Animal waste biomass processing using ionic liquids

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8.1 Introduction

The processing of materials, fuels, and food contributes to 50% of global greenhouse gas emissions and 90% of biodiversity loss, leading to the pollution of air, land, and water ("United Nations Environment Programme. Global Resources Outlook," 2019). With the global population continuing to rise, the demand for fundamental resources such as food, medicines, energy, and everyday consumer products is expected to grow considerably. This will generate a large volume of by-products and waste across various industrial sectors (Ajila et al., 2012), highlighting the urgent need to develop alternative and sustainable solutions for their proper disposal.

Livestock farming, for instance, generates a vast amount of agricultural waste, including feed residues, fat, blood, wool, and feathers (Phiri, Mavinkere Rangappa and Siengchin, 2024). Depending on their risk level and according to the European Union's regulatory framework (Regulation EC No. 1069/2009), animal waste can be categorized as follows ("1069/2009 of the European Parliament and of the Council of October 21, 2009 Laying down Health Rules as Regards Animal By-Products and Derived Products Not Intended for Human Consumption and Repealing Regulation (EC) No," 1774): *Category 1*—high-risk materials, requiring incineration or landfilling; *Category 2*—medium-risk wastes, amenable to be used as fertilizers; and *Category 3*—low-risk wastes, which have been used as feed for food-producing animals (Kee et al., 2023; Gutschmann et al., 2023). While European legislation allows for the disposal of animal by-products in properly regulated sanitary landfills, illegal practices such as open dumping, i.e., the uncontrolled discharge of waste directly into the environment, pose serious risks to the environment (Open dumping, 2025). In fact, landfilling and illegal open dumping currently account for approximately 60%–80% of common waste management practices (Ajila et al., 2012; Shekdar, 2009). However, both approaches share a major drawback: they are unsustainable due to uncontrolled greenhouse emissions and high management costs. In contrast, when treated appropriately, waste can be used to recover high-value compounds and simultaneously decrease their accumulation (Klintenberg et al., 2014).

Various biological, chemical, and mechanical approaches have been explored for waste valorization, including anaerobic digestion, fermentation (Bote, Naik and

Ionic Liquids in Biomass Processing. https://doi.org/10.1016/B978-0-443-33447-4.00001-4

Jagdeeshgouda, 2020), and solvent extraction (Acosta and De Vrieze, 2018). Nevertheless, many conventional methods are still challenged by low efficiencies, low selectivities, high cost, and high environmental impact (Phiri, Mavinkere Rangappa and Siengchin, 2024). Aiming to overcome these limitations, ionic liquids (ILs) have been approached as alternative and effective solvents for waste valorization (Anastas and Eghbali, 2010). ILs are a class of organic molten salts composed of an organic cation and an organic or inorganic anion that present negligible volatility at ambient conditions, high thermal stability, and nonflammability. Due to their remarkable structural diversity achieved through the combination of different ion pairs, their properties can be adjusted to match the requirements of specific applications, being thus referred to as task-specific fluids and designer solvents. If properly designed, they may present low environmental impact and offer high efficiency in extraction and separation processes (Kudłak, Owczarek and Namieśnik, 2015). Recently, Polesca et al. (2023a) used aqueous solutions of the IL 1-butyl-3-methylimidazolium acetate ($[C_4C_1im][C_1CO_2]$) to recover keratin from chicken feathers, obtaining a yield of 90 wt%. The recovered keratin was successfully used to produce a film for wound-healing applications. On the other hand, King et al. were able to dissolve chitin from shrimp shells using the IL 1-ethyl-3-methylimidazolium acetate—$[C_2C_1im][C_1CO_2]$. The dissolved chitin was further mechanically and biologically characterized, enabling its application for drug delivery and/or skin treatment (Catherine King et al., 2017). In order to valorize waste beef tallow from the tannery industry, Ranjitha et al. (Srinivasan et al., 2024) used an amino acid-based IL (L-valine amido ethyl methyl imidazolium bromide) [L-ValC2C1im]Br as a catalyst agent to enhance the transformation of waste beef tallow into lipids for biodiesel production. Collectively, these studies are representative of the potential of ILs to recover proteins, polysaccharides, and lipids from distinct animal waste sources and their valorization into biomaterials, which will be further discussed below.

Overall, this chapter presents the current state of the art in animal waste biomass valorization practices using ILs, focusing on assessing the recovery of proteins, polysaccharides, and lipids and their subsequent application in distinct scientific fields. First, a description of the current landscape of waste management methodologies is presented. This is followed by a discussion of relevant studies on the use of ILs for animal waste biomass valorization and their conversion into value-added materials and respective applications. Finally, the current challenges and opportunities in the field are addressed and complemented by an overview of the contribution of IL-based waste valorization approaches for advancing the United Nations Sustainable Development Goals (UN SDGs).

8.2 Current landscape of animal waste biomass production patterns

According to the UN, an increase in the population has been observed in the last decades that will continue to grow in the future, resulting in the intensification of

industrial processes and an increase in waste production (*United Nations. United Nations Department of Economic and Social Affairs Population Division*, no date). It is estimated that each individual produces an average of 0.74 kg of waste per day, leading to an estimated global municipal solid waste of 2.13 billion tons in 2020, with projections to rise to 3.78 billion tons by 2050 (Lenkiewicz et al., 2024).

Since the beginning of civilization, humans have been creating new ways to deal with different categories of waste. Landfilling is the longest-established method of waste disposal (Barati, Zafar and Wang, 2022). Nevertheless, this method is associated with significant drawbacks, including high maintenance costs and the production of greenhouse gases (e.g., methane and carbon dioxide), posing risks to soil, air, and water (Kircher et al., 2023). Recognizing these challenges, the European Union has implemented legislative and financial measures to promote waste valorization, which is in line with the first Principle of Green Chemistry (Anastas and Eghbali, 2010). However, despite these efforts, the EU's progress in reducing waste over the last decade has been modest, due to the continued reliance on a linear economic system (Fig. 8.1). To address this limitation, the concept of circular economy was introduced (Boulding, 2013), envisioning an economic model in which the waste generated during production and consumption is recycled to develop innovative products and processes (Velenturf and Purnell, 2021). In that regard, the European Commission proposed in 2015 the "Closing the Loop—New Circular Economy package," mapping out a series of actions for the circular economy, as well as four legislative proposals on waste to be met by 2030, containing targets for landfill, reuse, and recycling. According to the EU, moving toward a circular economy could deliver diverse environmental and economic benefits to different stakeholders, including industries and consumers (Leising, Quist and Bocken, 2018; Cobo, Dominguez-Ramos and Irabien, 2018).

The livestock and meat industries are among the largest contributors to environmental pollution, generating approximately 150 million tons of waste per year (Broman et al., 2017; Zhan, 2022). Materials such as skin, blood, bones, fatty tissues, hooves, shells, feathers, wool, and internal organs can be used to recover high-value products, such as keratin, chitin, and collagen (Putra et al., 2024;

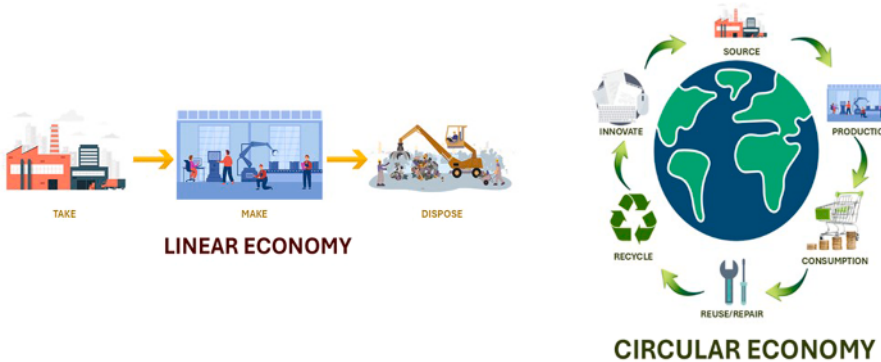


Figure 8.1 Schematic diagrams of a linear and a circular economy model.

Jayathilakan et al., 2012; Dhakal, Shrestha and Anal, 2017), with applications in pharmaceutics, cosmetics, biomedicine, textiles, agriculture, food industry, and bioplastics (Beaney, Lizardi-Mendoza and Healy, 2005).

8.3 Conventional methods for the valorization of animal wastes

Popular techniques to valorize animal wastes include mechanical (grinding, mixing, pressing, decanting, and separating), thermal (boiling, evaporating, and drying), chemical (solvent extraction), and biological processes (composting, anaerobic digestion, fermentation, and insect farming) (Iskakov and Sugirbay, 2023; Franke-Whittle and Insam, 2013).

The dissolution/extraction step plays a crucial role in these processes, which rely on traditional and emerging methods. Alkaline/acid hydrolysis and oxidation/reduction are representative of conventional methods, while more advanced techniques such as superheated water, steam explosion, microwave-assisted extraction, ultrasound-assisted extraction, and enzyme-assisted extraction are also documented (Ventura et al., 2017). Conventional methods are simple but require long times, extreme pH, and high temperatures (Franca-Oliveira, Fornari and Hernández-Ledesma, 2021), generally resulting in high-cost processes with low extraction yields. Although higher extraction yields are generally obtained with advanced techniques, these methods require specialized equipment or enzymes, driving up the associated costs (Zhang, Lin and Ye, 2018). To mitigate these issues, researchers have been searching for alternative approaches for biomass valorization, among which is the use of neoteric solvents such as ILs or deep eutectic solvents. Such an approach offers the additional advantage of promoting the selective extraction of valuable compounds under milder conditions without depending on harsh chemicals (Huang et al., 2023). When properly designed, low-cost and biocompatible ILs with high dissolution capability of a wide range of biomass matrices can be developed, offering as well the possibility of being recovered and reused in successive processing cycles (Mahmood et al., 2025).

8.4 Emerging role of ionic liquids in biomass valorization: basic concepts and features

The fifth Principle of Green Chemistry calls for the use of safer solvents and auxiliaries. In this line, ILs have shown promising potential toward the development of more sustainable processes and functional materials (Anastas and Warner, 2023; Nasirpour, Mohammadpourfard and Zeinali Heris, 2020). ILs are organic salts composed of a large and asymmetric organic cation paired with an organic or inorganic anion. They exhibit low volatility under ambient conditions, high thermal

stability, and are nonflammable. The wide range of possible ion combinations confers a remarkable degree of structural diversity, enabling their properties to be tailored to the requirements of specific applications (Anastas and Warner, 2023; Nasirpour, Mohammadpourfard and Zeinali Heris, 2020). Indeed, when properly designed, ILs with enhanced ability to dissolve a specific biomolecule may be prepared, overcoming the low dissolution ability of common organic solvents (Ventura et al., 2017). Between 2010 and 2024 (Fig. 8.2), more than 110,000 publications and over 7,500 patents have been registered, overall demonstrating the high interest in using ILs in distinct scientific fields, from synthesis, to dissolution/extraction, distillation, and catalytic processes.

Representative chemical structures of cations and anions commonly used as constituents of ILs are presented in Fig. 8.3.

