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From Food Protein Waste to Valuable Materials: Recovery and Valorization of Collagen and Keratin

TESI DI LAUREA MAGISTRALE IN
FOOD ENGINEERING
INGEGNERIA ALIMENTARE

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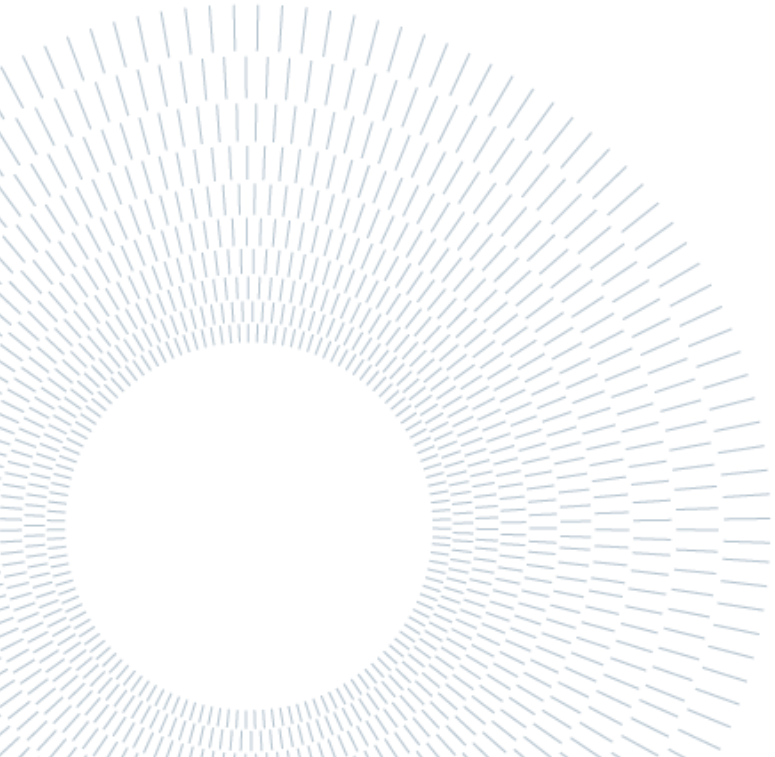


Abstract

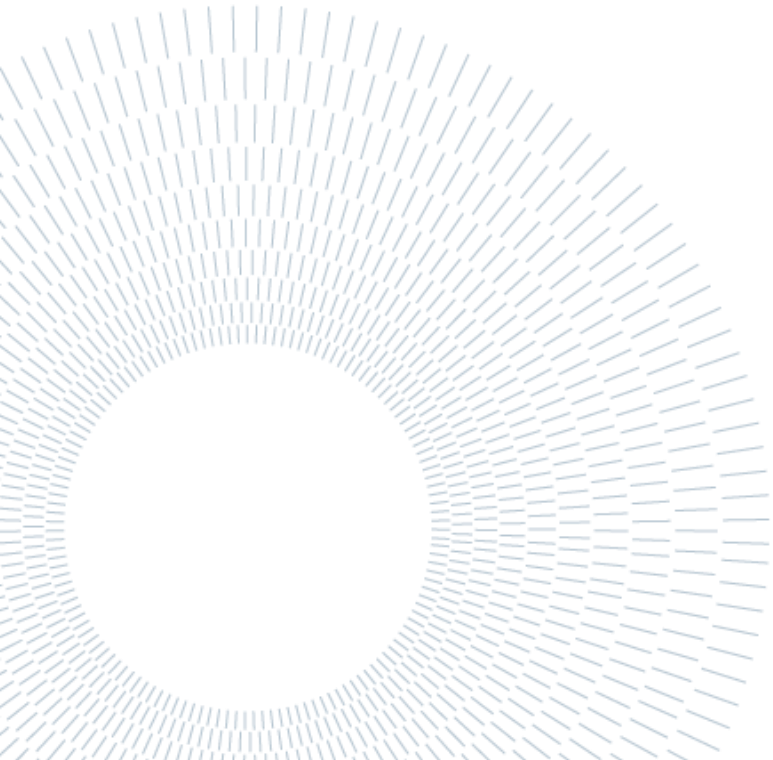
In recent decades, an accelerated increase in global food production has resulted in loss or waste of nearly one-third of produced food contributing significantly to environmental degradation, climate change, resource scarcity, and pollution. This issue does not only represent a major environmental concern, but it also has social and economic implications. Addressing this challenge requires a change towards an efficient global food production system and a reduction in food waste. To offer a compelling solution to mitigate the environmental impact of protein waste, this literature review explores the potential of a circular economy to recover and valorize food protein waste, focusing on collagen and keratin. These proteins can have diverse applications across different fields such as pharmaceutical, biomedical, food, cosmetics, and more. Various extraction methods for collagen and keratin are discussed, emphasizing the importance of employing environmentally conscious techniques to achieve optimal recovery. A comprehensive analysis of each extraction method is presented, providing insights into their specific purposes, inherent advantages, associated disadvantages, and overall performance. Furthermore, electrospinning is highlighted as a promising technology to transform recovered collagen and keratin into nanofibers. These nanofibers demonstrate enhanced physicochemical and mechanical properties which are particularly relevant in biomedical and pharmaceutical applications, especially in wound healing and tissue regeneration. Additionally, laboratory analyses like zeta-potential, FT-IR, dynamic light scattering, and optical microscopy under polarized light were conducted in an ongoing research project focused on the circular economy of keratin extracted from feather waste. All this comprehensive exploration contributes significantly towards a more sustainable and efficient circular economy by preventing the loss of valuable properties and reducing food waste.

Key-words: food waste, food protein waste, protein recovery, keratin, collagen, circular economy

Abstract in italiano

Negli ultimi decenni, un aumento accelerato della produzione alimentare globale ha comportato la perdita o lo spreco di quasi un terzo del cibo prodotto, contribuendo in modo significativo al degrado ambientale, al cambiamento climatico, alla scarsità di risorse e alla polluzione. Questo problema non rappresenta solo una grave preoccupazione ambientale, ma ha anche implicazioni sociali ed economiche. Affrontare questa sfida richiede un cambiamento verso un sistema di produzione alimentare globale efficiente e una riduzione degli sprechi alimentari. Per offrire una soluzione convincente per mitigare l'impatto ambientale degli scarti proteici, questa analisi della letteratura esplora il potenziale di un'economia circolare per recuperare e valorizzare gli scarti proteici alimentari, concentrandosi su collagene e cheratina. Queste proteine possono avere diverse applicazioni in diversi campi, come quello farmaceutico, biomedico, alimentare, cosmetico e altri. Vengono discussi vari metodi di estrazione del collagene e della cheratina, sottolineando l'importanza di utilizzare tecniche rispettose dell'ambiente per ottenere un recupero ottimale. Viene presentata un'analisi completa di ciascun metodo di estrazione, fornendo approfondimenti sui loro scopi specifici, vantaggi intrinseci, svantaggi associati e prestazioni complessive. Inoltre, si prende in esame l'elettrofilatura in qualità di tecnologia promettente per trasformare il collagene e la cheratina recuperati in nanofibre. Queste nanofibre dimostrano proprietà fisico-chimiche e meccaniche migliorate che sono particolarmente rilevanti nelle applicazioni biomediche e farmaceutiche, in particolare nel trattamento delle ferite e nella rigenerazione dei tessuti. Inoltre, analisi di laboratorio, come potenziale zeta, FT-IR, diffusione dinamica della luce e microscopia ottica sotto luce polarizzata sono state condotte in un progetto di ricerca in corso incentrato sull'economia circolare della cheratina estratta dagli scarti di piume. Tutto questo contribuisce in modo significativo a un'economia circolare più sostenibile ed efficiente prevenendo la perdita di proprietà preziose e riducendo gli sprechi alimentari.

