* 2020).

Bioplastics date back to the 1850s when a British researcher produced plastic materials using wood pulp cellulose (Muneer *et al.* 2021b). Bioplastics are plastics produced from natural and renewable raw materials, i.e., agro/food sources, materials such as starch, cellulose, protein, vegetable oils, fats, bacteria, and algae. Bioplastics could be disposed of in the environment where they are easily degraded by microbial activity (Demirbas 2007). Carbon atoms present in the polymer chain are degraded by the action of fungi and bacteria to produce organic material rather than ending up in landfills. Figure 1 shows the carbon cycle of plastics. The demand for the applications of bioplastics is growing rapidly because they are renewable, biodegradable, compostable, and environment friendly. Bioplastics release a negligible amount of greenhouse gases and are degradable and less toxic to the environment as compared with their synthetic counterparts derived from petrochemicals (Arikan and Ozsoy 2015).

Commonly, bioplastics are derived from raw materials that are either bio-based or biodegradable. Although bioplastics are safe and eco-friendly, they have some limitations such as high production cost or poor mechanical and barrier properties (Rahman 2019). However, inexpensive sources such as animal wastes (chicken feathers, exoskeletons of insects) to produce bioplastics can be used to overcome high production costs, and blends of two or more biopolymers are being used to deal with poor mechanical or barrier properties (Shubha and Srivastava 2019). Natural bio-based raw

materials from various renewable sources have been used to obtain biodegradable packaging and edible films. Chicken feathers contain approximately 90% of keratin as structural proteins. Keratin is insoluble in a majority of solvents and is resistant to proteolytic enzymes. The high cysteine content in keratin and the presence of three-dimensional cross-linked networks provide strength, hydrophobicity, and good thermal stability, which make it suitable to be used for the development of biodegradable materials (Tesfaye *et al.* 2018). Chitin is a linear polysaccharide of N-acetyl glucosamine, which upon hydrolytic deacetylation, yields chitosan. Both keratin and chitin are obtained from an abundant source of crustacean shells from organisms like shrimps and lobsters. Due to their unique chemical versatility, film-forming ability, low toxicity, and biodegradability, these are viable sources of bioplastic production (Abdullah *et al.* 2020)

Keratin- and chitosan-based bioplastic films are biocompatible and biodegradable, and can be molded into desired shapes to form different products such as bioplastic films and coatings. Due to the widespread availability of these wastes, these sources are sustainable and eco-friendly. Besides replacing petroleum-based plastics in order to protect the environment, these can also be used to produce valuable by-products (Ramakrishnan *et al.* 2018). Keratin can be used to generate bioplastic film by blending with other polymers or plasticizers to enhance its physical and mechanical properties (Wu *et al.* 2017). Developing biodegradable or bio-based polymers as an ecologically valuable replacement for synthetic plastics is an innovative area of polymer research.

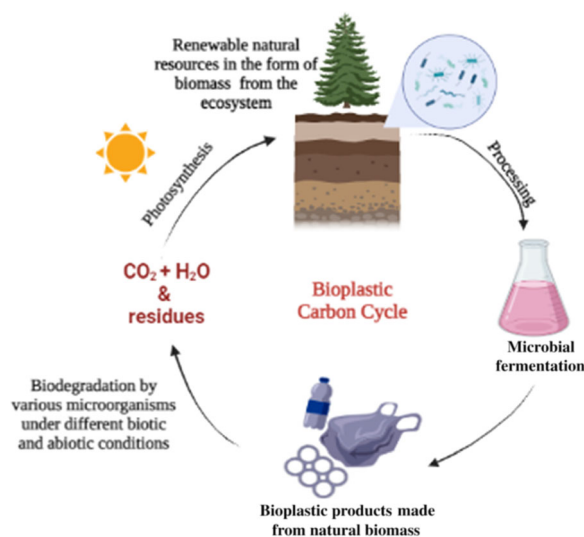


Figure 1. The carbon cycle of bioplastics.

2. Classification of bioplastics

Bioplastics are not just a single class of polymers, but it is a broader term to include polymers derived from a large number of renewable sources that are either bio-based and biodegradable or materials that are non-biodegradable and derived from renewable resources (Sidek *et al.* 2019). Based on the sources, bioplastics can be classified into the following three major classes (figure 2):

- (i) Bio-based and biodegradable polymers, i.e., biopolymers derived from natural biomass sources such as agro-polymers and polyesters from microorganisms
- (ii) Bio-based and bio-nondegradable
- (iii) Biodegradable polymers that are synthesized chemically (Kumar and Thakur 2017)

2.1 Bioplastics from plant polysaccharides

Polysaccharides are complex carbohydrates found abundantly in nature as bio-macromolecules (Ferreira *et al.* 2016). Bio-based plastics or bioplastics are derived from biomass from a wide range of living matter and organisms such as animals, plants, and agricultural sources. These sources include plant polysaccharides (cellulose, starch, chitosan, and gums)

and animal-derived proteins, e.g., soya, zein, casein, collagen, and keratin (Averous *et al.* 2012). A large number of polysaccharides and their derivatives have been known to be effective in the form of biodegradable films. These films and thin membranes are frequently used in the food and pharmaceutical industries. Polysaccharide-based biofilms have various mechanical and gas barrier properties depending on their structure, cross-linking agents, plasticizers, and the additives used to improve different characteristics (Freitas *et al.* 2014). Polysaccharide membranes have good mechanical and gas (O_2 and CO_2) barrier properties that make them highly desirable in bioplastic film production for food packaging materials (Yang *et al.* 2010).

2.1.1 *Starch*: Starch is the most abundant and commonly used renewable raw material. It can be extracted from a wide range of agricultural products such as wheat, corn, rice, and cassava (Amin *et al.* 2019). Starch is an attractive material for packaging applications due to its relatively low cost, biodegradability, availability, and thermoplastic behavior (Sanyang *et al.* 2016). Starch has poor resistance to moisture and mechanical properties that restrict its use. Hydrophilicity, hydrogen bonding, and intermolecular forces affect the thermoplastic behavior of starch polymers, resulting in high glass transition temperature (T_g) and low melting temperature (T_m) values (Zhang