Despite ILs have been receiving increasing attention in the last years for biologically related applications, several factors must be considered, such as the synthesis process, their potential recovery and reuse, and their toxicity and biodegradability profiles (Badgujar and Bhanage, 2015; Dennewald, Pitner and Weuster-Botz, 2011). Considering the questionable toxicity and high cost of traditional ILs, such as those based on the imidazolium cation, researchers started to study the synthesis of bio-based ILs (derived from renewable sources). The synthetic route is designed to align with green chemistry principles, emphasizing safer solvents and reduction in nonessential derivatization steps (Anastas and Eghbali, 2010). Following that, the recovery and reuse of ILs are critical for their industrial application, which should maximize the

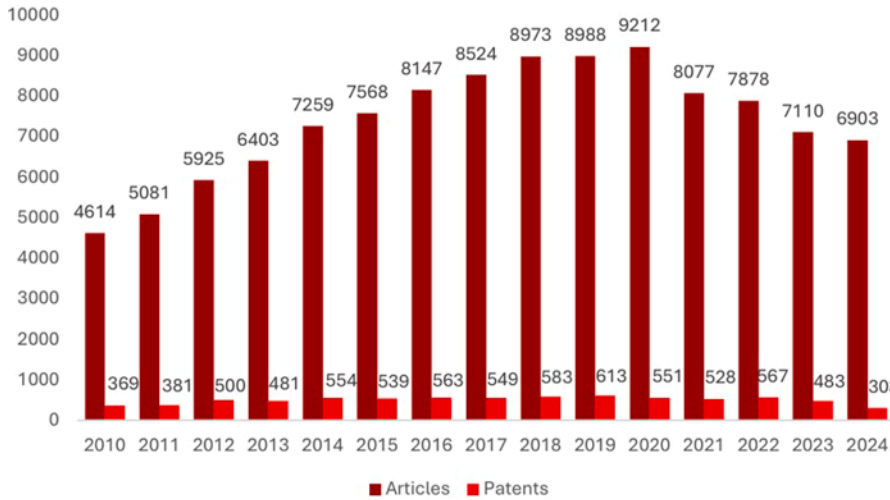


Figure 8.2 Number of scientific publications and patents registered between 2010 and 2024 involving the use of ILs in diverse scientific disciplines. Data retrieved from the European Patent Office (keywords: "Ionic liquids" or "ionic liquid") and Web of Science (keywords: "Ionic liquids" or "ionic liquid") (*European Patent Office*, no date) (Web of Science—Research Database, no date). This figure presents an overview of the number of scientific publications and patents related to the use of ILs across various scientific disciplines.

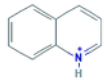
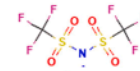
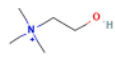
Cation	Chemical structure	Anion	Chemical structure
Imidazolium		Chloride	
Pyridinium		Tetrachloroaluminate	
Quinolinium		Hexafluorophosphate	
Ammonium		Tetrafluoroborate	
Morpholinium		Bis-(trifluoromethylsulfonyl)imide	
Phosphonium		Dichloroacetic acid	
Pyrrolidinium		Acetate	
Guanidinium		Methanesulfonate	
Choline		Trifluoroacetate	

Figure 8.3 Name and chemical structures of cations and anions usually applied as constituents of ILs (retrieved from PubChem) (Kim et al., 2025).

IL recovery yield with effective removal of impurities while maintaining the stability of the IL over multiple cycles of use. To achieve IL recovery, distillation, vacuum evaporation, column distillation, and molecular distillation are commonly used. Nevertheless, the cost of these processes represents a challenge for industrial scale (Mai, Ahn and Koo, 2014; Zhou et al., 2018). For instance, the techno-economic assessment of the valorization of chicken feathers for keratin recovery using cholinium acetate ($[N_{111(2OH)}][C_1CO_2]$) (80 wt% in water) by Polesca et al. (2023b) revealed that the IL recovery step using multiple-effect evaporators represents the main contribution to the overall cost of the process, underscoring the need to develop new methods for IL recovery and reuse. To achieve this, some researchers have investigated the application of supercritical CO_2 (sc-CO_2) or membrane-based separation methods (Alvarez-Guerra et al., 2014; Isci and Kaltschmitt, 2022). Another critical factor to consider when using ILs in large-scale applications is their biodegradability, as improper degradation of ILs can lead to environmental pollution. For example, cholinium-amino acid-based ILs (e.g., acid 1-ethyl-3-methylimidazolium glycinate $[C_2C_1im][Gly]$) exhibit high biodegradability due to their naturally occurring anions

or cations (Kirchhecker and Esposito, 2016). Similarly, fatty acid-derived ILs (e.g., 1-butyl-3-methylimidazolium oleate [C_4C_1im][Oleate]) leverage renewable, organic anions that serve as carbon sources for microbial communities, thus facilitating degradation (Vavina et al., 2024). Overall, the dissolution of animal biomass using ILs involving the recovery of keratin (e.g., from wool, feathers, and hooves) (Banasaz and Ferraro, 2024), chitin (e.g., from crustacean shells and squid pens) (Kumari et al., 2023), collagen (e.g., from skin, bones, and tendons) (Karami and et al., 2019), and lipid-enriched components (e.g., fats and skin trimmings) (Işler et al., 2010) and their subsequent valorization will be overviewed in the next section.

8.5 Valorization of animal biomass waste into protein-, polysaccharide-, and lipid-enriched fractions using ionic liquids

8.5.1 Keratin

With the increase in urbanization and protein-meal consumption, millions of tons of keratin-rich animal waste biomass are thrown away into the environment every year (Gerber, Opio and Steinfeld, 2008). The main sources of keratin in animal biomass include feathers, wool, hair, beak, nail, and hoof (Fig. 8.4). Around the world, over 2.5 million tons of wool and more than 65 million tons of feathers are produced each year (Tanabe et al., 2002). This biomass is currently treated as solid waste by incineration or landfilling, wasting resources and causing environmental harm. In fact, the resulting contamination of lands and water bodies will lead to uncontrolled eutrophication processes and soil acidification due to nitrogen deposition (Lu et al., 2014). To counteract these issues, some methods currently investigated for the

Figure 8.4 Sources of keratin from animal biomass. Representation of different sources of animal biomass from which keratin is extracted.

valorization of this waste, which is highly enriched in keratin, include applications in textiles and decorative products, their processing as feed materials for livestock or fertilizers, or the development of new materials for biomedical or water treatment applications (Tesfaye, Sithole and Ramjugernath, 2017).

Keratin is a fibrous protein, representing the third most abundant biopolymer in nature after chitin and cellulose, and the main structural protein found in epithelial cells (Coulombe and Omary, 2002). On a dry weight basis, feathers, hair, and wool are highly enriched in keratin (approximately 90% for feathers, 80% for hair, and 95% for wool) (Robbins, 2012), being also composed of lipids, fibers, and ash (Cardamone et al., 2009; Kajiura et al., 2006). According to its hierarchical structure, keratin can be organized into two different conformations: α-helix, which is connected by intramolecular hydrogen bonds, and β-sheets linked by interchain hydrogen bonds between amino and carbonyl groups. Usually, the α-keratin form is found in mammals and is the primary constituent of wool, hair, nails, hooves, and the outermost layer of skin (Cardamone et al., 2009). On the other hand, the β-sheet form is more common in hard avian and reptilian tissues, such as feathers, claws, and beaks of birds, as well as scales and claws of reptiles (Saravanan and Dhurai, 2012).

Depending on the amount of sulfur cross-links, keratins can be classified as soft and hard keratins. Due to the inter- and intramolecular disulfide bonds, keratin is poorly soluble in common organic solvents, and thus the recovery yield using these solvents is generally very low (Donato and Mija, 2020; Guiza et al., 2021; Nuutinen et al., 2019; Zhang et al., 2017). To efficiently recover and process keratin, the disulfide and hydrogen bonds must be destroyed, although the primary structure of the protein should be maintained for further applications. The most common methods to this end and their respective advantages and limitations are overviewed in Table 8.1 (Rajabinejad et al., 2018). In summary, most of these methods present limitations, including the use of hazardous chemicals, long processing times, and high costs. Additionally, these methods may promote protein degradation, which will impair the subsequent downstream applications of keratin (Robbins, 2012; Sturm et al., 2014; Zhang, Zhao and Yang, 2015; Ji et al., 2014).

The potential of ILs as designer solvents, achieved by combining different cations and anions that allow different intermolecular interactions, is one of the main advantages in the dissolution and processing of biomass, allowing them to overcome the low dissolution ability displayed by traditional solvents (Padinhattath, Shaibuna and Gardas, 2025). ILs also offer a significant advantage over traditional chemicals, as protein recovery after dissolution with ILs requires only the addition of an anti-solvent/coagulant to precipitate the protein (Mahmood et al., 2025).

Many ILs have been investigated for keratin recovery, frequently consisting of cations such as tetraalkylammonium, imidazolium, and pyrrolidinium, combined with chloride, bromide, and acetate anions. Since 2005, when Xie, Li and Zhang (2005) first demonstrated the potential of ILs to dissolve wool keratin, the interest in this area has steadily increased. Table 8.2 summarizes key studies on the use of ILs for animal waste biomass valorization and keratin recovery, including the main processing conditions and keratin recovery yields.

Table 8.1 Methods commonly used for keratin recovery from animal waste biomass and respective strengths and limitations.

Methods	Strengths	Limitations	References
Reduction	Effective in breaking the cysteine disulfide bonds	Makes use of hazardous and flammable solvents	(Robbins, 2012; Ji et al., 2014)
Oxidation	Simple process	Long dissolution time and low protein recovery yield	
Alkaline/acid hydrolysis	Simple process	Long dissolution time, makes use of hazardous and flammable solvents, and low protein recovery	
Microwave irradiation	Short dissolution time	Potential protein degradation and high industrial application costs	(Sturm et al., 2014; Zhang, Zhao and Yang, 2015)
Supercritical water	Short dissolution time	Potential protein degradation and high industrial application costs	
Steam explosion	Short dissolution time	Potential protein degradation and low protein recovery	
Enzymatic	Moderate temperature	High industrial application costs and long processing times	(Rahayu, Syah and Thenawidjaja Suhartono, 2012; Shavandi et al., 2017; Fang et al., 2013)

Table 8.2 Reported studies using ILs for keratin recovery from distinct animal waste biomass sources, the optimum operational parameters, and the obtained recovery yields (NA = no information reported).