Parole chiave: spreco alimentare, scarto proteico alimentare, recupero di proteine, cheratina, collagene, economia circolare.



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1 Food waste

The food production rate has increased in the last decades, producing enough food to feed 10 billion people, with an estimated one-third of food being lost or wasted [1]. A big impact on the environment, contributing to climate change, resource scarcity, and pollution has been generated due to food production, which represents a major environmental, social, and economic problem [2]. Notably, it is important to consider the need for an efficient global food production system and a reduction in food waste to maintain balance on the planet and avoid major negative impacts on climate change.

For instance, food waste stands for food, a fraction of food, and inedible parts of food that are not consumed and are thrown away by consumers [1]. In industrialized countries, approximately 40% of food waste occurs at retail and customer level, and 40% of it is generated during the manufacturing process in the food industries [2]. Most food waste is inevitable and happens during the transformation of raw ingredients into final products [2]. Moreover, food waste contributes to climate change with an annual amount of food wasted of approximately 1.3 billion tons, which represents 30% of the total food produced for human consumption. Furthermore, it also accounts for 8 to 10% of global greenhouse gases (GHG) emissions [2]. For this reason, good management is needed to ensure the recovery and reduction of food waste. Food waste reduction targets are included in the EU Action Plan with proposed measures and waste regulations. In 2018, EU countries committed to reduce in half retail and consumer food waste by 2030 and reduce food loss across the supply chain [1].

Furthermore, there is no longer the possibility of discarding food protein waste as the final product of a linear economy, and the efficient valorization into a circular economy has become critical in different aspects [2]. Circular Economy (CE) refers to the responsible management of resources and the reuse of by-products or anything that would normally be thrown away. It aims to reduce environmental impacts and improve waste management practices to encourage greater recycling, recovery, and reuse of waste, as well as more renewable resources (Figure 1) [1].

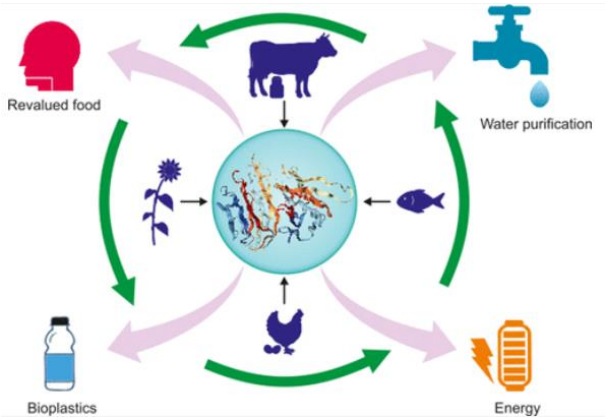


Figure 1. Circular economy of the transformation of food protein waste into sustainable technologies. Reproduced from reference [2].

This literature review explores the potential of a circular economy to recover and valorize food protein waste, with a focus on collagen and keratin. The review explores various extraction methods used for these proteins and their diverse applications across different fields. By getting to know about the recovery and valorization of collagen and keratin, the review gives light to innovative approaches that can be employed to maximize the recovery of food protein waste, contributing to a more sustainable and efficient circular economy.

A statistical analysis on the number of paper publications related to the topic was conducted by literature research on the scientific data base Web of Science for the last 5 years, regarding the keyword "food protein waste" alone. A result of 5215 publications was found from which 729 were for 2019, 974 for 2020, 1113 for 2021, 1182 for 2022, and 1217 for 2023. Subsequently, another research was conducted by using the keywords "food protein waste" AND "circular economy". As a result, 363 publications were found, from which 20 were for 2019, 58 for 2020, 75 for 2021, 91 for 2022, and 119 for 2023. It can be seen that every year shows an increase in the topics (Figure 2) providing updated information on them, and results for the year 2023 are expected to increase following the same trend.

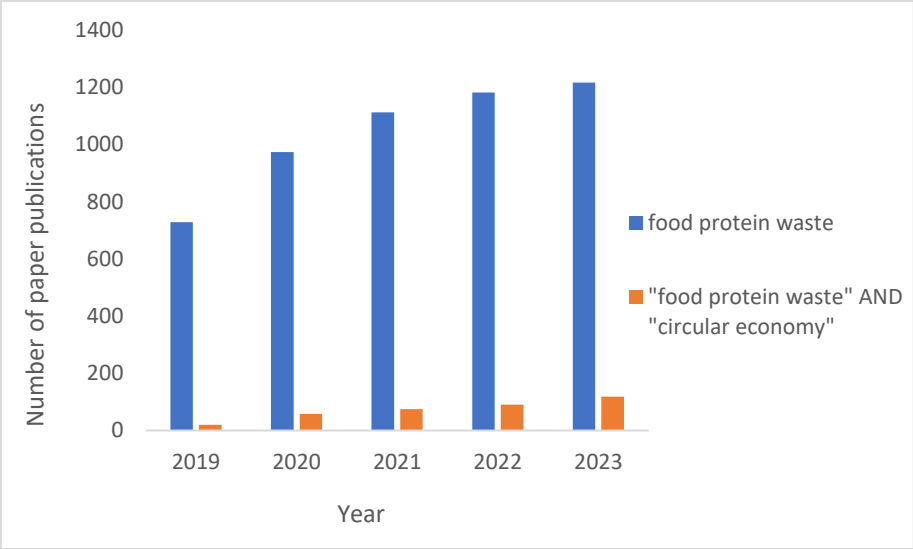


Figure 2. Statistical analysis results of the number of paper publications related to the topic on the scientific data base Web of Science for the last 5 years.