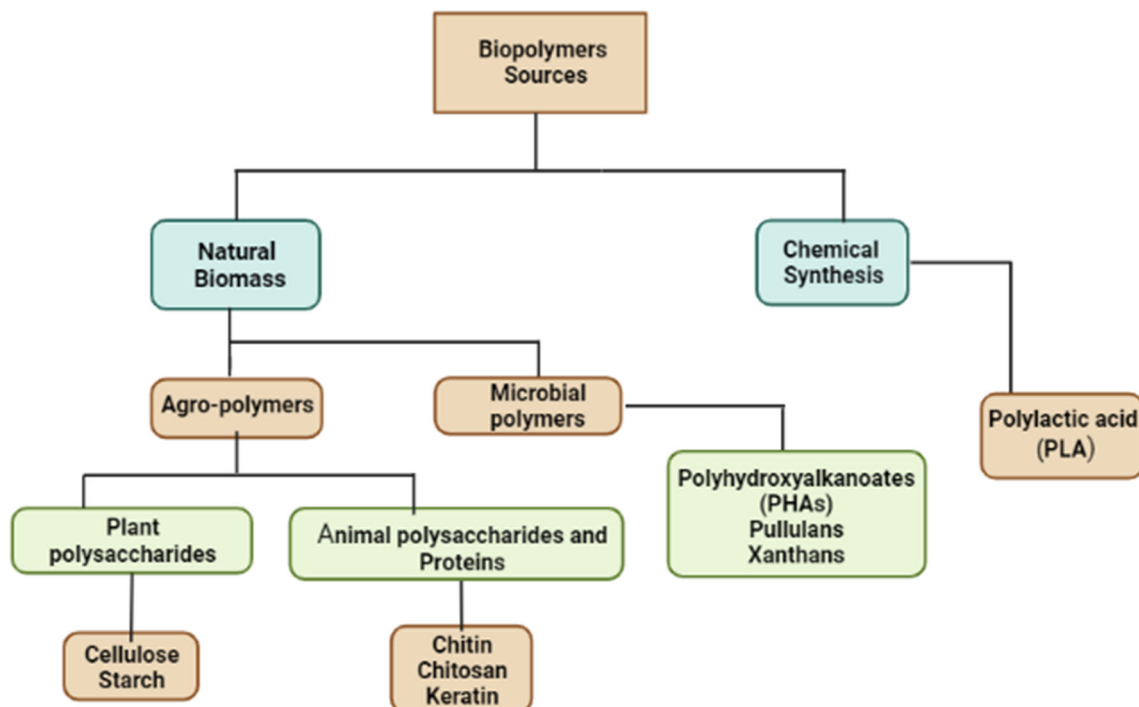


Figure 2. Classification of biopolymers based on their sources.

et al. 2014). These drawbacks can be overcome by using plasticizers that effectively enhance the chain mobility and flexibility of the material. Polyols like glycerol, glycol, and sorbitol are the most commonly used plasticizers that add mechanical support and physiochemical properties to bioplastic products (Babu *et al.* 2013).

2.1.2 Cellulose: Cellulose is the natural polymer abundantly present in the cell walls of plants, most commonly obtained from wood and cotton fibers (Muneer *et al.* 2021c). In general, cellulose due to its linear molecules, is a more suitable material for fibers and films (Demirbas 2007). Specific properties such as low mass, durability, cost-efficiency, non-toxicity, renewability, biocompatibility, chemical stability, biodegradability, and good film-forming performance make it a potential candidate for bioplastic materials (Duan *et al.* 2016). Due to its regular structure and array of hydroxyl groups, cellulose usually forms strong hydrogen-bonded crystalline microfibrils. Natural cellulose fibers, despite being inexpensive, non-toxic, and biodegradable, have limited applications in the industry due to their hydrophilic nature and transparency (Wang *et al.* 2013). The hard mechanical properties of cellulose can be improved by making blends with other polymers and plasticizers (Mohanty *et al.* 2003). However, to produce cellophane, which is biodegradable and has been used worldwide for packaging, cellulose can be treated with sodium hydroxide (NaOH) and sulfuric acid (H₂SO₄) to enhance its mechanical properties (Paunonen 2013).

3. Biopolymers from animals

3.1 Keratin

By-products from various animal sources (feathers, bones, fats, soft body parts, and blood) are used as fertilizers, livestock meals, and food for pets. Huge biomass waste is produced by the poultry industry from which a large amount is dumped, incinerated, or disposed in landfills, which causes environmental hazards, economical issues, and wastage of renewable materials (Ramakrishnan *et al.* 2018). Feathers are one of the most common waste constituents from the chicken industry, are inexpensive, and available in bulk. Therefore, using feather biomass is an effective way to not only protect the environment but also to produce value-added by-products. Poultry feathers are composed of 90% of keratin, which is a rich source of

hydrophobic amino acids such as cysteine, arginine, and threonine and has high nutrient potential (Sharma *et al.* 2018). Keratins are preferable proteins due to their characteristics like environmental stability, biodegradability, and biocompatibility. Various amino acids constitute protein polymers, and these amino acids hold unique side chains in the protein structure (Gupta *et al.* 2012). Keratin extracted from feathers is used as sponge fibers or films, alone or in combination with other natural or synthetic polymers in the form of blends. Utilizing feather biomass in bioplastic films is biodegradable or bio-based, and can be used in the biomedical, cosmetics, or textile industries and is a suitable way to avoid environmental pollution (Sharma *et al.* 2018).

3.2 Chitin/chitosan

Chitosan is a cationic polysaccharide and is obtained by the deacetylation of chitin. It is a copolymer of glucosamine and N-acetyl glucosamine units linked by a 1-4 glycosidic bond (Karua and Sahoo 2020). The majority of available chitin in the world is obtained from crustacean waste, and due to its biocompatibility, biodegradability, and good muco-adhesive properties, it has found potential applications in agriculture, the food industry, and medical field (Epure *et al.* 2011). Chitosan membranes are semipermeable to gases, and have a moderate water vapor barrier (Xu *et al.* 2005). To improve the performance of the chitosan-based film, glycerol is added to chitosan membranes and mechanical kneading is applied to obtain thermoplastic material having good mechanical properties (Ferreira *et al.* 2016). Thus, utilizing chitosan, which is easily available and inexpensive, offers a new pathway for the large-scale production of viable bioplastics that can be used to replace conventional and non-biodegradable plastics for commercial manufacturing.

4. Chemically synthesized bioplastics

4.1 Polylactic acid (PLA)

Polylactic acid (C₃H₄O₂)_n (the general structure in figure 3) is the first product obtained from renewable sources that are commercialized in the plastic industry (Sawyer 2003). PLA is a bio-based and biodegradable polyester obtained from corn, sugar beets, and potato starch (Rasal *et al.* 2010). PLA is a biodegradable and bio-absorbable, renewably derived thermoplastic

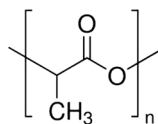


Figure 3. The general structure of polylactic acid.

polyester. L- and D-lactic acid (isomers of polylactic acid) are synthesized by the fermentation of starch or other carbohydrates and then polymerized to yield PLA (Gupta *et al.* 2007). Due to its biodegradability or biocompatibility, PLA is extensively used in the medical sector as medical implants, in food packaging, in environmental remediation films, and in the textile industry (Castro-Aguirre *et al.* 2016).

5. Polymers derived from microbes

Polyhydroxyalkanoates (PHAs), pullulan, xanthan, and several other microbial polysaccharides have film-forming abilities. Their mechanical strength, thin flexible layers, and unique antimicrobial activity make these biofilms excellent candidates in polymer science research with a focus on bioplastics. Bioplastics obtained from different sources have several properties as listed in table 1.