Source	IL abbreviation	IL name	Loading	Dissolution conditions (temperature, time)	Dissolution yield	Recovery conditions	Recovery yield (%)	References
Wool	$[C_4C_1im]PF_{6-}$	1-butyl-3-methyli-midazolium hexafluorop-hosphate	NA	130°C, 24 hours	No dissolution	Methanol	NA	Xie et al. (2005)
	$[C_4C_1im]BF_{4-}$	1-butyl-3-methylimidazolium tetrafluoroborate	NA					
	$[C_4C_1im]Br$	1-butyl-3-mehtylimidazolium bromide	2 wt%	130°C, 10 hours	Complete dissolution		NA	
	[aC1im]Cl	1-allyl-3-methyli-midazolium chloride	8 wt%	130°C, 10 hours			NA	
	$[C_4C_1im]Cl$	1-butyl-3-methyli-midazolium acetate	11 wt%	100°C, 10 hours 130°C, 10 hours				
			10 wt%	120°C, 30 minutes		Ethanol	78.54	Zhang et al. (2017)
				$[C_2C_1im][C_1CO_2]$			1-ethyl-3-methyli-midazolium acetate	15.77
				$[C_2C_1im]Cl$			1-ethyl-3-methyli-midazolium chloride	73.86
				$[C_2C_1im]DEP$			1-ethyl-3-methyli-midazolium diethylphosphate	70.20
				[aC1im]Cl			1-allyl-3-methyli-midazolium chloride	73.25
				$[C_4C_1im][C_1CO_2]$			1-butyl-3-methyli-midazolium acetate	
				120 °C, 24 hours		NA	NA	Liu et al. (2018)
	$[P_{4444}]Cl$	Tetrabutylphosphonium chloride	8 wt%	130 °C, 24 hours	No dissolution	NA	NA	Zheng et al. (2015)
	$[N_{4444}]Cl$	Tetrabutylphosphonium chloride						
	[BPy]Cl	1-butylpyridinium chloride						
	$[N_{2221}][DMP]$	Triethylmethylammonium dimethylphosphate		130 °C, 3 hours	Complete dissolution	Ethanol	71.13	

Duck feathers	[aC1im]Cl	1-allyl-3-methyli-midazolium chloride	5 wt%	120 °C, 1 hour	96.05 wt% Complete dissolution	Water	NA 75.1	Ji et al. (2014)
	[C_4C_1im]Cl	1-butyl-3-methyli-midazolium acetate			96.13 wt%	NA	NA	
	[C_4C_1im]Br	1-butyl-3-methyli-midazolium bromide			84.18 wt%			
	[C_6C_1im]CF_3SO_3	1-hexyl-3-methyli-midazolium trifluoromethanesulfonate			5.2 wt%			
	[SC_4C_1im]HSO_4	1-sulfobutyl-3-methyli-midazolium hydrogen sulfate			82.57 wt%			
Chicken feathers	[C_4C_1im] [C_1CO_2]	1-butyl-3-methyli-midazolium acetate	5 wt%	100 °C, 4 hours	Complete dissolution	Ethanol (solution: coagulant ratio 1:2 w/w), 5 °C, 1 hour	90.17	Polesca et al. (2023a)
	[$N_{111(2OH)}$] [C_1CO_2]	Cholinium acetate				20.25 wt% ethanol (solution: coagulant ratio 1: 1.45 w/w), 5 hours	93	Polesca et al. (2023b)
Turkey feathers	[DMEA] [HCOO]	N,N-dimethylethanolammonium formate	2 wt%	100 °C, 7 hours	Complete dissolution	Ethanol	63	Idris et al. (2014)
	[C_4C_1im]Cl	1-butyl-3-methyli-midazolium acetate	5 wt%	130 °C, 10 hours		Water	59	Idris et al. (2013)
	[aC_1im]Cl	1-allyl-3-methyli-midazolium chloride					57	
	$C_5H_{11}NO_2S$	Cholinium thioglycolate	45 wt%				51	
	$C_{18}H_{39}NO_2S$	Bis-(2-ethylhexyl)ammonium thioglycolate			No dissolution	NA	NA	
Bovine hoof	[C_2C_1im] [C_1CO_2]	1-ethyl-3-methyli-midazolium acetate	NA	80 °C, 11 days	Complete dissolution	Ethanol	12.70	Apostolidou (2020)

Considering the designer solvent character of ILs and the vast number of possible combinations of cations and anions, Liu et al. (2018) evaluated 621 ILs for dissolving three keratin models using the conductor-like screening model for realistic solvents (COSMO-RS) method and validated their findings experimentally. Among the investigated ILs and based on the logarithmic activity coefficient (ln γ), the software predicted that the IL 1-ethyl-3-methylimidazolium acetate ($[C_2C_1im][C_1CO_2]$) would be the most efficient to dissolve wool keratin. This finding was validated experimentally, being $[C_2C_1im][C_1CO_2]$ one of the most effective ILs for protein dissolution from wool at 120°C. Moreover, it was hypothesized that the enhanced dissolution ability of $[C_2C_1im][C_1CO_2]$ was due to the strong hydrogen-bond acceptor ability of the acetate anion. In fact, as more keratin–keratin hydrogen bonds are disrupted by the acetate anion, the protein structure starts to loosen and dissolves into the IL medium (Liu et al., 2018). This observation aligns with Zhang et al. (2017) who investigated the regeneration of wool keratin by immersing goat wool fibers in ILs with the same cation (1-ethyl-3-methylimidazolium) and different anions. Maintaining the same cation, ILs composed of the acetate anion ($[C_1CO_2]^-$) exhibited superior keratin dissolution capability compared to Cl^- and Br^- due to their stronger hydrogen-bonding acceptor properties (Zhang et al., 2017). Using the same source of keratin, Zheng et al. (2015) investigated the ability of tetrabutylphosphonium chloride $[P_{4444}]Cl$, tetrabutylphosphonium chloride ($[N_{4444}]Cl$), 1-butylpyridinium chloride ([BPy]Cl), and triethylmethylammonium dimethylphosphate ($[N_{2221}][DMP]$) ILs to dissolve 8 wt% wool keratin at 130 °C. The authors found that no dissolution occurred using [BPy]Cl $[P_{4444}]Cl$, and $[N_{4444}]Cl$ after 24 hours. However, the IL $[N_{2221}][DMP]$ was able to dissolve wool keratin in 3 hours. After dissolving the wool keratin, ethanol was used as a coagulant agent to precipitate the keratin from the solution, leading to a yield of 71.13% (Zheng et al., 2015).

Ji et al. (2014) observed that imidazolium-based ILs such as 1-allyl-3-methylimidazolium chloride ($[aC_1im]Cl$) and 1-butyl-3-methylimidazolium chloride ($[C_4C_1im]Cl$) achieved the highest duck feather dissolution rates (96.05% and 96.13%, respectively) at 120 °C for 1 hour (1:20 feather-to-IL ratio), outperforming the ILs 1-hexyl-3-methylimidazolium trifluoromethanesulfonate ($[C_6C_1im]CF_3SO_3$) and 1-sulfobutyl-3-methylimidazolium hydrogen sulfate ($[SC_4C_1im]HSO_4$). Post dissolution, a yield of 75.1% of keratin was obtained through precipitation with water. The IL ($[C_4C_1im]Cl$) was recovered and reused three times with high efficiency, achieving IL recovery rates from 95.5% to 96.9% and stable keratin yields, ranging from 74.8% to 75.1% (Ji et al., 2014).

More recently, Polesca et al. (2023a) studied seven ILs for chicken feather dissolution: $[C_2C_1im]Cl$ $[C_2C_1im][C_1CO_2]$ $[C_4C_1im]Cl$ $[C_4C_1im][C_1CO_2]$ $[C_4C_1im]Br$, 1-butyl-3-methylimidazolium thiocyanate ($[C_4C_1im]SCN$), and 1-butyl-1-methylpyrrolidinium ($[C_4C_1pyrr]Cl$). These assays were carried out at 100 °C for 4 hours in a solid:liquid ratio (chicken feathers:solvent) of 1:20 w/w. Firstly, the authors identified which pure IL could have a better dissolution efficiency under the same experimental conditions. According to the initial results of the IL screening, acetate-based ILs enabled total chicken feather dissolution (Polesca et al. (2023a)), since the acetate anion is a strong hydrogen-bond acceptor, interacting with the

hydrogen atoms in the amino and hydroxyl groups of the protein (Castner et al., 2011; Zhang et al., 2012; X. Liu et al., 2015). Previously, a study conducted by Passos et al. (2018) applied COSMO-RS to determine the hydrogen-bond basicity of a set of ILs by estimating their hydrogen-bond interaction energies (EHB), and found that ILs with more negative EHB exhibit higher hydrogen-bond basicity, with $[C_2C_1im][C_1CO_2]$ ($EHB = -20.21$ kJ mol^{-1}) and $[C_4C_1im][C_1CO_2]$ ($EHB = -19.83$ kJ mol^{-1}) showing the most negative values among the ILs studied. This high basicity enables the acetate anion to effectively disrupt keratin's structure across keratin sources and thus explains the findings from Polesca et al. (2023a). Following the first screening, Polesca et al. (2023a)) investigated the use of aqueous solutions of ILs (80 wt% and 60 wt% in water). They observed that the addition of 20 wt% water decreases the viscosity, increasing the mass transfer of the process, leading to the total dissolution of chicken feathers. Nevertheless, when water concentration increases from 20 wt % to 40 wt% (Polesca et al., 2023a), the solution cannot completely dissolve keratin since the water starts to compete with the hydrogen bonds responsible for feather dissolution (Zhang, Feng and Yang, 2021). Optimal dissolution conditions were achieved by resorting to 80 wt% $[C_4C_1im][C_1CO_2]$ for 4 hours at 100 °C. Then, different coagulants were tested to recover keratin: water, water/ethanol mixtures, ethanol, and acetone. Ethanol was found to be the most efficient coagulant for keratin recovery (90% recovery yield) at a solution:coagulant mass ratio of 1:2 w/w and for 1 hour at 5 °C. Polesca et al. (2023a)) also evaluated the recovery and reuse of ILs, demonstrating that the solvent can be efficiently reused for multiple cycles with minimal loss in its dissolution capacity, thereby improving the sustainability and cost-effectiveness of the process.

Idris et al. (2014) used the distillable protic IL N,N-dimethylethanolammonium formate ([DMEA][HCOO]) to dissolve turkey feathers at 100 °C for 7 hours. By adding excess methanol, keratin was recovered, affording a yield of as much as 63% based on the initial mass. In another work by the same research group, two imidazolium-based ILs ($[C_4C_1im]Cl$ and $[aC_1im]Cl$) and two thiol-based ILs, cholinium thioglycolate ($[C_5H_{11}NO_2S]$) and bis-(2-ethylhexyl)ammonium thioglycolate ($C_{18}H_{39}NO_2S$) were evaluated with the same goal in mind (Idris et al., 2013). The authors examined the amount of turkey feathers that each IL could dissolve at 130 °C for 10 hours. The imidazolium-based ILs successfully dissolved up to 50 wt% of the feathers, while $[C_5H_{11}NO_2S]$ dissolved 45 wt%, and $[C_{18}H_{39}NO_2S]$ failed to achieve dissolution within the proposed time. Following the dissolution process, room temperature water was added to precipitate the keratin extracted using the ILs $[C_4C_1im]Cl$ $[aC_1im]Cl$, and $[C_5H_{11}NO_2S]$. The precipitated keratin was then dried under vacuum at 60 °C for 3 days, resulting in regeneration yields of 59%, 57%, and 51%, respectively (Idris et al., 2013).

Considering the high cost and environmental concerns of most of the investigated ILs, Polesca et al. (2023b) developed a more sustainable process for keratin recovery from chicken feathers. Using an aqueous solution of 80 wt% cholinium acetate ($[N_{111(2OH)}][C_1CO_2]$), an IL with low toxicity and high biocompatibility, chicken feathers were dissolved at 100 °C, 650 rpm, and 4 hours. Response surface methodology (RSM) was applied to optimize keratin precipitation, revealing that the reduction of

ethanol content in the coagulant solution with an increase in water proportion enhanced the keratin precipitation yield. This is due to the preferential establishment of hydrogen bonding between water and cholinium acetate, which facilitates keratin precipitation. Furthermore, considering the scale-up of the process, a techno-economic analysis was performed, demonstrating that the minimum selling price of keratin is $22 per kg, making it viable for high-value applications such as in the biomedical field and personal care. According to Polesca et al. (2023b), to improve the economic feasibility of keratin extraction, further research into sustainable and energy-efficient IL recovery strategies is crucial. To achieve this goal, alternative IL regeneration approaches, such as water evaporation (Earle and Seddon, 2000; Zavgorodnya et al., 2017), carbene distillation (Earle and Seddon, 2000; Zavgorodnya et al., 2017), membrane-based separation techniques (e.g., reverse osmosis and nanofiltration) (Mai, Ahn and Koo, 2014; Zhou et al., 2018), and aqueous biphasic systems (Freire, 2016) could be tested to increase the competitiveness of the developed processes.