2 Food protein waste

Recently, an increase in the demand for high-protein foods has pushed industries to develop more protein-based products. Proteins are the most valuable macronutrients from an economic and human nutritional point of view [3]. The chemical structure of proteins consists of carbon, oxygen, nitrogen, hydrogen, sulfur, and phosphorus [4]. Proteins are highly complex polymers with a unique sequence of amino acids linked together through a covalent peptide bond [4]. Proteins play an important role in biological and food systems [3]. They are fundamental to human nutrition and necessary for cell growth and body's repair mechanisms [3]. The contribution of protein to healthy aging is increasingly recognized, as well as their role in a healthy diet [5]. The most important and basic functional property of food proteins is the solubility, which evaluates if protein preparation is appropriate for use in food technology and enables producers to create food products with desired, reproducible, and predictable characteristics [4]. Furthermore, food proteins have functional properties such as water holding, fat absorption, emulsifying, foaming, and gelation [6]. These properties make food proteins adaptable and valuable components in a variety of product development, formulation, and other applications [6].

Food proteins are important nutrients that can be divided into animal proteins and plant proteins. Animal proteins contain the necessary amino acids in a well-balanced manner, whereas plant proteins are deficient in certain amino acids [7]. From a general point of view, the most important sources of protein are meat, milk, wheat, and rice, followed by cereals [8]. Independently from their source, proteins will generate a carbon footprint and have a severe impact on planetary boundary systems.

Food industries produce food waste and by-products that are high in usable and recoverable proteins. Food protein waste plays a major role in the overall food waste landscape, as protein is one of the most valuable components [2]. Given that 5 to 10% of food waste is protein-based, millions of tons of proteins from both animal and plant sources could be better utilized [9]. Additionally, disposal of protein-rich products is difficult, costly, and highly regulated because an unappropriated disposal can contaminate the environment in a severe way, transforming into a serious threat to human health [10]. At the same time, the waste of protein-rich products contributes to air, soil, and water pollution. For this reason, reusing and decreasing protein waste is an ideal solution to reduce the resulting environmental and social impacts [2].

Figure 3 provides a graphical summary of the main sources and types of food protein waste that will be discussed below.

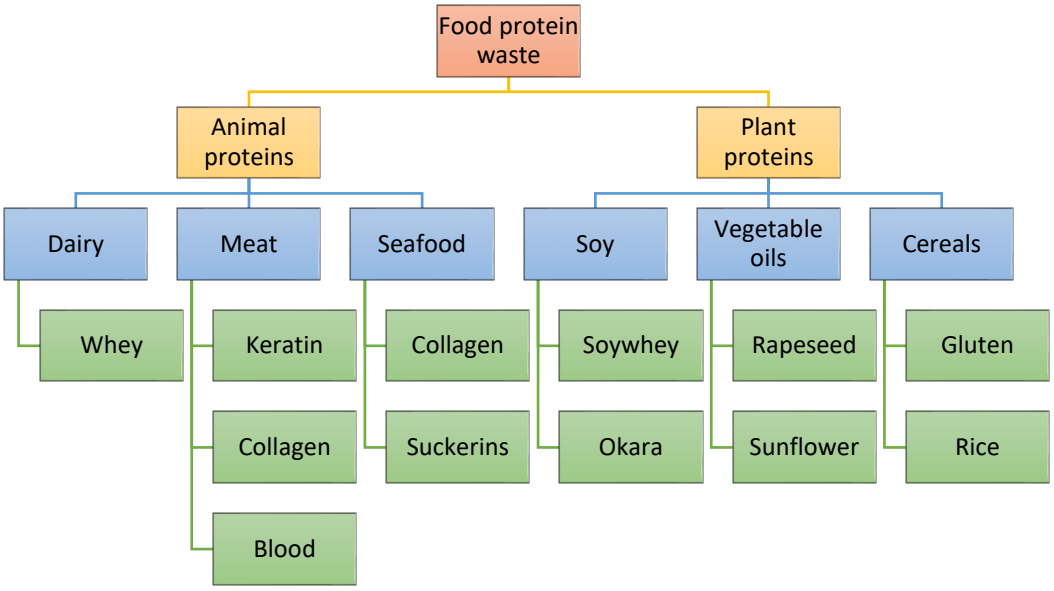


Figure 3. Graphical summary of the main sources and types of food protein waste.

2.1. Animal proteins

Animal proteins have a high nutritional value because they provide essential amino acids and are highly digestible [3]. The intensive production of animal proteins generates 12% of the total global GHG emissions and directly impacts climate change [5].

2.1.1. Dairy

Every year, approximately 150 million tons of milk are produced in Europe. During the production, 80 to 90% of fats and caseins are extracted and transformed into whey, which is a dairy by-product that includes 20% of proteins [2]. Effluents from dairy industry contain a high concentration of organic matter, which could make some problems appear, especially due to their organic load [11]. Pollution due to dairy industry affects the air, soil, and water quality, as well as being a cause for climate change [11].

Whey is a by-product found in milk, and approximately 240 million tons of whey are globally produced every year (Figure 4a), corresponding to almost 2 million tons of high nutritional value proteins [12]. The most abundant whey protein is β-lactoglobulin, which is rich in essential amino acids and provides an excellent nutritional value. The second major whey protein is α-lactalbumin, which synthetizes lactose in milk production, and has nutritive properties [12]. It is important to mention that 50% of the total whey produced remains unprocessed and is treated like waste and discharged into the environment [12].

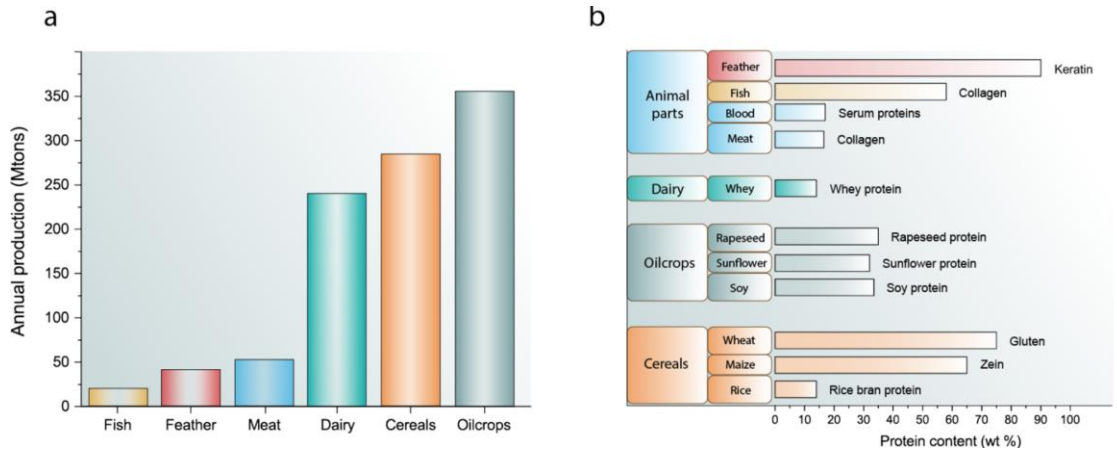


Figure 4. Global production of industrial protein-rich food waste and their protein content. (a) Global production of the different food waste sources. (b) Protein content in wt % for the main byproducts that are generated globally. Reproduced from reference [2].