5.1 Polyhydroxyalkanoates (PHAs)

Polyhydroxyalkanoates (PHAs) (general structure in figure 4) are optically active polymers produced by bacteria under conditions of excess availability of carbon sources and under limited supply of other vital nutrients (Lee 2000). The properties of PHAs depend on their carbon sources and the microbial strains involved in fermentation (Muneer *et al.* 2022). Due to similar physicochemical properties, PHAs can be used as alternatives to petrochemical-based plastics. (Keshavarz and Roy 2010). These polymers, alone or in combination with starch or synthetic plastics, make for excellent packaging films. Among more than 100 composites, polyhydroxybutyrate (PHB) is the most commonly found PHA, and is very similar to polypropylene formed by the polymerization of a 3-hydroxybutyrate monomer. It has been used in industrial, biomedical, agricultural, and domestic applications. PHB on degradation (under aerobic or anaerobic conditions) produces carbon dioxide and water. Properties like optical activity and insolubility in

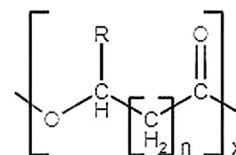


Figure 4. The general structure of PHA. Here, ‘n’ ranges from 1 to 4, and ‘x’ ranges from 100 to 30000.

water and gas barriers make PHB a potential candidate for packaging bioplastic materials (Rahman 2019).

5.2 Pullulan

Pullulan constitutes a maltotriose trimer made up of α -(1 \rightarrow 6)-linked (1 \rightarrow 4)- α -d-triglucosides with the chemical formula $C_6H_{10}O_5$. A generalized chemical structure of pullulans is shown in figure 5. Pullulan was isolated from the culture broth of *Aureobasidium* (Singh *et al.* 2017). It is biodegradable, innocuous, non-mutagenic, and non-odorous. Pullulan degrades at high temperatures (250–280°C) and has very low moisture absorbance (Singh *et al.* 2008). Pullulan membranes have excellent polymer characteristics: they are non-toxic and edible. These membranes are not only flexible and transparent but are also good barriers to oxygen. Some studies report anti-fungal properties of pullulans, making them a potential bio-based candidate for packaging (Trinetta and Cutter 2016). The high cost of pullulan has limited its use despite its excellent polymer properties. The physicochemical characteristics and mechanical properties of pullulan can be modified by forming its blends with other inexpensive polymers and additives which not only make it economically feasible but also improve its properties (Wu *et al.* 2013). Pullulans blended with different bio-based materials such as cellulose, alginates, and chitosan have shown improved thermo-mechanical and gas barrier properties (Trovatti *et al.* 2012). Pullulans and their derivatives are used widely in the medical, cosmetics, and food packaging industries (Ferreira *et al.* 2016).

5.3 Xanthan gums

Xanthan is an exopolysaccharide produced by the gram-negative bacterium *Xanthomonas campestris* (Kumar *et al.* 2018). It is a branched polysaccharide with side chains consisting of pentasaccharide units composed of mannose, glucose, glucuronic acid pyruvate, and an acetyl substitution group (figure 6) (Tang

Table 1. Properties of bioplastics obtain from plants, animals, or microbes

Biopolymer	Source	Properties	References
Cellulose	Plants (wood or cotton)	Non-toxic, bio-based, biodegradable, thermo-mechanical properties and sensitivity towards the water.	Campos <i>et al.</i> 2011
Starch	Cereals, nuts, cassava	Biodegradable, transparent, odorless, and tasteless	Chen and Lai 2008
Pullulan	Microbes	Biodegradable, transparent, edible, antifungal properties	Kanmani and Lim 2013
Xanthan gum	Microbes	Biodegradable, edible, water-soluble	Muneer <i>et al.</i> 2021b
PHAs	Microbes	Biodegradable, biocompatible and thermostable	Muneer <i>et al.</i> 2020
PLAs	Chemically synthesized (plant or carbohydrate source)	Biodegradable, high tensile strength	Sha <i>et al.</i> 2016
Chitosan	Animal	Biodegradable, antibacterial, biocompatible and non-toxic	Kerch 2015
Keratin	Animal protein	Hydrophilic, non-burning, and biodegradable	Saha <i>et al.</i> 2019a

et al. 2012). Xanthan gums are hygroscopic and safe polymers, and can be used as thickeners and polymeric stabilizers in blended forms. These polysaccharides behave as pseudo-plastics and are being used in the food, pharmaceutical, textile, and petroleum industries (Petri 2015; Ferreira *et al.* 2016).

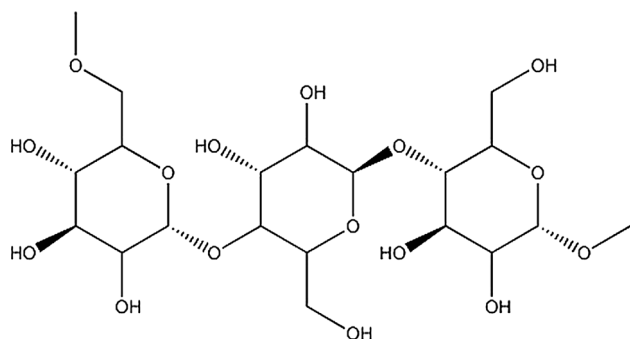
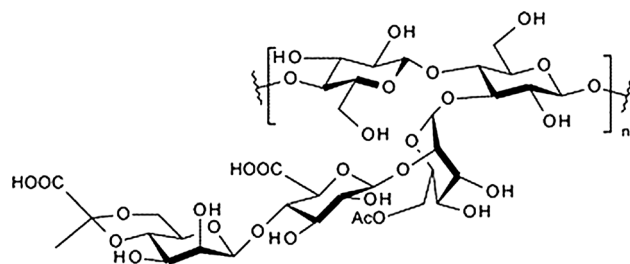
6. Production of bioplastics from animal resources

6.1 Chicken feathers: Source of keratin protein

It has been estimated that 2–3 billion tons of chicken feather waste are discarded annually from the poultry industry and slaughterhouses (Aluigi *et al.* 2007). A small portion of it is used in livestock feed while the disposal of the remaining bulk poses a serious threat to the environment. Poultry feathers have high keratin content, which has great film-forming ability due to its high mechanical strength (Reddy and Yang 2007). Keratin-based biomaterials have shown great potential to effectively replace synthetic plastics due to their

biodegradability, biocompatibility, mechanical durability, and natural abundance (Tesfaye *et al.* 2018). Therefore, due to economic and environmental concerns, it is required to mechanize lucrative and effective processes to use these sources for the development of naturally derived biomaterials (Wang and Cao 2012).