Keratin recovered fractions from waste can be used to prepare several types of biomaterials by taking into account keratin's properties, biocompatibility, and the capacity to promote cellular proliferation (Maclaren, 1987). Accordingly, keratin has been used to develop various biomaterials with diverse geometries (e.g., films, hydrogels, nanoparticles, and fibers) with applications ranging from tissue engineering, wound healing, drug delivery, cosmetics, and water treatment, respectively, sketched and summarized in Fig. 8.5 and Table 8.3.

Aiming to illustrate the potential of keratin-based materials in the biomedical field, Polesca et al. (2023a) prepared a keratin film intended for wound-healing applications using a keratin solution (15 wt%) extracted from chicken feathers while resorting to the solvent casting method. To assess the biocompatibility and wound-healing

Figure 8.5 Applications of keratin-based biomaterials and representative geometries of the resulting biomaterials.

Table 8.3 Applications of keratin-based biomaterials using IL-assisted extracted keratin from animal waste biomass.

Keratin source	Optimum extraction conditions				Application	Highlights	References
	IL	IL concentration	Temperature (°C)	Time (hours)			
Chicken feathers	$[C_4C_1im][C_1CO_2]$	80 wt%	100	4	Keratin-based films for biomedical applications	Antioxidant activity and anti-inflammatory effects (ROS inhibition); Slight activation of innate immune response (CD83 and CD54); Enhances wound healing via keratinocyte and fibroblast proliferation; Bioactivity associated with RGD and LDV motifs	Polesca et al. (2023a))
	$[C_4C_1im]Cl$	100 wt%	100	48	Keratin-based adsorbent for the removal of Cr(IV) ions in water	Increased adsorption efficiency with removal efficiencies ranging from 63.5% to 87.7%; High sorption capacity as indicated by the Freundlich constant; Electrostatic interactions between cationic amino groups and anionic Cr(VI) enhance ion binding and facilitate efficient removal from aqueous solutions.	Sun, Liu and Liu (2009)
				-	Keratin-based adsorbent for the removal of metal ions from wastewater and oil sand process-affected water	Efficient removal of V(V) and Ni^{2+} from wastewater; Enhanced adsorption in acidic conditions; Effective adsorption in complex matrices (oil-sand process-affected water)	Zahara et al. (2023)
	$[C_2C_1im][C_1CO_2]$	100 wt%	130	2.5	Sustainable keratin–cellulose filament for textile application (alternative to cotton fiber)	Tensile strength, Young's modulus, elongation, and stiffness significantly improved with 10% keratin loading in the filament, indicating that with 10% keratin loading. Filament thickness and titer decrease, potentially enabling lighter fibers	Kammiovirta et al. (2016)
	$[C_2C_1im][C_1CO_2]$	80 wt%	100	4	Keratin-based film for wastewater treatment	Keratin-based composite films were prepared with cellulose or chitin at different ratios; the keratin-chitin blend provided the highest tensile strength among all the formulations Pure keratin-based films performed better at adsorption of RB4 compared to the keratin–cellulose and keratin–chitin composite films.	Polesca et al. (2024)

potential, biological tests were performed, including cell viability assays (using monocytes, macrophages, keratinocytes, and fibroblast cells), the evaluation of its anti-inflammatory and antioxidant effects, and in vitro wound-healing experiments using keratinocytes and fibroblasts. A hydrophilic and homogeneous film was successfully prepared, presenting no toxicity for the cells under study. Additionally, the keratin film presented good antioxidant and anti-inflammatory effects, improving the proliferation of the cells and accelerating wound healing after 16 hours, in comparison with the control group at the same time point (Polesca et al., 2023a).

Some researchers have also explored the capability of keratin-based materials for the removal of pollutants from the environment. For instance, Sun, Liu and Liu (2009) evaluated the capacity of keratin extracted from chicken feathers to efficiently remove chromium (VI) (Cr(VI)) anions from wastewater. The recovered keratin exhibited an excellent adsorbent capacity for removing the Cr(VI) ion in water under low concentrations from 10 to 80 ppm. Due to its hydrophilic character, it enables the formation of cations, thus promoting binding to the negatively charged Cr(VI) ions. Additionally, Zahara et al. (2023) used keratin-based biopolymers derived from chicken feathers to extract metal ions from water due to the active sites of the keratin (e.g., containing functional groups such as –SH, –COOH, –OH, and − NH2). By measuring the residual metal ion concentrations in multi-metal synthetic wastewater, the authors noted that the keratin-based material exhibited a strong affinity for oxyanions, which was enhanced in an acidic environment. To further validate their findings, the authors applied this material to oil-sand process-affected water, which contains a complex matrix of inorganic and organic compounds. Again, the KBM-IL (keratin-based material–ionic liquid blend) exhibited a good performance to remove the oxyanions (vanadium (V), chromium (VI), and arsenic (III)). These results highlight the potential of IL-modified keratin-based materials for wastewater treatment (Zahara et al., 2023).

To improve its properties, keratin can also be combined with other types of polymers to produce composite materials such as magnetic hydrogels and fibers. For instance, Kammiovirta et al. (2016) dissolved keratin and cellulose in $[C_2C_1im][C_1CO_2]$ to prepare cellulose filaments with and without keratin, using the method of wet-jet spinning. After drying, mechanical testing revealed that the small addition of keratin recovered from chicken feathers significantly improved the strength of the material when compared to cellulose filaments, underscoring the effectiveness of the composite material (Kammiovirta et al., 2016).

In the same context, Polesca et al. (2024) recovered keratin from chicken feathers and processed films, either pure or blended with cellulose and α-chitin, for dye removal. Through the optimization of the blend ratios and pH and by adding glycerol as a plasticizer, films with hydrophilic behavior, stable up to 160 °C, and with promising potential in the removal of the toxic dye reactive blue 4 (RB4) were obtained (Polesca et al., 2024).

Overall, valorization approaches of keratin-rich animal waste not only represent an effective strategy to manage waste but also offer an opportunity to develop efficient and sustainable biomaterials with value-added for several applications.

8.5.2 Collagen

Collagen is the most abundant structural protein found exclusively in animals, especially in the flesh and biological tissues of mammals, including mammalian skin, bones, cartilage, fish skin/scales, and even chicken feet (Chai et al., 2010). It is a structural, rigid, and inextensible fibrous protein primarily found in tendons, cartilage, bones, skin, and blood vessels (Wenger et al., 2007). Although mainly used to give strength to structures in the body, 28 different forms of collagen are already known and described, exerting different functions such as protecting delicate organs and contributing to the proper alignment of cells (D. Liu et al., 2015). Collagen consists of three distinct α-chains, which are coiled around each other to form a triple-helix structure (Chai et al., 2010). The main collagens include type I, II, III, V, and XI collagens. Type I collagen is the most abundant and forms more than 90% of the organic mass of bone, providing stiffness. From a biological point of view, inter- and intramolecular covalent links are essential to provide strength to collagen fibers, presenting significant challenges when it comes to dissolving and recovering collagen (Bielajew, Hu and Athanasiou, 2020).

Collagen can be extracted from bones and skin of bovine and porcine animals, although the latter represents a safer source since the type of collagen is similar to that from humans, not causing any allergic response (Cortial et al., 2006; Cao et al., 2016). However, due to certain religious concerns, collagen from porcine sources is not widely used. Currently, the safer sources of collagen are from marine origin, which include marine vertebrates and invertebrates such as cuttlefish, jellyfish, sea anemone, octopus, starfish, and prawn (Krishnan and Perumal, 2013; Sugiura et al., 2009). Fish collagen differs from that of mammalian sources, particularly in the amino acid composition, with a notably lower hydroxyproline content that can influence its thermal stability and fibril assembly (Safandowska and Pietrucha, 2013). Nevertheless, marine sources often have high collagen content, no risk of disease transmission like bovine collagen sources, and no religious and ethical conflicts like the ones found with porcine sources (Kıyak, 2024).

The methodology of extracting collagen (Fig. 8.6) is contingent on the collagen source, and the process usually begins with a pretreatment step. Extraction methods are generally categorized as chemical, enzymatic, and assisted by ultrasound. Depending on the extraction method, collagen is classified into acid-soluble collagen (ACS) (Duan et al., 2009), salt-soluble collagen (SSC) (Liang et al., 2014), ultrasound-assisted collagen (UAC) (Kaewbangkerd, Hamzeh and Yongsawatdigul, 2023), and pepsin-soluble collagen (PSC) (Jeevithan et al., 2014). Nevertheless, these traditional chemical methods share the same drawbacks when applied to both keratin and collagen, requiring long processing times and the use of hazardous and flammable solvents and potentially leading to the degradation of the target protein. Although enzymatic treatments have the advantage of using milder conditions, they can cause permanent alterations to the collagen structure through enzymatic breakdown and present a high cost for industrial applications (Oliveira et al., 2021). Aiming to overcome these challenges, the use of ILs as alternative solvents has been investigated for collagen recovery, as analyzed in the next subsection.

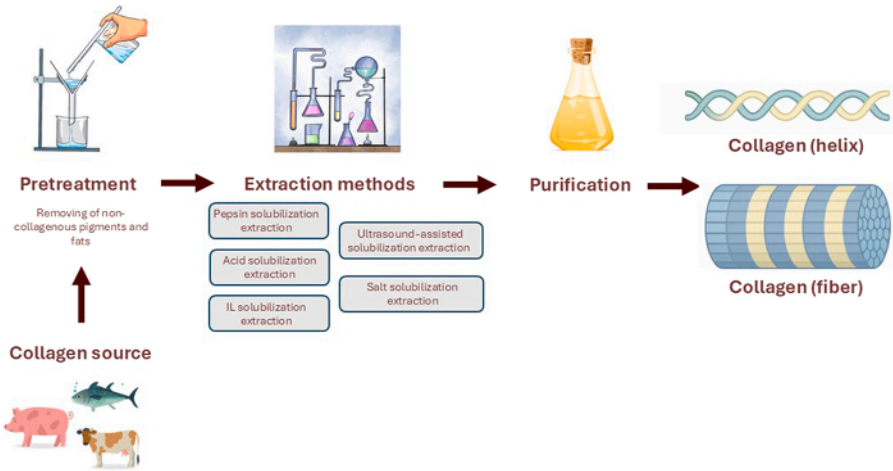


Figure 8.6 Schematic representation of collagen extraction methodologies. This figure aims to depict the conventional strategies used for collagen extraction.

Collagen has the repetitive sequence Glycine-(Gly)-X-Y, where X and Y represent amino acids other than glycine, while proline and 4-hydroxyproline are commonly found in the X and Y positions, respectively (Li and Wu, 2018). The collagen structure is stabilized by the presence of strong inter- and intramolecular hydrogen bonds, ionic bonds, van der Waals forces, and hydrophobic bonds between polar and nonpolar groups. Within the collagen recovery process using ILs and upon heating, IL cations and anions may interact with the ester groups in the collagen structure, thus starting the dissolution process of the protein source (Meng et al., 2012). Several studies have been performed to address the potential of ILs for collagen recovery, as presented in Table 8.4.