2.1.2. Meat

Due to the increase in global population, meat consumption is also increasing, as it is the most abundant source of proteins for the human diet [2]. Unfortunately, the intensive production of animal proteins generates a biodiversity loss and a direct impact on climate change. Every year, near 330 million of animals are slaughtered in EU resulting in the generation of more than 18 million tons of meat by-products that need to be managed in a sustainable way [13]. Overall, it is reported that approximately 52.6 million tons of meat byproducts are generated globally, representing a 20% of the annual meat production (Figure 4a) [2]. The meat industry generates large volumes of by-products like blood, bones, skin, cartilage, hair, and more, which are rich in nutrients, such as proteins, essential amino acids, vitamins, and minerals, as well as in keratin and collagen (Figure 4b) (More about these proteins in section 2.3) [2]. The disposal of these by-products is challenging and expensive because of regulations and considering that an inappropriate disposal can contaminate the environment in a severe way [2]. For these reasons, an efficient revalue and reuse of the byproducts have been considered.

The annual global blood production is estimated to be approximately 2.5 billion liters, containing enough proteins to fulfill the annual protein requirement for 17 million adults [14]. This by-product has not been completely reevaluated to its potential, because less than 4% of these proteins are valorized for human consumption [14]. In blood, around 35% of proteins are found in red blood cells, and 8% in plasma [14]. Protein recovery methods are still costly, so a sustainable alternative to valorize the by-products is still needed [2].

2.1.3. Sea food

During the last decades, the consumption of fish and seafood has increased, generating quantities of by-products of around 70% of the fish and shellfish volume [15].

Approximately 160 million tons of seafood are processed globally, which include 60% of protein content [2]. Seafood waste generates serious economic and ecological concerns, but they represent a valuable source of compound with high added value, such as lipids, enzymes, oils, vitamins, polysaccharides, and an average protein content of 60%, as shown in Figure 4b [15]. Animal bodies have a high content of collagen because it is what provides the structural stability, and is the principal component of fish skin, scales, fins, bones, and swim bladder, constituting around 30% of the total amount of protein in vertebrates [15]. Polysaccharides like chitin and chitosan are other valuable components that can be extracted from the skeletons of crab and shrimp [2]. Moreover, structural proteins, called suckerins, are found in marine animals. These proteins make up almost the entire sucker-ring teeth of squid and cuttlefish, have an excellent mechanical performance, and are as strong as synthetic polymers [2].

2.2. Plant proteins

Due to climate discussions, alternatives to animal proteins have increased in the last years. A wide variety of plant sources can be used for protein production, but the most common ones have been soy and wheat [3]. At the same time, recent studies have been focusing on finding new plant sources for the same purpose. On the other hand, plant protein production has immediate implications for nitrogen, and phosphorus cycles and impacts the loss of biodiversity by transforming forests into exploitable lands for agriculture [2]. Furthermore, they generate an increase in pressure on global freshwater use, change in land use, and chemical pollution, both in terms of crops and livestock [2].

2.2.1. Soy

Soybeans are the world's most popular plant protein source and most abundant cultivated crop in the world with 400 million tons each year [16]. The major by-products of soy are soy whey and soybean pulp. Even though they contain great amounts of nutrients, they require several pretreatment steps before their disposal, contributing to the environmental impact of soy production [2].

Soy whey is the wastewater generated by soaking and boiling soybeans [17]. Depending on the source and processing conditions, soy whey contains proteins in the range of 0.3 to 8.2 g/L, and a high concentration of organic compounds such as minerals, carbohydrates, and soy isoflavones [18]. This by-product has a limited 1-day shelf life, and its disposal is still challenging due to its organic load [18].

Soybean pulp residue, called okara, is another major byproduct obtained from soymilk and tofu production and contains around 20.9 to 39.1% of proteins [19]. As it undergoes a rapid putrefaction process and has a high drying cost, it is directly disposed of by incineration or dumped in landfills [19].

2.2.2. Vegetable oils

For a long period of time, seeds have been the main source of vegetable oils for human and animal consumption. More recently, vegetable oils provide valuable materials for industrial and fuel purposes, which generates a demand increase for vegetable oil worldwide [20]. During the refining of oils, around 364 million tons of waste are produced, in the form of meal or cakes (Figure 4a) including valuable components such as proteins, polysaccharides, phenolic compounds, cellulose, and more [21]. Depending on the type of plant waste, residual cakes can reach up to 50% protein content, which primarily consist of seed storage proteins that function as a source of nutrients during growth and germination such as nitrogen, oxygen, carbon, and minerals [22]. These types of by-products are still considered to be low-value materials and are directly disposed of in landfills or incinerated [2].

Rapeseed is the second most cultivated oilseed crop in the world after soybean, with a production of approximately 24 million tons in Europe and 74 million tons globally [21]. Approximately, 40 million tons of rapeseed by products are produced every year globally [23]. Depending on the processing conditions, these by-products have protein content of 28 to 31% when they are cold-pressed; and 38 to 45% (Figure 4b), when they are hot-pressed [24]. Even though these by-products are rich in proteins, due to the presence of antinutritional elements such as glycosylates, fibers, and phenolic compounds, their utilization in human nutrition is limited [24].

Sunflower is the third major cultivated oilseed crop in the world [21]. Sunflower meal is the main by-product of the sunflower oil industry, with 20 million tons produced globally [25]. The composition of this by-product depends on different factors, such as a crop variety, growing conditions, and processing techniques. On average, it contains 20 to 30% of proteins [25]. Although this type of byproduct has a potential nutritional value, it still remains underutilized and is often disposed of in landfills or used as solid fuel [2].

2.2.3. Cereals

Cereals, in general consist, of 8 to 11% proteins and are rich in amino acids [2]. Corn is the world's most cultivated cereal crop with a global production of 1100 million tons per year with it mainly used as maize flour and meal [2]. Corn contains approximately 10% of proteins with zein being the predominant protein of numerous by-products such as corn gluten meal [9]. Corn gluten meal contains digestible fibers, starch, and around 25% of proteins, making it a valuable source of nutrients [26]. The corn kernel contains around 9 to 12% of proteins, but only half of it is considered an industrially valuable zein protein [2].

Gluten is a proteinaceous blend of glutenin and gliadin, which results from the isolation of starch or corn flour, and it is one of the most abundant plant proteins being

a major by-product of starch [27]. It is widely available and relatively inexpensive, as well as it possesses good thermoplastic behavior and processability [28].

Rice is one of the most important food sources in the world with a production above 600 million tons [29]. The main by-products as a result of the rice milling process are rice husk and rice bran, which represent 25% of the total weight of the kernel, with more than 70 million tons being produced globally [30]. Rice kernel outer layers are rich in nutrients, and bioactive compounds, which include a 16% of protein content [29]. A main limitation for the valorization of these by-products is the presence of antinutrient compounds and a fast oxidation, which would require a rapid and efficient stabilization treatment [31].