6.1.1 Keratin structure: Keratin is a complex protein found in the nails, hooves, wool, hair, and feathers of various animals (Deivasigamani and Alagappan 2008). Keratin is a polypeptide containing various amino acids with inter- and intra-molecular bonding of cysteine with polar and non-polar groups. A successive folding of keratin fibers is due to the presence of side chains present in the polypeptide backbone (Saha *et al.* 2019a). Elasticity in keratin is due to the presence of α -helices and β -sheets in the macromolecule. Keratins are differentiated into α -keratins and β -keratins. Soft tissues such as wool, hair, and skin have α -keratin, while hard β -keratins are found in nails, bird feathers, and fish scales. α -Keratin has a high molecular weight and is rich in cysteine, whereas β -keratin has a low molecular weight and is rich in amino acids like alanine and glycine (Sharma and Gupta 2016; Kamarudin *et al.*

**Figure 5.** Chemical structure of pullulan.**Figure 6.** Chemical structure of xanthan.

2017). Fifty percent of chicken feathers are composed of fiber and quills. Keratin fiber is composed of 41% α -helix and 38% of β -pleated sheets. However, the quill contains more than 51% β -sheets in its structure. Ninety percent of the keratin present in feathers is a small-sized insoluble protein having a molecular weight of 10 kDa (Wattie *et al.* 2018). Its structure contains a central carbon atom linked to various functional groups including amine [-NH₂] and carboxylic groups [-COOH]; the hydrogen atoms and the R-group (sulfur) make it chemically reactive under suitable conditions. It is a hard protein found in nature and contains hydrophobic amino acids, β -sheet crystallites, and is highly cross-linked (approximately 7%) by cysteine (Acda 2010; Sharma *et al.* 2018). Cysteine-containing amino acids tend to cross-link with one another by forming disulfide linkages and hydrogen bonds, and provide strength, stiffness, and solubility with good thermal and insulating properties to the keratin structure (Sharma *et al.* 2019). A schematic representation of keratin components is shown in figure 7. Keratin is insoluble and has low chemical reactivity; however, its solubility increases at acidic pH, high temperature, and in the presence of reducing agents like Na₂SO₃ or Na₂S. Keratin has been studied widely due to its biodegradable and non-toxic nature, as this versatile biopolymer can be used as biofilms, gels, and beads with a wide range of applications in the cosmetics, food, and pharmaceutical industries (Khosla and Amanullah 2013).

6.1.2 Extraction of keratin: Keratin extraction from various biomass sources is the main step to utilizing it sustainably. Extraction of keratin from chicken feathers without deformation of its secondary structure is a challenging task that requires great expertise and

efficient techniques (Sharma *et al.* 2017b). Various methods and techniques including chemicals, enzymes, and ionic solutions are used to extract functional keratin from waste biomass (Sharma *et al.* 2018). During the extraction of keratin, extreme care is needed as acidic hydrolysis can destroy its secondary structure, which can render it useless due to polymer property loss. On the other hand, enzymatic hydrolysis is a slow process making it commercially infeasible (Sharma *et al.* 2017a). Cross-linking cysteine with peptide chains imparts stability to the protein structure by forming disulfide bonds (Korniłowicz-Kowalska and Bohacz 2011). The strength of keratin in feathers can be minimized by breaking these disulfide bonds. Reagents that can break the bonds present in keratin also decreases the strength of these fibers (Terzopoulou *et al.* 2015). Using oxidizing agents for this purpose yields a slow process. Despite that, a more effective method to extract proteins without any chemical alteration is using reducing agents that quickly dissolve the hard protein (Fernández-d'Arlas 2019). Urea, sodium sulfide (Na₂S), and sodium hydroxide (NaOH) are some oxidizing agents used for this purpose. Sodium sulfide solution shows the best result and gives a high yield of protein (Kumar and Thakur 2017). Factors such as pH, temperature, and the ratio of reducing agents are considered while performing the extraction procedure. The procedure is usually conducted by dissolving chicken feathers using different reducing agents and later extracting the protein from chemicals for further preparation of bioplastic films (Gupta *et al.* 2011). The protein extracted from chicken feathers can be refined into films using different techniques such as the wet processing method (casting) or thermo-mechanical processing (dry pressing) (Verbeek *et al.* 2010). The basic steps involved in the extraction process are shown in figure 8.

6.1.3 Film characterization: Films processed by using purely protein sources are fragile. To improve their mechanical properties, plasticizers and other additives such as starch and cellulose are blended in to make them acceptable for commercial applications (Moore *et al.* 2006; Alashwal *et al.* 2020). Plasticizers are considered to enhance film flexibility by reducing inter- or intra-molecular attractive forces (Barone *et al.* 2005). Keratin-based films can be characterized by their morphological structure, mechanical properties such as Young's modulus, tensile strength, and elongation-to-break ratio, thermal properties, water solubility, and biodegradability in the natural environment. Morphological studies of bioplastic film showed undissolved particles in the absence of plasticizers.

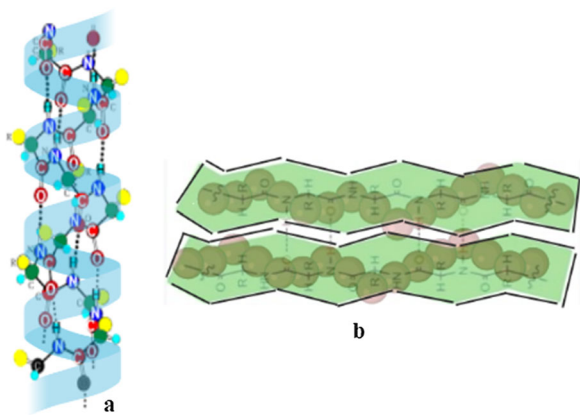


Figure 7. Different components of keratin: (a) α -helix and (b) β -sheet conformation of keratin protein.

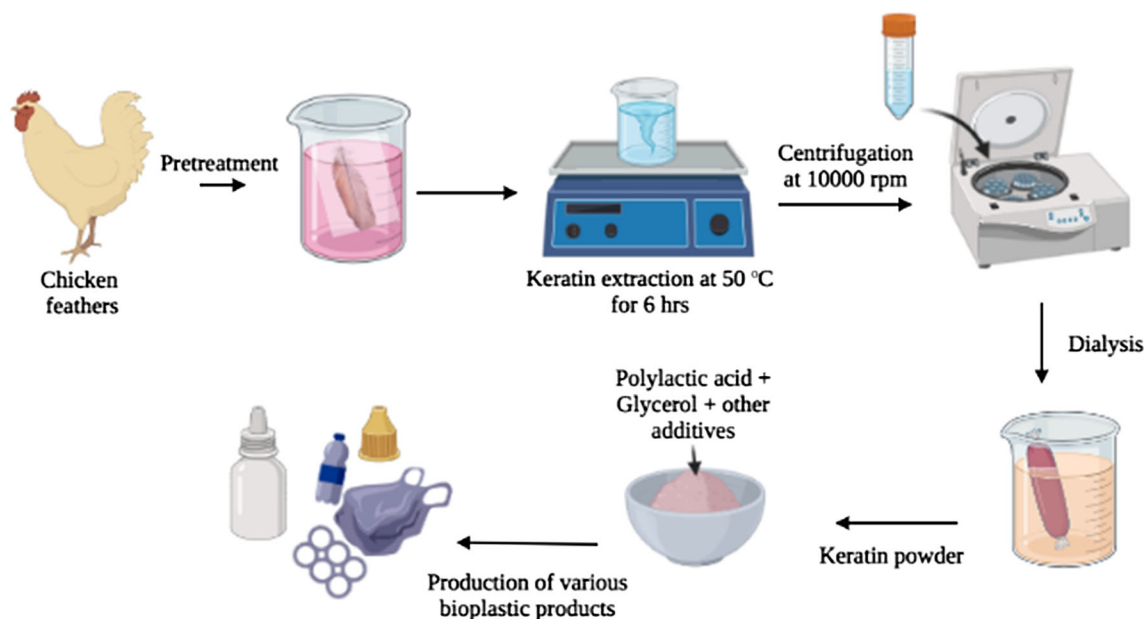


Figure 8. Basic steps involved in keratin extraction using chicken feathers and production of bioplastic products.