Luo et al. (2024) introduced the first application of the IL–aqueous biphasic system (IL-ABS) for collagen extraction from bovine hides, using tetra-*n*-butylammonium hydroxide (TBAH) and potassium tartrate as phase-forming agents. Dissolution occurred at room temperature with a 40% TBAH aqueous solution. Additionally, the authors used potassium tartrate ($K_2C_4H_4O_6$) (around 32.25 wt%) as a chelating agent. The rationale behind this choice was based on previous evidence regarding its unique potential to cleave the covalent bonds between collagen and trivalent chromium (Cr(III)) (Malek, Hachemi and Didier, 2009). In optimized conditions, the separation of chromium and collagen was achieved by a solution consisting of $K_2C_4H_4O_6$, ultrapure water, and TBAH. After 4 hours at room temperature, the system undergoes phase separation, where the collagen is isolated from the upper phase, and the lower phase consists of chromium impurities. After adding ethanol for collagen precipitation, TBAH was regenerated and recovered through an alkali alcoholate-based (AAB) process, enabling its reuse in subsequent cycles (Luo et al., 2024).

Table 8.4 Reported studies using ILs for collagen recovery from distinct animal waste biomass sources, the optimum operational parameters, and the obtained recovery yields (NA = no information reported).

Source	Abbreviation	IL name	Loading	Dissolution conditions (temperature, time)	Dissolution yield	Recovery conditions	Recovery yield (%)	References
Native skin collagen	$[C_4C_1im]Cl$	1-butyl-3-methylimidazolium chloride	6 wt%	100 °C, 2 hours	NA	IL solution casted with deionized water at 4°C, renewed every 0.5 hours for 3 times	NA	Meng et al. (2012)
Bovine hides	$[C_4C_1im]Cl$	1-butyl-3-methylimidazolium chloride	5 wt%	120 °C, 30 minutes	Complete dissolution	Ethanol (solution: coagulant ratio of 1:1)	16.71	Luo et al. (2024)
	$[C_4C_1im]Br$	1-butyl-3-methylimidazolium bromide					22.43	
	TBAH	Tetra-*n*-butylammonium hydroxide	10.25 wt%	25 °C, 30 minutes			51.22	
	$[C_2C_1im][C_1CO_2]$	1-ethyl-3-methylimidazolium acetate	5 wt%	80 °C, 30 minutes			19.90	
Carp fish scales	$[C_2C_1im][C_1CO_2]$	1-ethyl-3-methylimidazolium acetate	5 wt%	100 °C, 12 hours	Complete dissolution	Water and NaCl (ratio of IL solution to coagulant of 1:1)	3.10	Muhammad et al. (2017)
Porcine skin collagen	$[C_2C_1im][C_1CO_2]$	1-ethyl-3-methylimidazolium acetate	10.50 wt%	45 °C, 24 hours		Water (ratio of water:IL solution 0.3:1)	NA	Hu et al. (2013)
Native collagen (type I)	$[C_4C_1im]C_1CO_2]$	1-butyl-3-methylimidazolium acetate	8 wt%	25 °C, 24 hours		IL solution casted in PTFE plate soaked in deionized water		Liu et al. (2014)

To illustrate the designer capacity of ILs and understand the effect of the cations and anions as constituents of ILs for collagen recovery, Muhammad et al. (2017) investigated the interaction of 80 imidazolium-based ILs with collagen polymers using COSMO-RS. According to the authors' results, $[C_2C_1im][C_1CO_2]$ is a promising solvent for the dissolution and recovery of collagen from fish scales. The collagen sigma-profile (σ-profile) revealed two peaks, both in the polar region, indicating its capacity to establish hydrogen bonding with ILs. Similarly, the σ-profiles of 1-ethyl-3-methylimidazolium acetate showed complementary features—the acetate anions exhibited a peak in the polar region due to its oxygen atom and a nonpolar region peak from its methyl group, enabling strong interactions with collagen's hydrogen-bond donors (Muhammad et al., 2017). Hu et al. (2013) explored the dissolution of lyophilized porcine skin collagen using the same IL ($[C_2C_1im][C_1CO_2]$) but with a sodium solvent system. In addition to the IL, supplementation with sodium-based additives (sodium sulfate—Na_2SO_4, sodium chloride—NaCl, trisodium phosphate—Na_3PO_4, sodium hydrogen phosphate—$NaHPO_4$, and sodium bisulfate—$NaHSO_4$) was carried out to modulate the solution behavior. Among these, Na_2HPO_4 and $NaHSO_4$ significantly enhanced collagen solubility by shifting the pH away from collagen's isoelectric point, inducing electrostatic repulsion between fibrils and promoting swelling. A solubility of 10.5% was achieved at 45 °C during 24 hours in the IL-Na_2HPO_4 system, with water being introduced at a 0.3:1 (v/v) water:IL ratio to precipitate collagen, demonstrating the potential of pH-tunable IL-salt systems for biopolymer processing (Hu et al., 2013).

Using a similar IL, Liu et al. (2014) investigated the potential of $[C_4C_1im][C_1CO_2]$ at room temperature as a solvent for type I collagen dissolution during 24 hours. To monitor dissolution efficacy, the researchers used polarizing optical microscopy (POM) observations, which visually confirmed the progressive breakdown of collagen fibrils. This approach enabled them to achieve a collagen solubility of 8 wt% (Liu et al., 2014). In summary, despite the numerous anions available to design ILs, the results presented to date highlight the potential of acetate-based ILs for collagen recovery, with emphasis on $[C_4C_1im][C_1CO_2]$ and $[C_2C_1im][C_1CO_2]$, due to their high dissolution ability. Nevertheless, to the best of our knowledge, the use of an IL with high biocompatibility and low cost (e.g., cholinium acetate) has not been investigated yet for collagen recovery.

Collagen presents high potential for biomedical applications, specifically for tissue engineering, as drug delivery systems, and as healthcare devices, as illustrated in Fig. 8.7 (Sorushanova et al., 2019; Picker et al., 2022). Type I collagen has been mainly used to produce surgical sutures and hemostatic sponges (Alcaide-Ruggiero et al., 2021), while type III collagen has been used for wound dressing applications (Table 8.5) (Kuivaniemi and Tromp, 2019). Due to their emulsion stabilization properties, collagen-based biomaterials can be additionally used in the cosmetic industry. They are also applied in papermaking, where they improve tensile strength, and in the cleaning industry, due to their amphiphilic properties (Sionkowska et al., 2020; Wang, Yu and Wang, 2012). Furthermore, this biopolymer can be combined with other biomaterials, such as hydroxyapatite (HAp), to enhance functionality by mimicking the natural composition of bone (Ielo et al., 2022). HAp can form composites with

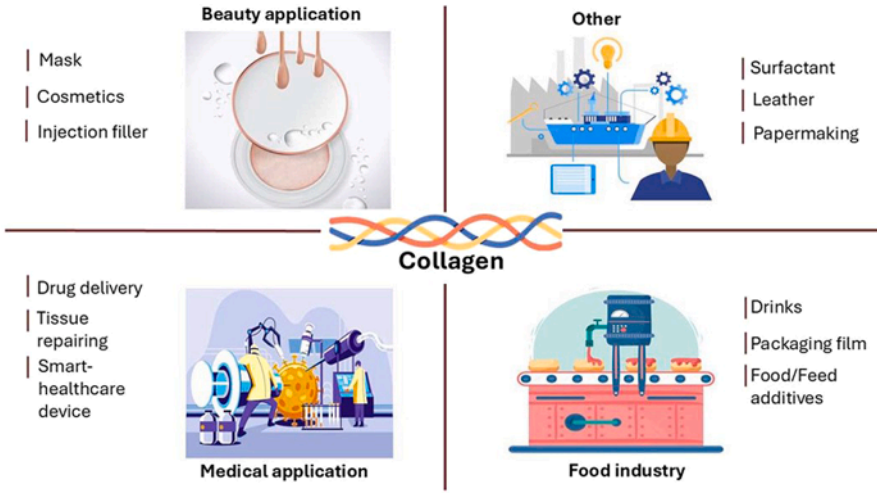


Figure 8.7 Applications of collagen-based biomaterials. This figure intends to represent the application of collagen-based biomaterials across distinct fields.

collagen that are valuable in orthopedic and dental applications such as implants and tissue engineering scaffolds (Sadat-Shojai et al., 2013).

To illustrate the potential of collagen for biomedical applications, Zhai et al. (2018) investigated the effect of ILs on the fibrillogenesis and gelation properties of collagen extracted from grass carp (*Ctenopharyngodon idellus*) skin using 0.5 M acetic acid. The authors revealed that the ILs $[C_2C_1im]Br$ $[C_2C_1im]Cl$, and $[C_2C_1im][C_1CO_2]$ significantly slowed fibrillogenesis kinetics, which can lead to a controlled assembly resulting in thicker fibrils. Moreover, acetate-based ILs were the most effective in delaying assembly, leading to a denser packing and enhanced thermal stability. The thermal stability of collagen also increased from 38.86 °C to 50.09 °C, suggesting that the introduction of ILs can improve the properties of collagen-based biomaterials.

Li and Fan (2019) produced for the first time collagen-based hydrogels in the presence of an IL $[C_2C_1im][C_1CO_2]$ and a microbial transglutaminase (MTGase), an enzyme that catalyzes covalent cross-linking between primary amines and proteins. The researchers used human-like collagen (HLC) for the composite hydrogels, combining it with the IL and the MTGase enzyme. While the MTGase-HLC system alone failed to form a stable hydrogel, the addition of the IL in the system enabled gelation at physiological temperature (37°C). Infrared (IR) spectra confirmed that the IL promoted covalent cross-linking between the collagen molecules. The network chains in the IL-MTGase-HLC system become curled instead of stretched, as seen in the MTGase-HCL, which gives both flexible and tough properties to the hydrogel. Additionally, the IL-containing hydrogels were capable of increasing the anti-inflammatory levels during longer periods than without the IL, demonstrating the benefits of this IL for tissue engineering applications.

Table 8.5 Applications of collagen-based biomaterials using IL-assisted extracted collagen from animal waste biomass.

Collagen source	Optimum processing conditions		Application	Highlights	References
	IL	IL concentration (%)			
Grass carp skin	$[C_2C_1im][C_1CO_2]$	100	Collagen/IL-based hydrogels	Collagen triple helix is preserved in all tested ionic liquids; ILs induced thicker and thermally stable collagen fibrils, particularly with $[C_2C_1im][C_1CO_2]$	Zhai et al. (2018)
Human-like collagen from *E. coli*				$[C_2C_1im][C_1CO_2]$-containing gels show higher toughness, compressibility, slower degradation by collagenase, less inflammation, and reduced immune response.	Li and Fan (2019)

In summary, although ILs have demonstrated significant efficiency for collagen recovery and application, the number of studies with collagen-based biomaterials is still low, emphasizing the need for more research on this topic.

8.5.3 Chitin

After cellulose, chitin is the second most prevalent polysaccharide in the world (Mohan et al., 2022). Although structurally similar to cellulose, chitin's C-2 carbon atom carries an acetamide group instead of a hydroxyl, as observed in cellulose. Chitin is a linear biopolymer composed of β-(1,4)-linked 2-acetamido-2-deoxy-β-D-glucose monomer units, also known as N-acetylglucosamine, and can occur in three forms—α, β, and γ-based on their degree of hydration, unit cell size, and chitin chains. Chitin is a biocompatible, antimicrobial, and biodegradable polymer (Wu et al., 2008) that has been investigated for the development of functional materials (Synowiecki and Al-Khateeb, 2003). Nevertheless, despite the huge annual chitin production (estimated to be around 10^{10}–10^{11} tons), this biopolymer still remains an unexplored resource (Gortari and Hours, 2013; Gopalan Nair and Dufresne, 2003).