2.3. Collagen and keratin

Among the many proteins that can be recovered from food waste, the focus will be given to two of the most abundant ones: collagen and keratin. These proteins possess unique properties that make them highly applicable in a wide range of industries, including pharmaceutical, biomedical, food, cosmetic, and more. For this reason, recovering collagen and keratin from food waste represents a valuable opportunity to generate high-quality and eco-friendly protein products that can meet the growing demand of these proteins in a sustainable way, create new economic opportunities, and contribute to a more circular economy.

2.3.1. Collagen

Collagen is the main constituent of meat by-products, animal extracellular matrix, and the most abundant protein in mammals [32]. Collagen is made up of three polypeptide chains twisted around each other to form a triple helix structure (Figure 5) stabilized by hydrogen bonds [33]. Collagen consists of a bundling fibrillar structure and imparts unique mechanical strength and stability to different body parts [32]. However, collagen has a low nutritional profile and lacks essential amino acids, so collagen-rich byproducts are not valorized for nutritional purposes, but they are for the extraction of bioactive peptides [34]. There are around 29 types of collagens, but the most common ones are the type I, II, and III [35]. To exploit its functional properties in food and other applications, several purification techniques have been developed to isolate this protein into gelatin either in its native form or partially hydrolyzed [32].

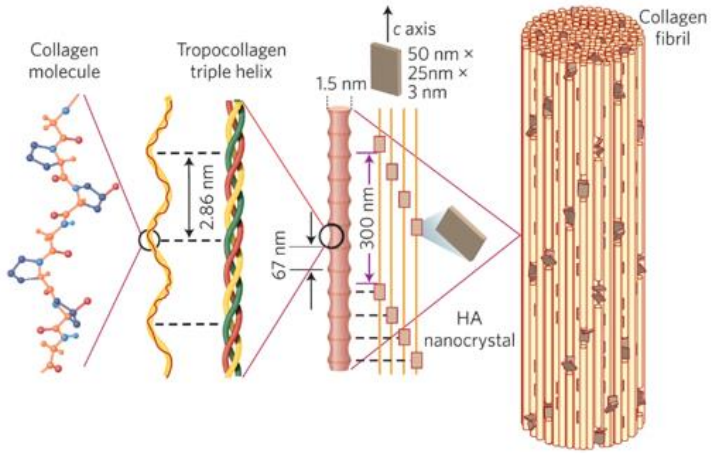


Figure 5. Scheme of collagen's fibril, triple helix, and molecule. Reproduced from reference [36].

2.3.2. Keratin

Keratin, like collagen, is the most abundant structural protein characterized by its high cysteine content [37]. It has a significant presence in animal components like hair, nails, feathers, horns, claws, and wool [37]. Keratin is a fibrous protein which structure mainly consists of a polypeptide chain of different amino acids with inter- and intra-molecular bonding, such as hydrogen, ionic, and disulfide bonds (Figure 6) [38]. Keratin's filamentous structure is characterized by high mechanical and chemical stability that provides extreme resistance to most physical and biological agents [39]. Every year, 40 million tons of keratin-rich waste products are generated, bringing some challenges regarding the waste management due to a lack of effective disposal procedures [2]. The conventional incineration of keratin waste produces toxic gases, which are rich in sulfur and are characterized by slow biodegradation rates generating an accumulation of the byproducts and presenting an uncontrollable issue for the environment and human health [40].

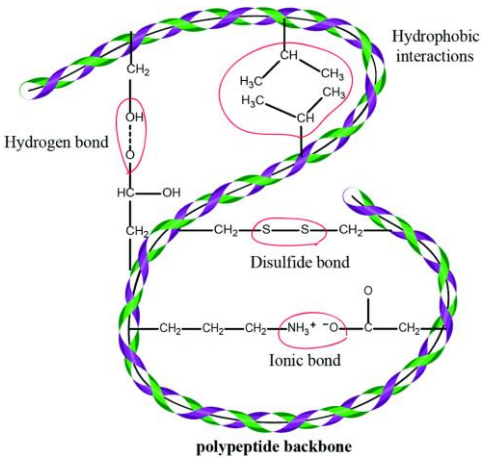


Figure 6. Scheme of main noncovalent interactions involved in the tertiary structure of keratin. Reproduced from reference [41].

3 Food protein waste recovery techniques

Food waste generated during food processing can be recovered and have the potential to be turned into value-added products due to the high content of useful macromolecules that it contains, such as proteins [42]. Numerous techniques are used to recover proteins, and each type of protein has a preferred recovery method. As collagen and keratin are the most common proteins found in food waste, the focus will be on their extraction techniques.

3.1. Collagen extraction methods

Different extraction methods can be applied based on the source and the type of product from which the protein has to be extracted [43]. Collagen extraction typically involves a pretreatment of the source, the extraction, and further purification (Figure 7) [44].

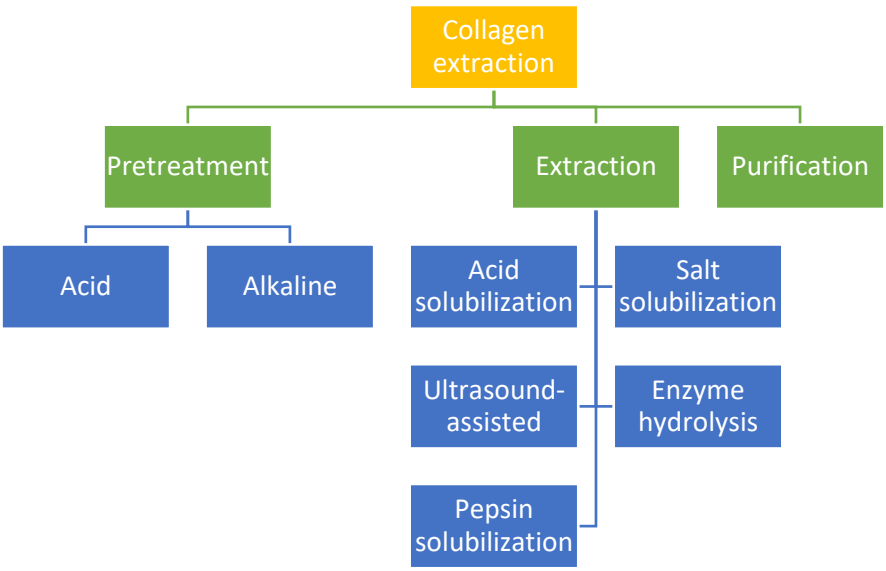


Figure 7. Graphical summary of collagen extraction methods.