However, adding glycerol makes the surface uniform and homogenous (Dou *et al.* 2016).

Plasticizers are used to enhance the film's malleability. At high concentrations, it decreases the ability of the film to bear stress and its Young's modulus. However, its presence increases the elongation at break and adds free volume by dissolving the hydrogen bond present between the protein matrix (Fakhoury *et al.* 2012). Thermal properties include melting temperature and glass transition temperature (T_g). With the increase in inter- and intra-molecular forces of the protein, derived biofilms, and matrix, the glass transition temperature increases. Thus, the presence of glycerol reduces the glass transition temperature (T_g) by reducing inter- and intra-molecular interactions (Amanullah and Wu 2013; Ramakrishnan *et al.* 2018). The effect of different concentrations of glycerol is presented in table 2. However, the solubility content in the film can be enhanced by using starch/keratin blends (Tsfaye *et al.* 2018). The keratin-based film can be fully degraded within 24 h by adding a high concentration of glycerol (Wahyuningtyas and Suryanto 2017). Due to these reliable mechanical and thermal properties, keratin-based films are considered suitable and can be used for commercial applications. The mechanical properties of keratin blends with other additives are summarized in table 3.

6.2 Crustacean shells

Large amounts of by-products from marine crustaceans such as lobsters, crabs, and shrimps are discarded as

waste by seafood processing industries. The outer coverings of these by-products are an excellent source of chitosan, a derivative of chitin, which is a source of great interest after cellulose (Kumar *et al.* 2020). It comprises co-polymers of glucosamine and N-acetyl glucosamine, and is considered a favorable alternative to conventional plastics because the films obtained from this polymer have bio-stability, biodegradability, semi-crystalline structure, and are eco-friendly, inexpensive, and abundant in nature. Therefore, extraction of raw materials from these remnants is a sustainable and economical way to minimize waste and obtain value-added by-products with a wide range of industrial and commercial applications (Hamed *et al.* 2016).

6.2.1 Structure of chitin and chitosan: Chitin is a polysaccharide that can be obtained from the exoskeletons of arthropods and the cell walls of microorganisms. It is a linear β -(1-4) polymer of N-acetyl-D-glucosamine. The acetamide group present at the carbon-2 position instead of the hydroxyl group differentiates it from animal cellulose. It is present in various forms such as α -chitin, β -chitin, and γ -chitin. Of all, α -chitin is the most abundant in nature and has stable antiparallel strands as compared with β -chitin and γ -chitin. This configuration provides resistant structures in the exoskeletons of shrimp, lobsters, and the cell walls of yeast and fungi (Srinivasa and Tharanathan 2007). Chitin exhibits excellent properties of bio-stability and the ability to degrade in the environment, but its hydrophobic nature limits its applications. The firmness of chitin is due to strong hydrogen

Table 2. Effect of glycerol concentration on thermal and mechanical properties of keratin-based biofilms

Glycerol conc./g keratin	Film solubility	Transition temperature (T _g)	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)	Reference
0.00	30.6	71	16.56	10.1	1.7	Moore <i>et al.</i> 2006
0.01	N.R.	N.R.	6.3	2.0	11.8	
0.03	37.1	68.5	7.5	2.1	13.9	
0.05	40.6	64.5	5.4	1.2	19.7	
0.07	48.2	56.3	2.1	0.9	30.56	

N.R., not reported.

Table 3. Mechanical properties of keratin blends

Polymer blends	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)	Reference
Starch/keratin	7.3	N.R.	26.20	Tesfaye <i>et al.</i> 2018
Cellulose/keratin	3.6	1.52	15.8	Sharma <i>et al.</i> 2018
Citric acid/keratin	1.8	18.2	24.5	Reddy <i>et al.</i> 2013
Sorbitol/keratin	3.4	28	52.7	Martelli <i>et al.</i> 2006
Polyethylene oxide/keratin	6.1	30	46.2	Aluigi <i>et al.</i> 2008

N.R., not reported.

bonding (Fernando *et al.* 2016). Chitin deacetylation influences its physical and chemical properties. The degree of deacetylation normally referred to as the glucosamine/N-acetyl glucosamine ratio has a great effect on its solubility. Chitosan is an N-deacetylated form of chitin that causes a reduction in molecular weight (figure 9). Chitin has a molecular weight of 1.03 to 2.5×10^6 Da, but upon N-deacetylation, it reduces to 1.0 to 5×10^5 Da (Srinivasa and Tharanathan 2007). Chitosan is a water-soluble polymer of β -(1-4)-linked glucosamine (2-amino-2-deoxy-O-glucose) units. A positive charge on its amino group makes chitosan a hydrophilic cationic polymer. The presence of D-glucosamine makes chitosan insoluble at alkaline or neutral pH; however, at acidic pH, it is water-soluble. Because of its unique characteristics such as low oxygen permeability, biodegradability, biocompatibility, and desirable thermo-mechanical properties, chitin-derived polymers are safe and effective to use in food packaging, biomedicine, agriculture, and cosmetics (Dekker *et al.* 2019; Muneer *et al.* 2021c).

6.2.2 Extraction of chitin and chitosan production:

Extraction of chitin from crustacean waste is the most critical step to obtaining a high-quality polymer. The extraction method used for chitin determines its purity rates. Some methods yield more purified chitin when compared with others. Two types of methods (chemical and biological) are used for the extraction of chitin.

Chemical extraction of chitin is completed in three steps: deproteination, demineralization, and decoloration. For depolymerizing the biopolymer, it is important to break the bond present between the polymer and the protein. Sodium hydroxide, potassium hydroxide, sodium carbonate, and bicarbonate are the reagents used for deproteination. The demineralizing (removal of minerals such as calcium carbonate) step is carried out by using sulfuric acid, hydrochloric acid, or formic acid. Hydrochloric acid is the most commonly used reagent for this purpose. The decoloration step is an auxiliary step that is required to obtain a colorless product (Manni *et al.* 2010; Sami 2010). Although chemical methods have many drawbacks (they are not environmentally friendly) and affect the properties of chitin, they are still preferred over biological methods because of their short processing time. The chemical extraction method has some drawbacks as its acidic nature can destroy the polymer and its properties; therefore, a more sustainable and eco-friendly method, the biological method, is used to overcome the limitations of chemical extraction. In the biological method, enzymes or microbes are utilized for the deproteination and demineralization of animal shells for the extraction of chitin. Deproteination of chitin followed by proteolytic enzymes yields high-molecular-weight chitin (Sami 2010). Moreover, lactic-acid-producing bacteria can be used for chitin extraction: minerals such as calcium carbonate are converted by

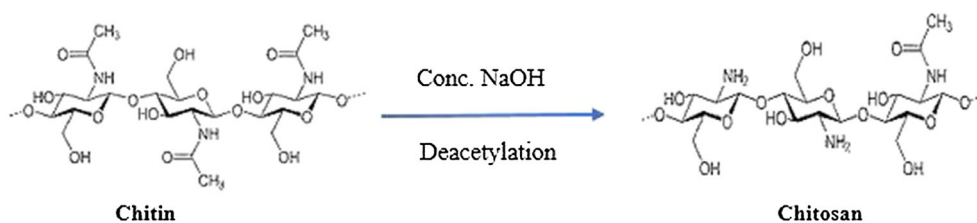


Figure 9. Deacetylation of chitin into chitosan.

the action of lactic acid to calcium lactate and are removed in the form of precipitates (Sini *et al.* 2007). The chemical and biological methods with their advantages and limitations are summarized in table 4. Chitin extracted either by a chemical or biological method is converted into chitosan by using alkali or acid treatment. At varying temperatures, NaOH is used followed by acid treatment to clear out impurities, resulting in the formation of chitosan. The acetyl group is removed and N-acetyl-D-glucosamine units are converted into D-glucosamine units with free [-NH₂]-groups (Yadav *et al.* 2019). A summary of the extraction process is shown in figure 10.