The most prevalent type of chitin, α-chitin, is mostly present in insect cuticles, yeast and fungal cell walls, shrimp, lobster, and crab shells and tendons (Kaur and Dhillon, 2014). While γ-chitin, a rare form of chitin, is found in some insects' cocoons and mostly linked to squid pens (João, Silva and Borges, 2015). Chitin recovery from animal waste has been performed by chemical, biological, or physicochemical extraction, as summarized in Fig. 8.8. A first pretreatment step of the raw materials through rinsing them several times with boiling water is essential to eliminate all other related impurities in chitin-rich by-products. Following this first step, the biomass is subjected to demineralization, usually with HCl treatment to remove calcium carbonate ($CaCO_3$), and then encompasses a deproteinization step. The dense composite structure of chitin, along with the hydrogen-bond network, makes its recovery from biomass sources challenging, often requiring very harsh conditions for processing, like alkaline and acidic solvents with high temperatures to dissolve calcium carbonates and proteins (Yong, 2018).

Dissolution of chitin depends on three main factors: degree of acetylation (DA), molecular weight (MW), and crystallinity. Chitosan, a derivative of chitin, is obtained through the deacetylation of chitin (Sivashankari and Prabaharan, 2017). A higher DA increases hydrophobic interaction and hydrogen bonding between chains, making the dissolution of chitin harder (Radhakrishnan and Panicker, 2025). The length of the chitin chain is determined by its MW. Higher MW polymers have more extensive inter- and intramolecular hydrogen bonding, which can make dissolution difficult (Minagawa et al., 2007). Nevertheless, most of the investigated methods are time-consuming, make use of hazardous solvents (Cushing et al., 1954), and can also lead to the degradation of the polymer (Ravi Kumar, 2000). Additionally, a large volume of hazardous chemicals that are subsequently released into the environment is commonly required, underscoring the necessity to develop sustainable and more effective ways for chitin recovery (Younes et al., 2014). Looking for biological extraction, the addition of enzymes alongside a mild alkali treatment is considered

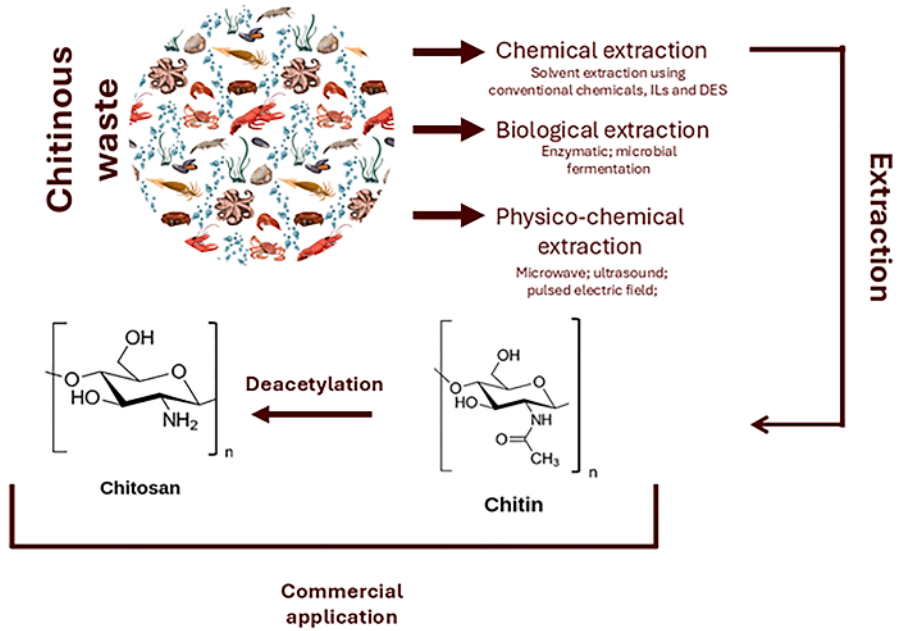


Figure 8.8 Overview of chitin recovery processes from animal waste biomass and valorization approaches. This figure represents conventional methods for chitin extraction from animal waste biomass and valorization approaches.

one of the greener extraction methods for biomass valorization (Xing et al., 2024). Rakshit et al. (2021) used shrimp waste for chitin extraction by identifying protease-producing bacterial strains, *S. marcescens*, *Bacillus pumilus*, and *Pseudomonas aeruginosa. A* high rate of deproteinization (74.76%) was obtained. However, biological extraction is limited to small-scale production (Mohan et al., 2022), since enzymatic methods require long processing times and have a high cost for industrial applications, thus demanding more efficient alternatives for chitin recovery from animal waste.

Properly designed ILs allow efficient biomass dissolution, resulting in a high chitin recovery (Feng et al., 2020; Shamshina et al., 2014, 2017; C.A. King et al., 2017). Noteworthy, Wineinger et al. (2020) highlighted that, on average, chitin extracted by ILs possesses an MW 2.5 times higher than that of commercially available practical-grade chitin (PG-chitin), being thus more suitable for materials processing. However, due to the different polymorphic forms of chitin (α-, β-, γ-) and the different sources of biomass, the same IL and operational conditions can present different extraction or dissolution quantities with different properties (e.g., different MW and DA), emphasizing the need for a detailed investigation in the field (Shamshina, 2019). The studies reporting the use of ILs for chitin recovery are summarized in Table 8.6.

Table 8.6 Reported studies using ILs for chitin recovery from distinct animal waste biomass sources, the optimum operational parameters, and the obtained recovery yields (NA = no information reported; *solubility studies using chitin commercial standards).

Source	Abbreviation	IL name	Loading	Dissolution conditions (temperature, time)	Dissolution yield	Recovery conditions	Recovery yield (%)	References
Chitin*	$[C_4C_1im]Cl$	1-butyl-3-methylimidazolium chloride	10 wt%	110 °C, 5 hours	NA	Water or methanol	NA	Xie, Zhang and Li (2006)
N-α-chitin*	$[C_4C_1im][C_1CO_2]$	1-butyl-3-methylimidazolium acetate	4 wt%	110 °C, 2 hours			6	Wu et al. (2008)
N-β-chitin-L*			1 wt%				6–7	
N-β-chitin-H*			1 wt%				3	
Chitin from shrimp shells	$[C_2C_1im]Cl$	1-ethyl-3-methylimidazolium chloride	5 wt%	105 °C, 48 hours	3 wt%	Water (IL-Solution: coagulant ratio of 1:5 w/w)	NA	Jaworska and Gorak (2016)
	$[C_2C_1im]Br$	1-ethyl-3-methylimidazolium bromide			12 wt%			
	$[C_2C_1im]I$	1-ethyl-3-methylimidazolium iodide			11 wt%			
Shrimp shells	$[C_2C_1im]C_1CO_2]$	1-ethyl-3-methylimidazolium acetate	10 wt%	100 °C, 19 hours	46 wt%	Water	94	Qin et al. (2010)
PG-chitin					15.20 wt%		87.40	
Pure chitin			25 wt%		80 wt%		40.20	
Lobster shells			2 wt%	2–3s pulses of microwave irradiation for a total of 10 minutes	NA	Water (solution: coagulant ratio of 1:2 w/w)	8.30	Achinivu, Shamshina and Rogers (2022)

Continued

Table 8.6 Reported studies using ILs for chitin recovery from distinct animal waste biomass sources, the optimum operational parameters, and the obtained recovery yields (NA = no information reported; *solubility studies using chitin commercial standards).—cont'd

Source	Abbreviation	IL name	Loading	Dissolution conditions (temperature, time)	Dissolution yield	Recovery conditions	Recovery yield (%)	References
Crab shells							14.90	
Shrimp shells				2- to 3-second pulses of microwave irradiation for a total of 5 minutes			32.60	
Fly larvae							14.00	
Crab shells	[aC_1im]Br	1-allyl-3-methylimidazolium bromide		10 wt%	100 °C, 24 hours	Aqueous citric acid solution 0.0078M (IL solution: citric acid ratio of 3:1 w/w)	7.50	Setoguchi et al. (2012)
Shrimp shells	[$N_{(i3)\ (i3)00}$] [C_1CO_2]	Diisopropylmethylammonium acetate		6.66 wt%	110 °C, 36 hours	Aqueous citric acid solution	14.80	Tolesa et al. (2019)
	[$N_{(i3)\ (i3)00}$] [C_2CO_2]	Diisopropylmethylammonium propionate			100 °C, 30 hours		13.70	
	[N_{0114}][C_1CO_2]	Dimethylbutylammonium acetate					11.50	
Squid pens	[$N_{111(2OH)}$] [C_1CO_2]	Cholinium acetate		5 wt%	100 °C, 2 hours	Ethanol	>60.00	Nakasu et al. (2024)

In 2006, Xie, Zhang and Li (2006) studied the dissolution of commercially available chitin in $[C_4C_1im]Cl$. The dissolution was conducted at 110 °C in an oil bath for 5 hours (Table 8.6). Under these conditions, 10 wt% chitin was completely dissolved, as confirmed by wide-angle X-ray diffraction (WAXD), which indicates that the crystalline domains of chitin have been completely disrupted by the IL. In a different study, Wu et al. (2008) investigated the use of the ILs $[C_4C_1im][C_1CO_2]$ $[aC_1im]Cl$, and $[C_4C_1im]Cl$ for the dissolution of fully acetylated α-chitin from crab shells (N-α-chitin) and two types of β-chitins from squid pens. Due to its basicity, acetate anions exhibit favorable interactions with the hydrogen-bond networks in chitin by deprotonating amino (-NH) and hydroxyl (-OH) groups. As a consequence, the compact crystal structure of chitin is disrupted, leading to the solubilization of the polysaccharides (Bonhôte et al., 1996; MacFarlane et al., 2006). Qin et al. (2010) also investigated the use of $[C_2C_1im]Cl$ $[C_4C_1im]Cl$, and $[C_2C_1im]C_1CO_2]$ to dissolve and extract chitin from pure chitin, PG-chitin, and raw shrimp shells. In line with previous studies, the acetate anion proved to be the most efficient for chitin recovery, while coupling a microwave irradiation step enables a more time-efficient process with a total time of 2 minutes. The solution was then introduced into a water bath to coagulate the polymer, and after the application of centrifugation/filtration, the following yields of recovered chitin were obtained from shrimp shells (94%), PG-chitin (87.4%), and pure chitin (40.2%) (Qin et al., 2010). The use of $[C_2C_1im][C_1CO_2]$ under microwave irradiation looks suitable for extracting chitin from other sources (e.g., crab shell and lobster shell) as well. For instance, Achinivu et al. (2022) used this IL in a microwave-assisted extraction of chitin from crab shells, shrimp shells, fly larvae, and lobster shells. The authors then used water to precipitate the chitin from the IL solution, yielding 8.30% chitin from lobster shell and 14.90% from crab shell.