3.1.1. Pretreatment

The pretreatment consists of the preparation, extraction, and recovery, and aims to isolate the protein from the waste matrix [43]. The purpose of performing pretreatment on the raw materials is to eliminate impurities, enhance the quality of the collagen, and

break covalent intermolecular crosslinks between the collagen molecules [45]. These covalent intermolecular crosslinks play a crucial role by facilitating the structural and mechanical function of collagen in tissues and organs [46]. Due to their slow breakdown in water, different chemical treatments are employed to partially hydrolyze the collagen, break the crosslinks, and preserve the collagen chains [46].

The preparation consists of washing, cleaning, separating, and cutting into smaller sizes [43]. After the preparation, a gentle chemical pretreatment is carried out to enhance the efficiency of the extraction method and eliminate the substances without collagen [43]. The pretreatment approach used may vary depending on the raw material and the extraction technique, which can be either acidic or alkaline [43].

3.1.1.1. Acid pretreatment

For acid pretreatment, the washed and cut pieces are immersed in diluted acid at a controlled temperature [45]. This causes the skin to swell up to 2 or 3 times its original size and breaks down the crosslinks in the skin [43]. This type of treatment is suitable for fragile skins with a lower degree of fiber intertwinement, such as fish skins [45].

3.1.1.2. Alkaline pretreatment

The alkaline pretreatment involves the application of diluted sodium hydroxide or calcium hydroxide for a few days or even up to several weeks, depending on the thickness of the treated material [43]. Using sodium hydroxide is more convenient because it swells the skin significantly, allowing a deeper diffusion of the alkali into the tissue matrix, which facilitates collagen extraction due to an increase in the transfer rate [47]. This type of pretreatment is effective on extracting collagen from thick and hard materials and helps to hydrolyze unwanted proteins without collagen and other organic materials [45].

The efficiency of the treatment is affected by various conditions such as temperature, duration, and concentration of the alkali [45]. A concentration of 0.05 to 0.10 M of sodium hydroxide and a temperature between 4 to 20°C retains much of the acid soluble collagen and its structure in the tissue [45]. However, a concentration higher than 0.20 M can lead to substantial loss of acid soluble collagen, and a concentration of 0.50 M may degrade its structure [45]. Moreover, prior to the extraction, a demineralization of the raw materials is required in order to increase the collagen extraction efficiency with body parts with a high amount of minerals such as, bones, cartilage, and scales [43]. The most common agent to be used in this step is a chelating agent called ethylene diamine tetra acetic acid (EDTA) or HCl in order to demineralize the proteins and remove inorganic components [48].

3.1.2. Extraction

Due to the stable inter- and intra-molecular hydrogen bonds and crosslinks present in collagen fibers' triple helix structure, collagen fibers are insoluble in water [43]. Therefore, specific extraction techniques are necessary to increase collagen protein solubilization and isolation [43]. To minimize collagen degradation, the extraction temperature is typically low, around 4°C [45].

The extraction methods can be adapted on the desired yield and properties of the final product [45]. The specific extraction method used can affect collagen properties, such as the average length of the polypeptide chains, solubility, viscosity, thermal stability, emulsifying capacity, and water retention [49]. Based on the commonly used extraction methods, collagens are classified into acid soluble collagen (ASC), salt soluble collagen (SSC), ultrasound assisted collagen (UAC), and pepsin soluble collagen (PSC) [44].

3.1.2.1. Acid solubilization extraction

Acid solubilization extraction involves the application of organic acids such as acetic, chloroacetic, citric, and lactic acids, which break collagen crosslinks more effectively and lead to a higher extraction efficiency [45]. These acids hydrolyze the triple helix of collagen and solubilize its single chains, and the depolymerization of proteins into shorter peptides takes place [43]. The collagens obtained after acidic treatments are called acid soluble collagen (ASC) [44].

3.1.2.2. Salt solubilization extraction

When collagen is extracted by salt solubilization, it is referred to as salt soluble collagen (SSC) [44]. Salt solubilization extraction is used for collagen extraction from tissues such as bones, cartilage, skin, and scales [50]. The method involves the use of neutral salt solutions such as citrates, phosphates, sodium chloride, and Tris-HCl [45]. Type I collagen dissolves in salt concentrations less than 1 M and requires continuous stirring for 24 hours [50].

3.1.2.3. Ultrasound-assisted extraction

Collagen extracted from an assisted ultra-sonication is called ultrasound assisted collagen (UAC) [44]. Ultrasound-assisted extraction involves the use of ultrasound waves to generate bubble cavitation in the biological matrix that collapses with high temperature and pressure [50]. The technique is simple, rapid, safe, reliable, environmentally friendly, and economical, and it reduces processing time while improving the extraction yield [49]. The yield of collagen depends on the amplitudes and duration of the treatment, and a higher yield is obtained with higher amplitude and short time [50]. Studies have shown that ultrasound-assisted extraction can recover higher yields of collagen, compared to acid extraction [51].

3.1.2.4. Enzyme hydrolysis

Enzymatic hydrolysis is a preferred method for collagen extraction because it offers higher reaction selectivity and is less harmful to collagen, which ultimately increases the yield and purity of the extracted products [45]. Although enzymes are more expensive than acids, alkalis, and salts, they can be used under mild conditions, are less corrosive to processing equipment, consume less energy, and produce less waste [50]. Proteolytic enzymes of animal, plant, or microbial origin are typically used in collagen extraction, such as trypsin, pepsin, bromelain, papain, collagenase, and others [45].

3.1.2.5. Pepsin solubilization extraction

Pepsin is the most commonly used endopeptidase enzyme in collagen extraction, particularly for seafood sources, as it specifically targets the non-helical portion of the peptide chain of collagen and leaves the structurally important helical portion intact [45]. In addition, pepsin enzymatically breaks down all non-collagenous proteins during extraction, resulting in a higher purity of the extracted pepsin-soluble collagen (PSC) [50]. Pepsin can be used by itself or combined with different concentrations of acetic acid to enhance collagen solubility in an acidic solution and increase the yield [50].

3.1.3. Purification

After collagen extraction, a purification step is necessary to remove neutral salts and non-collagen proteins that may be present [45]. The most commonly used methods for collagen purification are salting out, dialysis, centrifugation, and filtration, often used in combination to achieve optimal purification results [52].

3.1.4. Collagen's Extraction method performance and comparison

Among the various methods for extracting collagen, acid solubilization and enzymatic hydrolysis are the most commonly used [35]. Collagen is widely used in several applications due to its favorable functional properties [35]. Therefore, it is essential to analyze and compare the effects of different extraction methods on their results for extraction rate, physiochemical properties, and biological activity [35]. This is summarized in Table 1.

Cao *et al.* conducted a comparison of collagen extraction from discarded bones of spent hens applying an alkaline pretreatment, acid solubilization with acetic acid, salt solubilization with sodium hydroxide, and pepsin solubilization [35]. An extraction yield of 3.4% for the ASC was obtained, 9.6% for the PSC, and 2.4% for the SSC [35]. PSC resulted in a higher yield, possibly due to specific peptide cleavage facilitating extraction from the fibrillary matrix [35]. The protein content was also higher for PSC (87.6%) compared to ASC (83.37%) and SSC (81.37%) [35]. Amino acid composition

analysis revealed that all three extraction methods exhibited typical collagen characteristics [35].