6.2.3 Film formation and characterization: Similar to keratin-based films, dry and wet methods are used to obtain chitosan-based films. The melt processing method including extrusion and kneading under thermo-mechanical treatment is usually preferred for the production of large-scale polymer films. Acceptance of this method remains challenging for processing polysaccharide-based materials due to the low thermal stability of chitosan. However, the solvent-casting method (wet route) is currently considered the only process with the ability to produce chitosan films (Dean *et al.* 2013). Chitosan-based films are characterized by their thermo-mechanical properties (Maria *et al.* 2016; El-Aidie 2018). Chitosan has unique properties but its film-forming capabilities are compromised by its poor water vapor barrier characteristics. The physiochemical properties of chitosan-based films have been improved by forming blends with other polymers or plasticizers (de Moraes Lima *et al.* 2017). The brittleness of chitosan films is usually overcome by forming blends with plasticizers such as glycerol and polyethylene glycol (PEG). Glycerol improves the mechanical properties of films by reducing the intermolecular forces among the keratin molecules present in the film. Glycerol at high concentration decreases the tensile strength and increases the flexibility/elongation of the film (Butler *et al.* 1996). Moreover, the tensile strength and Young's modulus of the chitosan-based

film increases with the addition of starch at high concentration, and the presence of a glycosidic bond between the starch units make the film completely biodegradable (Hasan *et al.* 2018). Chitosan itself has great antimicrobial properties, and yet it has been reported that blending chitosan with essential oils such as ginger, clove, and eucalyptus augments the antimicrobial property of the film and can be used for packaging purposes (Kumar *et al.* 2020). However, chitosan blends with polyvinyl alcohol (PVA) show an increase in film solubility which is 33.2% enriched with 10% ethyl lauroyl arginate (LAE), thus acting as an antimicrobial agent (Haghighi *et al.* 2020). The presence of the free amine group in the chitosan structure makes it suitable for chemical modification in order to enhance its physico-chemical and biological properties. Due to this, efforts have been made to form chitosan blends with natural extracts or plasticizers to make chitosan-based films acceptable for commercial applications. The mechanical properties of chitosan film blended with different additives as listed in table 5.

7. Applications

The interest in developing bio-based materials obtained from renewable resources has increased due to the environmental impact caused by non-degradable plastic materials. Keratin and chitosan are biopolymers that are obtained from chicken feathers and crustacean shells (Vásconez *et al.* 2009). Because of their biological origin, non-toxicity, and biodegradability, they are considered safe and their use as a biomaterial has no negative impact on the environment. They are versatile biopolymers, with film-forming abilities that can be modified and used in the form of sponges, gels, films, beads, and nano/micro-particles (Khosha and Amanullah 2013). Keratin and chitosan can be blended with other polymers to modify their chemical and physical properties and broaden the scope of their applications. The versatility of applications varies from the food industry, agriculture, biomedicine, tissue

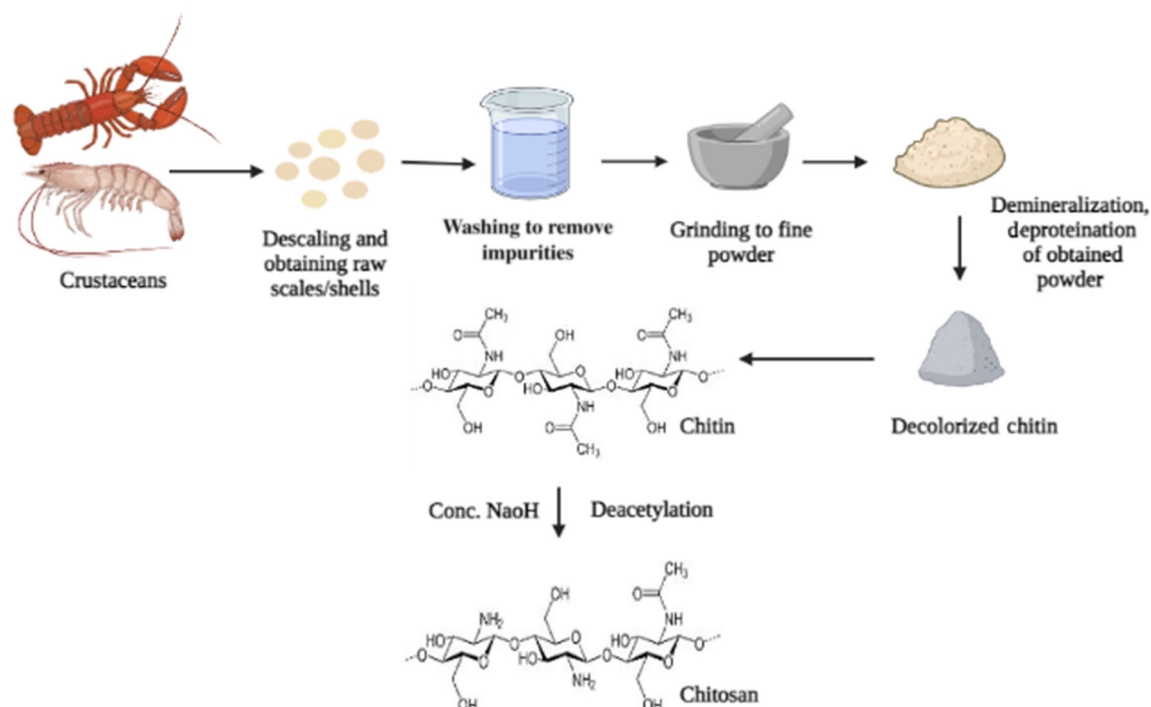


Figure 10. Extraction of chitin from crustacean shells and chitosan production by acetylation process.

engineering, and cosmetology to wastewater treatment, as shown in figure 11 (Morin-Crini *et al.* 2019; Khumalo *et al.* 2019).