As seen from Table 8.6 $[C_2C_1im][C_1CO_2]$ seems to be one of the preferred solvents to extract chitin from animal biomass waste (Shamshina, 2019), due to its high dissolution ability, linked to its availability, low toxicity, and partial biodegradability (Chemical Hazard Classification and Labeling: Comparison of OPP Requirements and the GHS, 2015). Considering the efficiency of acetate-based ILs and aiming to investigate another class of ILs, Tolesa et al. (2019) synthesized three protic ammonium-based ILs—dimethylbutylammonium acetate ($[N_{0114}][C_1CO_2]$), diisopropylmethylammonium acetate ($[N_{(i3)(i3)00}][C_1CO_2]$), diisopropylmethylammonium propionate ($[N_{(i3)(i3)00}][C_2CO_2]$)—to recover chitin from shrimp shells. By varying the chemical structure of IL, temperature (70–100 °C), and time (18–36 hours), chitin yields from 11.5% to 14.8% were obtained. Considering the average chitin content in biomass to be around 20%–30%, the chitin yield after 24 hours of extraction was about half of that available in the biomass. After extraction, the IL-chitin solution was centrifuged in a citric acid solution to demineralize. Subsequently, the chitin was converted to chitosan via alkaline deacetylation (40% NaOH, 1:10 w/v ratio, 100 °C, 12 hours), yielding a highly deacetylated product (93%) suitable for biomedical applications (Tolesa et al., 2019). In the search for more biocompatible and low-cost ILs, Nakasu et al. (2024) developed a sustainable biorefinery strategy for valorizing squid pens using $[N_{111(2OH)}][C_1CO_2]$. A 20% aqueous solution of

$[N_{111(2OH)}][C_1CO_2]$ allowed for an optimal selective fractionation, achieving 95% pure β-chitin and 90% pure protein streams. The optimized process involved treating powdered squid pens (5 wt% solids loading) in the IL-aqueous solution at 100 °C for 2 hours. Then, the addition of ethanol induced β-chitin precipitation via solvation competition, while the subsequent addition of water triggered protein salting-out by disrupting IL–protein interactions. Furthermore, the recovery and reuse of the IL were achieved with an average yield of 98% for five consecutive cycles (Nakasu et al., 2024). A techno-economic analysis indicated that a competitive process should imply a minimum protein selling price of $6.6/kg with co-production of β-chitin, even though solvent-intensive steps contributed to 4.27 kg CO_2/kg product emissions, emphasizing the need for process integration to enhance sustainability (Nakasu et al., 2024). In agreement with the analysis performed by Polesca et al. (2023b) for keratin extracted from chicken feathers, the results obtained by Nakasu et al. (2024) indicate that the costs associated with IL recovery are predominant, representing 64% of the total costs of the process, followed by fixed costs at 17%. As previously highlighted for IL-mediated keratin extraction processes, there is room for improvement in IL recovery steps, which will allow decreasing the costs associated with chitin recovery from biomass waste.

Deacetylated and acetylated chitin products have many applications in various sectors such as agriculture, cosmetics, and the biomedical field, as sketched in Fig. 8.9 and summarized in Table 8.7.

Catherine King et al. (2017) evaluated the topical and transdermal administration of caffeine using chitin films, thus illustrating their potential for drug delivery purposes. The authors observed that the properties of the film could be tuned by adjusting the loading of chitin, film thickness, and drying methods. With a 2.5 w/w% chitin loading and by using sc-CO_2 drying, they successfully retained caffeine in wet-chitin films. This approach showed a burst-release of caffeine (80%) in the first 20 minutes. The remaining caffeine was slowly released during 36 hours from the chitin film (Catherine King et al., 2017).

To explore the potential of creating blends of chitin with other biopolymers, Silva et al. (2012) used $[C_4C_1im][C_1CO_2]$ to prepare hydrogels of chitin and chitosan blended with silk. After individually dissolving each component at 95 °C, mixtures of chitosan/silk fibroin at the ratios 50/50 and 30/70 were then homogenized and transferred to specific polystyrene molds. To eliminate the IL from the chitosan-silk/IL solution, Soxhlet extraction with ethanol was applied. The authors observed that the blend of chitosan-silk fibroin hydrogels provided a good environment for human dermal fibroblast attachment and growth, thus favoring their application as wound dressings. Shamshina et al. (2014) explored the properties of biomaterials made from chitin extracted with ILs from shrimp shells. After redissolving the chitin in $[C_2C_1im][C_1CO_2]$, the authors developed microfibers by dry-wet jet spinning. The authors observed that the produced fibers had high mechanical strength and antibacterial properties that could suppress bacterial growth (Shamshina et al., 2014; Barber et al., 2014). These properties enable its application in drug delivery and for targeted skin treatments at the site of inflammation, thus reducing side effects associated with systemic delivery.

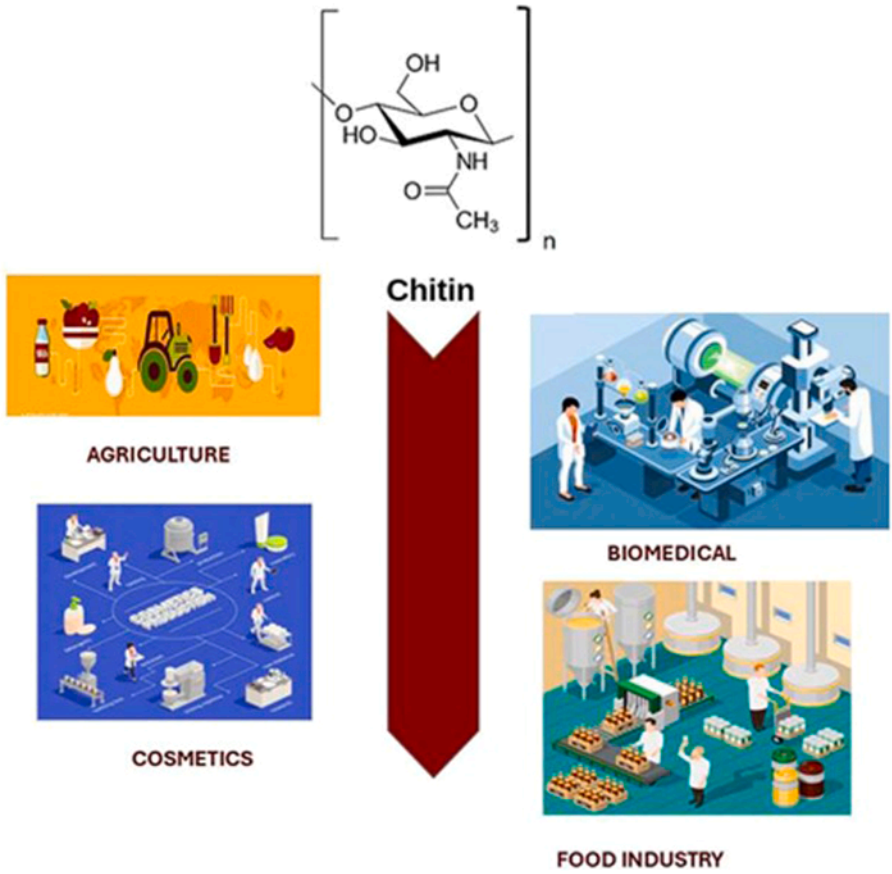


Figure 8.9 Overview of applications of deacetylated and acetylated chitin in various sectors.

As proved by the mentioned studies, chitin has several applications, emphasizing its potential to produce chitin-based materials, pure or blended with other polymers. The variability in chitin's polymorphic form (α, β, and γ-chitin) and its diverse natural sources present both unique opportunities and challenges for its recovery and processing. Greener extraction methods, notably the use of ILs, enable efficient dissolution and extraction of chitin. However, the yield and quality of the recovered chitin remain highly dependent on its polymorphic form and biomass source.

8.5.4 Lipids

Tannery and slaughterhouse wastes, such as fats, skin trimmings, hair, and protein-rich residues, are treated by conventional disposal methods (e.g., landfilling or incineration), which raise environmental and economic concerns (Işler et al., 2010). Nonetheless, the lipid fraction present in these wastes can be recovered for biodiesel

Table 8.7 Applications of chitin-based biomaterials using IL-assisted extracted chitin from animal waste biomass.

Chitin source	Optimum extraction conditions				Application	Highlights	References
	IL	IL concentration	Temperature (°C)	Time			
Shrimp shells	$[C_2C_1im][C_1CO_2]$	95 wt%	90	3 hours	Chitin-IL film for controlled caffeine delivery	Caffeine-loaded chitin/IL film, prepared with 2.5% w/w chitin and dried using sc-CO2, exhibited a biphasic release profile, characterized by an initial burst release, followed by a sustained release up to 36 hours.	Catherine King et al. (2017)
			Microwave radiation	2-second pulses during 3–4 minutes	Chitin-calcium alginate fibers for potential wound-healing application	Chitin and chitin-calcium alginate fibers prepared via wet spinning exhibited smooth morphology and high tensile strength, with chitin-only fibers reaching 256 MPa. In vivo assays confirmed biocompatibility and enhanced wound-healing performance, with chitin-calcium alginate fibers with over 95% wound contraction achieved in 10 days.	Shamshina et al. (2014)
Squid endoskeleton	$[C_4C_1im][C_1CO_2]$	100 wt%	95	-	Chitosan-silk fibroin hydrogels for possible wound-healing applications	The IL was used as a common solvent for chitosan-silk fibroin, enabling the development of hydrogels with viscoelastic and swelling behaviors. The formulation with 30% silk fibroin showed promising potential for wound-healing applications.	Silva et al. (2012)

production. However, their direct use in energy generation (biodiesel) can face barriers due to challenges such as compositional variability, seasonal availability, and physicochemical instability (Alptekin, Canakci and Sanli, 2012). The conventional disposal methods (i.e., landfilling and incineration) raise environmental concerns, reinforcing the need for sustainable alternatives that align with the circular economy principle (Velenturf and Purnell, 2021).

Most of the globally produced biodiesel comes from first-generation feedstock (e.g., sugarcane or corn) (Senatore et al., 2019). Reliance on edible, high-quality oils can increase production costs, making biodiesel less competitive than fossil fuels (Wan Osman et al., 2024). In that regard, low-cost alternatives such as rendered animal fats, waste cooking oils, and tannery by-products offer a sustainable and cost-effective solution (Binhweel et al., 2023). Discarded animal waste can be processed by chemical, thermochemical, or biochemical processes to be used as an energy source, especially as biodiesel fuel (Shirzad et al., 2019). These lipid-rich wastes can be processed into biodiesel, which shares similar properties with conventional diesel and can be directly used in common engines, offering a notable advantage due to the higher oxygen content, which contributes to lower carbon monoxide emissions (Ndiaye et al., 2020). Despite these benefits, conventional biodiesel production via transesterification process relies on hazardous solvents and energy-intensive steps (Chanakaewsomboon et al., 2020; Toldrá-Reig et al., 2020). Furthermore, animal fats typically contain high levels of saturated fatty acids, which results in a relatively higher density, viscosity, cloud point, and pour point (Nagappan et al., 2021). Additionally, free fatty acids and phosphoglycerides in animal tissues make the conversion process difficult, hindering the conversion of fatty acids into alkyl esters or biodiesel (Fig. 8.10).

Common lipid extraction techniques can present some challenges. Thermal rendering using dry or moist heat, microwave-assisted (Costa and Bragagnolo, 2017), ultrasound-assisted (Chemat et al., 2017), and supercritical fluid extraction (Ilias et al., 2023), may impair lipid quality due to the high contact temperatures that are achieved between the heat source and the sample (Dufour and Iribarren, 2012). On the other hand, solvent-based extraction, such as the Folch and Bligh and Dyer methods, heavily relies on toxic organic solvents like chloroform and methanol, raising safety and environmental risks (Khan et al., 2021).

In this context, the valorization of fatty tissue waste using ILs emerges as a greener and more effective approach, using more environmentally friendly approaches without impairing the lipid content and quality.