Thermal stability is a crucial factor for determining the application range of collagen [53]. Cao *et al.* found that PSC exhibited the best thermal stability, with a very slight difference compared to ASC (106.58 °C and 106.41 °C respectively), while SSC had the lowest thermal stability (103.76 °C) [35]. The difference in the result may be due to the effect of salt on the collagen structure and the variation in amino acid composition [35]. Furthermore, PSC demonstrated the highest solubility and SSC the lowest solubility, which might be due to increased ionic strength affecting protein chain interactions or competition between salt and protein water to increase a hydrophobic interaction and aggregation [35]. These results align with the research conducted on collagen extracted from catfish skin by different methods by Tan and Chang [54], as well as the research from Song et al. on skin of the Nile tilapia [55].

Biological activity is a crucial factor that determines the application of collagen in food, medicine, cosmetics, and other fields. Sensory quality, which encompasses attributes such as color and odor, serves as a common intuitive indicator for evaluating collagen's commercial quality [35]. In the research by Cao *et al.*, it was observed that PSC and ASC had resulted in the highest brightness, while SSC had a yellow color probably caused by the amide bonds in collagen molecules and the oxidation of -NH_2 under alkaline conditions [35].

Considering the results, PSC exhibited a better in vitro antioxidant activity through radicals, thermal stability, and sensory acceptability. Thus, with all the results, it can be said that PSC is the most favorable extraction method, and it also has a wider application prospect in food and medicine due to its complete and stable structure, higher yield, thermal stability, processability, antioxidant activity, and sensory acceptability [35].

Table 1. Comparison results for collagen extraction methods.

Extraction methods	Yield (%)	Protein content (%)	Thermal stability (°C)	Solubility	Color	Reference
Acid solubilization (ASC)	3.40	83.37	106.41	Low	White	[35]
Pepsin solubilization (PSC)	9.60	87.60	106.58	High	White	
Salt solubilization (SSC)	2.40	81.37	103.76	Very low	Yellow	

3.1.5. Collagen extraction method purpose, advantages, and disadvantages

Based on the results on research and experiments done, Table 2 shows the comparison of the most commonly used extraction methods for collagen in order to make decisions of the most suitable approach for the desired result by understanding their strengths and limitations.

Table 2. Collagen extraction method purpose, advantages, and disadvantages.

Extraction method	Purpose	Advantages	Disadvantages	Applications
Acid solubilization	Higher extraction yield [45].	Effectively cleave collagen crosslinks resulting in higher extraction yield [45].	May require careful handling due to corrosive nature [45].	Large quantity of collagen production like gelatin for food and pharmaceutical [56].
Salt solubilization	Lower extraction yield and high salt concentration [57].	Maintain stability in critical salt concentration, and structural integrity preservation due to gentle extraction [57].	Limitations apply and requires careful control of salt concentration [58].	Certain biomedical applications that require maintaining collagen's stability and functionality [44].
Enzyme hydrolysis/Pepsin solubilization	Maximize yield and purity [45].	Better reaction selectivity, less damaging to collagen, can maximize yield and purity, leaves structurally important helical portion intact [45]. Better specificity, degree of control of hydrolysis, moderate action conditions, less corrosive to equipment, and less waste [50].	Expensive method [45].	When high-quality collagen is needed like in tissue engineering, wound healing, and cosmetics [59].
Ultrasound-assisted extraction	Improve yield and reduce extraction time [45].	Better yield and reduction of extraction time [45]. Simple method, reduction of corrosive chemicals, economical, and safe [60].	May damage collagen structure during alkali treatment [61].	Large-scale production of collagen-based materials [62].

3.2. Keratin extraction methods

Finding an efficient, environmentally friendly, and economically viable way to extract keratin is crucial since it is a difficult process. The manufacturing of keratin-based materials has increased due to recent improvements in its extraction and characterization [37]. The methods generally applied for keratin extraction are redox processes, chemical hydrolysis, enzymatic and microbial treatment, dissolution in ionic liquids, microwave technique, steam explosion technique, and thermal hydrolysis (Figure 8) [37].

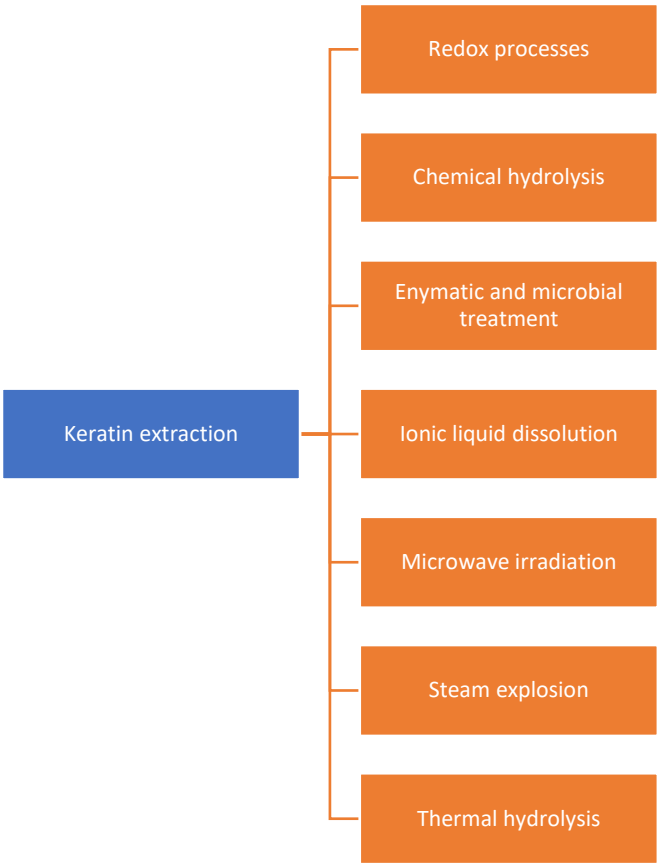


Figure 8. Graphical summary of keratin extraction methods.

3.2.1. Redox processes

An oxidation method is typically used to extract keratin from hair and wool. This method involves applying 2% peracetic acid, mild ammonia, and hydrochloric acid [37]. In addition, the oxidation method involves the use of two other oxidizing agents: sodium thioglycolate and hydrogen peroxide. These agents work by breaking the inter- and intra-molecular crosslinks within keratin, converting cystine into cystic acid residues, and oxidizing various functional groups such as aldehydes, ketones, thiols, and sulfides [63].

On the other hand, reduction can be achieved through the use of certain denaturing agents such as 2-mercaptoethanol or sodium metabisulfite and urea, which target the disulfide bonds, hydrogen bonds, and salt linkages of the keratin fibers [63]. Although these methods have been traditionally employed for extracting keratin from keratinous materials, most of the chemical agents used are toxic, making it challenging for industrial application [64]. Moreover, these techniques have other limitations such as low extraction yield, prolonged processing times, irreversible oxidation of amino acids, and loss of protein integrity [63].