7.1 Biomedical applications

Keratin has the potential to be used as wound dressing material due to its bio-compatibility and capability of supporting cellular attachment (Dias *et al.* 2010). The ideal wound dressing material is bio-compatible, cost-

effective, prevents microbial infections, and maintains a humid environment (Hoeksema *et al.* 2013). Keratin blends with sodium alginate and chitosan are used as a dressing material for wound treatment. This fabricated material has non-toxic behavior, the ability to support cell growth, and can quickly heal excision wounds (Shanmugasundaram *et al.* 2018). Similarly, the anti-fungal and antimicrobial properties of chitosan make it suitable to be used in wound treatment. Chitosan-based biomaterials can help in wound healing and support dermal regeneration when used as nano-fibers and bio-

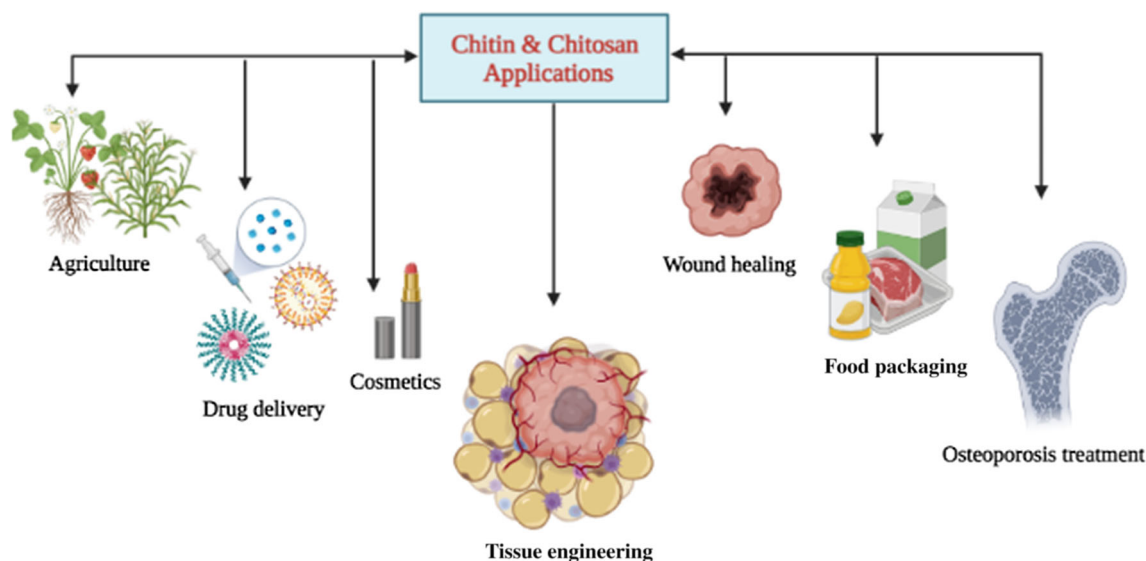
Table 4. Comparison between biological and chemical methods used for chitin extraction

Extraction method	Treatment	Advantages	Limitations	Reference
Chemical method	Demineralization with decalcifying agents such as acetic acid, sulfuric acid, or hydrochloric acid Deproteinization by alkaline treatment using NaOH	Used at commercial scales for chitin preparation The simple and short duration of the process	Environment unfriendly, expensive, effect chemical properties of chitin and removal of minerals and proteins	Pal <i>et al.</i> 2014 Pachapur <i>et al.</i> 2016
Biological method	Lactic acid treatment: Demineralization by using <i>Lactobacillus</i> sp. Treatment with proteases: Deproteinization with proteases	Eco-friendly, economical, non-toxic Soluble proteins might be used afterward as nutrients	Can only be performed in the laboratory and risk of contamination	

Table 5. Mechanical properties of chitosan blends with other polymers

Polymer blends	Film thickness (mm)	Tensile strength (MPa)	Young's modulus (MPa)	Elongation at break (%)	Reference
Chitosan/glycerol	250	23.9	N.R.	37.67	Leceta <i>et al.</i> 2013
Chitosan/starch	N.R.	6.78	6.093	13.451	Hasan <i>et al.</i> 2018
Chitosan/xanthan	0.091	14.07	N.R.	9.03	de Morais Lima <i>et al.</i> 2017
Chitosan/PVA	44.8	42.5	1570.1	54.2	Haghighi <i>et al.</i> 2020
Chitosan/cellulose	0.32	52.34	0.468	60	Bhuvaneshwari <i>et al.</i> 2011
Chitosan/PLA	N.R.	43.38	983 ± 55	6.63	Torres-Hernández <i>et al.</i> 2018
Chitosan/ginger oil	71	18	186	35	Souza <i>et al.</i> 2017

N.R., not reported.

**Figure 11.** Uses of keratin and chitosan in various aspects of daily life.

sponges. Some examples of chitosan-based biomaterials that help in wound healing include commercially available products such as HemCon® Bandage and Reaxon® (Medovent, Germany). HemCon® is a chitosan acetate-based bandage designed for hemostatic dressing (Ahmed *et al.* 2018). Reaxon® is a flexible, bio-based nerve guide that helps minimize the drawbacks and limitations of auto-grafts.

7.1.1 Tissue engineering: Tissue engineering is a very advanced and rapidly growing technique. Damaged body parts can be repaired and even replaced by transplanting appropriate cells and biomolecules for tissue regeneration (Ahmed *et al.* 2018). To overcome the drawbacks of synthetic polymers, there has been great interest in using biodegradable porous scaffolds to regenerate tissues. Keratin has been shown in

different studies to accelerate mammalian cell growth by supporting fibroblast and keratinocyte cell growth. Scaffolds fabricated with keratin and agar are used in tissue-engineering applications and have shown good bio-compatibility rates against myofibroblast cell lines present in mammals, which promote wound healing and support the re-epithelialization of the human skin. Their hydrophilic nature, good degradability, and mechanical properties make these porous scaffolds competent candidates for biomaterial applications (Nayak and Gupta 2015).

Chitosan has intrinsic antimicrobial, bio-compatible, and biodegradable properties, and is therefore considered a versatile bioactive polymer to be used in tissue engineering (Qi *et al.* 2010). Methods such as phase separation and 3D printing are used to fabricate chitosan scaffolds for cell proliferation during tissue

regeneration. The instability and low mechanical strength of chitosan limit its applications. Chitosan scaffolds have to be combined with other natural and synthetic polymers such as vinyl alcohol, poly-acrylic acid, and poly-ethylene glycol to enhance their mechanical and biological properties (Pandey *et al.* 2017).

7.1.2 Drug delivery agents: Keratin, due to its bio-compatible nature, is modified as a drug delivery carrier. Incorporating nanotechnology in biomaterials is the best option for increasing the efficiency of a particular drug (Khumalo *et al.* 2019). Keratin-based nanoparticles, having the potential to target tumor sites, have been proven effective against cancer treatment (Vasconcelos and Cavaco-Paulo 2013). They also exhibit antibacterial or antioxidant activity. Keratin-based micro-needles are used to deliver medicinal drugs, serums, and proteins such as albumin in a more efficient way to desired locations in the body, which make their use potentially safe during treatments (Vasconcelos and Cavaco-Paulo 2013).