Biodiesel production from animal fat using ILs as catalysts: Applications of lipid-based biomaterials using ILs as catalysts to process waste animal fat (WAF) are shown in Table 8.8. Ranjitha et al. (2020) used ethanol as a renewable solvent and an amino acid-based IL catalyst, L-valine amido ethyl methyl imidazolium bromide ([L-ValC_2C_1im]Br) to convert waste beef tallow into biodiesel. The IL was selected due to its ability to suppress saponification reactions in the case of high free fatty acid (FFA) content. The optimized reaction parameters (animal fat to ethanol molar ratio of 1:7.5%, 20% IL, 75 °C during 2.6 hours) resulted in a near-complete

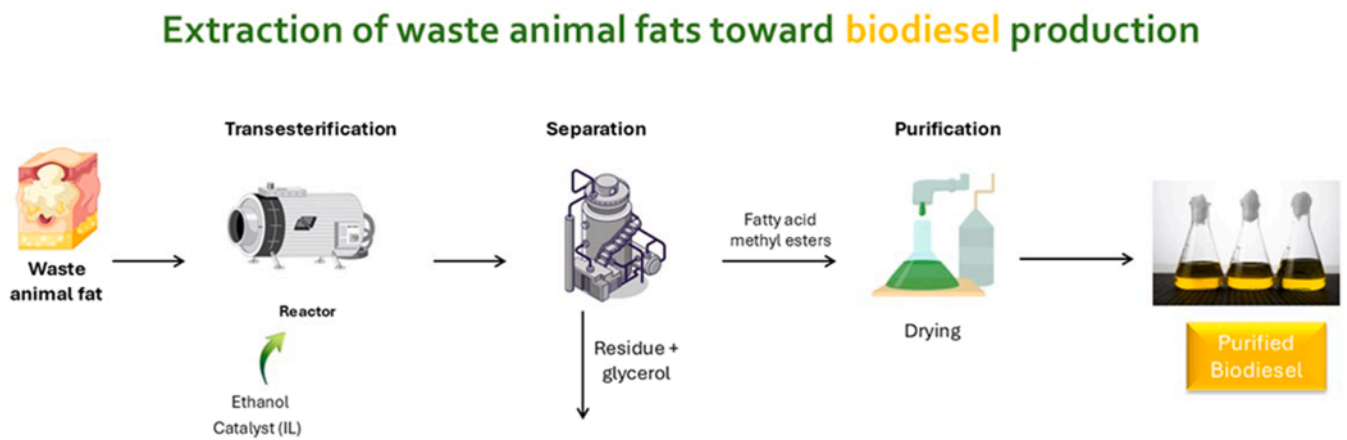


Figure 8.10 Schematic representation of a common process to produce biodiesel from waste animal fat.

Table 8.8 Applications of lipid-based biomaterials using ILs as a catalyst to process waste animal fat.

Lipid source	IL function	Application	Highlights	References
Beef tallow Waste animal fat	[L-ValC_2C_1im] Br as a catalyst agent [D-ValC_2C_1im] Cl as a catalyst agent	Biodiesel production for CI engine	B20 exhibited enhanced performance and combustion characteristics with a reduced emission level.	Ranjitha et al. (2020) Srinivasan et al. (2024)

transesterification. The resulting biodiesel was blended with ordinary diesel, presenting a total concentration of 20% of biodiesel in the total diesel system (commonly described as B20). The fuel was tested in a compression ignition (CI) engine and confirmed its viability as a fossil fuel substitute with enhanced performance and combustion along with less hazardous emissions compared to a total ordinary diesel system (Ranjitha et al., 2020).

Similarly, Srinivasan et al. (2024) employed an amino acid-based IL, D-valine amido ethyl methyl imidazolium chloride ([D-ValC_2C_1im]Cl), as a catalyst to understand how fatty acid composition in WAF governs both the production process and end-use performance of biodiesel. The obtained results revealed that, under optimized conditions (1:6 M ratio, 10 wt% IL, 75 °C, 1.5 hours), WAF-derived triglycerides, rich in long-chain saturated fatty acids (e.g., palmitic and stearic acids) and monounsaturated oleic acid, achieved a yield of biodiesel of 97.36%. The chloride anions facilitated nucleophilic attack on triglycerides, influencing fuel properties while increasing both the density and the viscosity. The higher density allows for a greater amount of fuel to be delivered in the same injection cycle, partially compensating for its lower heating value. Nevertheless, these characteristics contribute to safer fuel storage and transportation. Additionally, the reuse of the IL was effective throughout 10 cycles (Srinivasan et al., 2024). In summary, these studies highlight ILs as promising catalysts for tannery waste valorization, bridging green chemistry and circular economy principles.

8.6 Challenges and opportunities of waste valorization approaches using ILs

Converting animal by-products into value-added biomaterials will allow addressing health, environmental, and economic concerns, thus playing a critical role in

advancing several SDGs from the UN 2030 Agenda. By reducing uncontrolled disposal waste through waste valorization approaches in the context of circular economy approaches, contributions to successfully achieving the following SDGs (United Nations. Sustainable Development Goals, 2015) are foreseen: SDG 3 (Good Health and Wellbeing), SDG 7 (Affordable and Clean Energy), SDG 9 (Industry, Innovation, and Infrastructure), SDG 12 (Responsible Consumption and Production), and SDG 13 (Climate Action). By recovering and processing materials like keratin, collagen, and chitosan, it is possible to create alternatives to replace synthetic or petroleum-derived materials, promoting sustainability. Certain animal by-products, especially tallow and fat, can be converted into substrates to produce biodiesel and renewable energy. Processes like green catalysis or enzymatic transesterification can contribute to the diversification of renewable energy options while helping with waste management (Table 8.9).

Traditional methods for protein, polysaccharide, and lipid recovery/valorization rely on high temperatures or harsh chemicals. The integration of ILs into animal waste valorization offers a more sustainable pathway to valorize animal waste biomass, as ILs facilitate biomass dissolution and extraction of biopolymers under milder conditions. As seen, imidazolium-based ILs such as $[C_4C_1im][C_1CO_2]$ enable the solubilization of keratin and chitin under moderate temperatures, allowing the recovery of the target product with high quality and enhanced extraction efficiencies. Being derived from natural sources, bio-based ILs (e.g., cholinium-based ILs) with improved biocompatibility profiles can contribute to designing greener processes and are starting to be increasingly used. From the current state of the art, it becomes clear that acetate-based ILs are highly efficient for the recovery of biomolecules from different waste sources due to the high hydrogen-bond basicities. Nevertheless, the diversity of

Table 8.9 Contribution of animal waste valorization to SDGs.

SDG	Contribution for animal biomass valorization	References
SDG 3 (good health and well-being) SDG 7 (affordable and clean energy) SDG 9 (industry, innovation, and infrastructure) SDG 12 (responsible consumption and production) SDG 13 (climate action)	Recovery of biomaterials for biomedical use Conversion of animal fats into biofuels, reducing fossil energy sources Development of biorefineries and novel processing routes with greener solvents Transformation of animal residues into high-value products, reducing landfilling and incineration Reduction in methane emission from organic waste and production of low-emission fuels	United Nations. Sustainable Development Goals (2015)

Table 8.10 Opportunities and challenges in animal waste biomass valorization using ILs.

Opportunities	Challenges
High solubilizing power; Potentially recyclable and low-volatility solvents; Enable milder processing conditions, yielding a product with higher quality. Preservation of native-like structures of extracted biomolecules.	Optimization of processing conditions for each biomass type; Concerns about toxicity and biodegradability of ILs; High cost of IL synthesis (can be mitigated by reusing the IL); Risk of residual IL contamination, affecting downstream processes; Need for cost-effective IL recovery methodologies; Regulatory gaps and a few successful examples of products in the market.

chemical structures of ILs studied remains largely unexplored and may open doors to developing more competitive processes. To increase the cost-effectiveness of these processes, the recycling and reuse of ILs across multiple cycles has been demonstrated. This stage, however, accounts for a significant fraction of the total process costs and deserves further investigation. A summary of current opportunities and challenges in waste biomass valorization approaches using ILs is presented in Table 8.10.

The type of value-added compounds to be extracted is very diverse in terms of structure and properties, as well as the biomass source, which requires the optimization of operational parameters for each process and target compound. However, the use of computational tools such as COSMO-RS and artificial intelligence methodologies may help in identifying the most relevant ILs for a given application before any experimental data. The design of ILs should additionally consider their synthetic route, as it will directly influence the cost and their toxicity and biodegradability profiles, critical issues not only for industrial implementation of these processes but also for the application of the resulting materials in different fields. Regulatory gaps regarding the application of ILs in animal waste valorization, particularly in the pharmaceutical, food, and cosmetic industries, must be cautiously considered.

Overall, the valorization of animal by-products using ILs affords a unique opportunity for advancements toward a more sustainable future, allowing a more efficient use of resources while improving waste management methodologies with benefits for the general society and the environment.

8.7 Conclusions

The valorization of animal waste biomass using ILs represents an innovative approach to address global sustainability challenges, such as greenhouse gas emissions, biodiversity loss, and resource inefficiency. This chapter provides a survey of IL

applications as versatile solvents for recovering high-value biopolymers (keratin, chitin, collagen, and lipids) from distinct animal waste sources and their conversion into value-added biomaterials/products. Among the various biopolymers investigated for recovery from animal-derived waste, keratin has received the greatest research attention due to the high availability and low cost of these residues. Based on the reviewed work, acetate-based IL (e.g., $[C_2C_1im][C_1CO_2]$) stands out as one of the most effective solvents for extracting diverse biopolymers, irrespective of the biomass origin. The great performance is correlated with the ability of the acetate anion to form strong hydrogen bonds with biopolymer structures, therefore enabling effective disruption and dissolution of biomass and biopolymer solubilization. Additionally, given the tunable nature of IL, exploring different ion combinations may help design more competitive waste valorization processes.

Despite the progress achieved to date, challenges remain. Most studies focus on biopolymer recovery, with limited research on processing these biopolymers into functional products for various applications. The environmental and economic viability of IL-based processes largely relies on solvent recovery, revealing that IL recovery and reuse are major limiting factors, emphasizing the need for other IL recovery techniques with lower cost. Furthermore, the environmental footprint of ILs requires further investigation, as bio-based ILs offer promising potential in terms of favorable toxicity and biodegradability profiles. Also, it remains important to evaluate the scalability of these processes, a crucial factor to make them competitive in relation to petroleum-based solvents.

In the future, researchers must prioritize three key areas: (1) expanding potential applications of recovered biopolymers; (2) investigating the application of more sustainable and cost-effective ILs, such as bio-based ILs derived from renewable feedstocks; and (3) performing comprehensive assessments, such as life-cycle analysis and techno-economic analysis, to enlighten on the application of these products at an industrial scale. In summary, ILs have the potential to transform animal waste into high-value biomaterials for several applications, contributing to a zero-waste future.

Acknowledgments

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, UID/50011/2025 (DOI 10.54499/UID/50011/2025) & LA/P/0006/2020 (DOI 10.54499/LA/P/0006/2020) & UID/PRR/50011/2025 (DOI 10.54499/UID/PRR/50011/2025), financed by national funds through the FCT/MCTES (PIDDAC). C. Polesca acknowledges FCT—Fundação para a Ciência e a Tecnologia for the PhD grant with the reference UI/BD/151282/2021 (DOI 10.54499/UI/BD/151282/2021). Augusto Q. Pedro acknowledges FCT for the research contract CEECIND/02599/2020 (DOI 10.54499/2020.02599.CEECIND/CP1589/CT0023) under the Scientific Employment Stimulus—Individual Call.

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