Chitosan is a non-toxic, bio-compatible, and biodegradable polymer. It has gained much attention in diverse pharmaceutical and biomedical applications. Chitosan-based drug delivery systems can release drugs at a controlled rate at the specific target site (Shariatnia 2019). Chitosan can be used in various ways as a drug delivery agent; for example, it can be employed as hydrogels, microspheres, and nanoparticles. In oral medication, it can be administered as an adjuvant in tablet formulations. High-molecular-weight chitosan delays the release of active ingredients while enhancing the side effects of high-potency medicinal compounds administered as oral tablets. Chitosan conjugated with anti-tumor agents exhibits better antitumor effects and reduced side effects compared with the original drugs (Cheung *et al.* 2015). Chitosan nanoparticles are also used for the encapsulation of levofloxacin antibacterial agents to treat ocular infections (Shariatnia 2019).

7.2 Food packaging

Keratin-based food packaging materials are an environmentally friendly alternative to synthetic plastics. Food packaging materials must have good mechanical and barrier properties, water resistance, and low processing cost (Hurley *et al.* 2013). It is necessary to modify the physicochemical properties of keratin by blending it with different polymers or

plasticizers to make it suitable for packaging applications. For instance, keratin films blended with citric acid show good mechanical and antibacterial properties with high flexibility, which can improve the shelf life of food (Ramirez *et al.* 2017). Keratin/aldehyde starch blends are also used as stable packaging films in the food industry. Currently, keratin has no commercial applications but it might have the potential to be used as active packaging in the food industry (Hefft 2017).

The US Food and Drug Administration (FDA) has approved chitosan as a food additive, dietary supplement, and functional ingredient. Its bioactive nature and cationic character make it suitable to be used as a nutritional ingredient, antimicrobial agent, and nutraceutical. It can be used as a food packaging or coating biomaterial in the form of fibers, films, gels, beads, or nanoparticles (Gutiérrez *et al.* 2018). Chitosan films and blends as packaging materials show good mechanical properties and reduce the use of harmful pesticides in food protection due to their antimicrobial properties. Chitosan-based functional foods or dietary fibers are now commercially available. ChitoseenTM-F, ChitoClear®, and MicroChitosan are some of the commercially available cholesterol-lowering agents (Morin-Crini *et al.* 2019).

7.3 Cosmetics

Keratin hydrolysate is most commonly used in today's skin- and haircare products. It is found in shampoos, conditioners, lotions, and nutritive serums for hair, as well as mascaras, nail polish, and eye makeup agents (Mokrejš *et al.* 2017). Its compatibility with a hydrophilic environment makes it useful as a cosmetic product. Keratin-based shampoos protect hair from damage, provide strength, and rebuild the damaged hair. Keratin in skincare products enhances the moisture content of the skin because it can penetrate through a layer of the epidermis and gives a soft and healthy appearance to the skin.

CurasanTM, HydamerTM, ZenvivoTM, and chitosan MM222 are some of the chitosan-based products that are commercially available for cosmetic use. Chitosan's cationic character, antibacterial property, and moisture-retaining feature make it suitable for application in cosmetics. It has found applications in shampoos, dyes, hair color, hairspray, beauty creams, and cosmetics such as nail paint and lipsticks. It is well tolerated by the skin and is a safe ingredient for consumer use (Morin-Crini *et al.* 2019).

7.4 Agriculture sector

Chicken feathers, being a rich source of keratin, have the potential to be converted into nitrogen-rich organic fertilizer (Sinkiewicz *et al.* 2017). Feather waste, upon microbial transformation and suitable conditions, provides important bio-products that enhance soil fertility and overall plant health. Bio-hydrolysates of keratin can be used for the preparation of fertilizers and soil amelioration and as a protein source in animal feedstock (Kucinska *et al.* 2014). Treatment of feathers with *Chryseobacterium* spp. enhances the utilization of waste feathers from poultry as bio-fertilizer by increasing the release of nitrogen. Treating waste feathers with the Thermophilic actinomycetes strain results in bio-fertilizers used for the cultivation of ryegrass (Sharma and Gupta 2016). Keratin microbial hydrolysate is considered a long-lasting plant growth-promoting fertilizer that maintains the quality of the soil.

Chitosan-based products can be used as effective bio-controlling agents for controlling the release of agrochemicals. These products and their blends have proved effective as insecticides and seed coating (Pandey *et al.* 2018). They also inhibit the effect of viruses and viroids in plants. Plant seeds can be soaked in certain chitosan-based blends to protect them from microbes as these products have shown antimicrobial activities. Chitosan has shown active behavior as a bio-pesticide, forming blends with starch, gums, and alginate to enhance its property for delayed release of pesticides (Morin-Crini *et al.* 2019).

7.5 Wastewater treatment

Pollutants and contaminants in wastewater can be removed by functionally modified keratin that can act as a bio-sorbent. These modifications are usually done in the tertiary structure, which results in the formation of a more porous network and increases the chemical reactivity of the macromolecule (Saha *et al.* 2019a). Keratin is considered an effective material to remove heavy metals like cadmium (Cd), nickel (Ni), chromium (Cr), zinc (Zn), and arsenic (As) from wastewater. Cr uptake of 44.2–81.4% was observed using keratin cross-linked with epichlorohydrin. However, keratin modification with methyl alcohol (CH₃OH) showed the highest arsenic uptake in the range of 85–90% (Saha *et al.* 2019b). Hydroxyl and amino functional groups impart various absorbent characteristics to chitosan for the removal of pollutants and contaminants from wastewater. Nanoparticle-coated chitosan membranes act as a filter for improving water quality. Chitosan

nanostructures have shown efficacy for the removal of a large number of heavy metals from the water including lead, chromium, and arsenic. Nano-composites of chitosan and activated carbon have been shown to absorb as much as 52.63 mg/g of cadmium. Zinc oxide nanoparticles and chitosan blends remove almost 99% of colored pollutants from water (Yadav *et al.* 2019).

8. Conclusion

The uncontrolled and rapid use of synthetic plastics has had a severe impact on the environment and health. Therefore, researchers are left with no choice but to replace fossil-based plastics with biopolymers. Keratin and chitosan are versatile biopolymers that are abundantly available as by-products of poultry and marine industries. Due to their biodegradability, bio-compatibility, and natural abundance, they have a key role in solving environmental pollution. These biopolymers have the potential to play an important role in agriculture, biomedicine, tissue engineering, food industry, cosmetics, wastewater treatment, and pharmaceuticals. It is of great importance to recover important proteins, polysaccharides, and by-products from marine and poultry wastes, as these are crucial for maintaining environmental balance and financial gains. Extraction of biopolymers from waste biomass is a challenging task and is done by biological and chemical methods. These polymers undergo further modification by forming blends with other polymers or additives in order to enhance their chemical and biological properties, so that they can be used for various applications. The most important concern while researching such polymers is lower fabrication cost and higher efficiency. However, these polymers have a promising future when challenges regarding the control of mechanical and physical properties are overcome. Modifications to enhance physicochemical properties and polymer behavior with improved thermo-mechanical characteristics are currently under investigation. The biotechnological applications of these bio-based materials have shown promising results in anti-carcinogenic agents, drug delivery agents, biocatalysis, cosmetics, textiles, bio-imaging, and bioplastic production.

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