3.2.2. Chemical hydrolysis

Acidic hydrolysis is highly efficient, but it results in a residue with low nutritional value due to the loss of amino acids, like tryptophan, during the process, while alkaline hydrolysis results in a lower loss of amino acids [37]. The yield of the hydrolysis process depends on various factors such as pH, temperature, reaction time, and the type and concentration of the acid or base used [37]. For instance, alkaline hydrolysis conducted at 80°C with 2% sodium hydroxide for 3 hours can yield up to 25% [65]. However, chemical hydrolysis usually requires high temperatures to ensure a high yield, which can also destroy some of the amino acids [38].

3.2.3. Enzymatic and microbial treatment

Keratin extraction is aided by various microorganisms that secrete keratinolytic and proteolytic enzymes, such as keratinases [38]. Examples of such microorganisms include mesophilic fungi, actinomycetes, and certain species of Bacillus bacteria [37]. Enzymes are environmentally safe catalysts commonly used on a commercial scale for keratin hydrolysis, consuming less energy and having milder treatment conditions compared to other commonly used chemical methods [38]. Enzymatic hydrolysis requires minimal energy, but it needs certain reducing agents that can break down keratin's disulfide bonds [37].

3.2.4. Ionic liquid dissolution

Ionic liquids are mainly composed by large cations and organic or inorganic anions [38]. The most common ones used for keratin extraction are 1Butyl-3-methylimidazolium chloride [Bmim]Cl, 1-allyl-3-methylimidazolium chloride [Amim]Cl, and 1-Butyl-3methylimidazolium bromide [Bmim]Br (Figure 9) [41]. These liquids are also considered safe and recyclable solvents, which makes them ideal for use as catalysts, solvents for various polymers, and ion-conductive media [66]. Compared to other anions like BF_4^-, PF_6^-, and Br^- ionic liquids containing chloride as counterion were found to be the most effective solvents for keratin dissolution resulting in a yield of 57% when heated for 30 minutes at 120°C [38]. They are considered the best solvents due to their high nucleophilic activity and chloride ion

concentration which help break down hydrogen bonds [37]. After dissolution, the keratin is separated by precipitation using ethanol or water, and the regenerated keratin consists mainly of beta-sheet structures as the alpha-helix structure is destroyed during the extraction process [38].

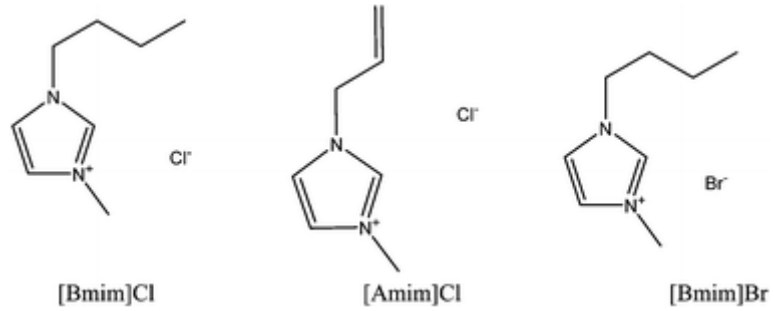


Figure 9. Chemical structures of ionic liquids used for keratin extraction [Bmim]Cl, [Amim]Cl, and [Bmim]Br. Reproduced from reference [41].

3.2.5. Microwave irradiation

The microwave irradiation method is used to extract keratin by reducing the activation energy required and providing uniform heating to the keratinous mass [37]. This method is highly effective as it breaks down and extracts keratin uniformly in a short period of time, but it has a significant drawback of causing a degradation of amino acid sidechains [38]. Unlike conventional heating methods, microwave-assisted heating reduces the activation energy required for keratin extraction, providing uniform heating that allows the molecules in the reactor to absorb energy evenly as the temperature rises [38]. Using this method for 1 hour at a temperature of 180°C can result in an extraction yield of 60%, whereas using it for 20 minutes at a temperature of 200°C can result in a yield of 72% [37]. Thus, it can be said that increasing the temperature and reducing the time can lead to higher extraction yields.

3.2.6. Steam explosion

Steam explosion is a hydrothermal process that involves using high-pressure saturated steam for 1 to 10 minutes to treat keratinous biomass, which is kept at temperatures between 180 and 230 °C in a reactor [37]. When the treatment is almost complete, the pressure drops quickly, causing the rigid fibers in the biomass to break explosively [37]. The result of the process is influenced by a number of factors, including temperature, resistance time, particle size, and moisture [38].

The steam flash explosion is a more advanced version of the steam explosion and was created to improve the ability of feathers to dissolve in water and other solvents before further treatment [37]. This technique involves maintaining steam pressure between 1.4 and 2 MPa for 30 seconds to 5 minutes, which is then followed by an abrupt explosion [37]. Compared to traditional steam explosion methods, it offers quicker

processing time, increased digestibility, dissolubility, and low-temperature usage. Nevertheless, the quality of the by-product may be compromised [38].

3.2.7. Thermal hydrolysis

Thermal hydrolysis is a method of breaking down biomass using water, heat, and pressure to convert protein into oligopeptides, and its rate of dissolution depends on the temperature and duration of the treatment [37]. It involves two steps: first, the denaturation of the keratin protein network of the intermediate filaments, and second, the breakage of the disulfide bonds that connect the keratinous fibrils together [67]. This two-step process results in a keratin recovery yield of around 70% [67].

3.2.8. Keratin extraction method performance and comparison

Different methods of keratin extraction can be used depending on the desired outcome. In an experiment conducted by Shavandi, *et al*. various methods including alkali hydrolysis, reduction, oxidation, and ionic liquids were used to extract keratin from wool for use as a biopolymer, and the results are summarized in Table 3 [68]. The highest extraction yield was obtained with the reduction method (54%), followed by the ionic liquid (51%), alkali (25%), and oxidation method (5%) [68]. The oxidation method had the lowest yield, but it showed the highest content of cysteine-S-sulfonated residues, and it also has the advantage of separating the extracted keratin into α, β, and γ keratoses fractions allowing it to be more soluble to be used in products that require high solubility [68]. The extraction yield observed with the ionic liquid method can be attributed to the loss of water-soluble keratin protein that remained in the solution and did not precipitate [68].

The ionic liquid method had the highest amount of protein (97%), followed by the oxidation (93%), reduction (86%), and alkali method (63%) [68]. The alkali method resulted in lower extraction yield and protein content, likely due to extensive hydrolysis and degradation of amino acids [68]. The characterization showed different structures for each method, such as flake and fibrous for reduction, plate-like for alkali, porous and granular for oxidation, and highly granular with a dense microstructure for ionic liquids [68]. The keratin extracts were mostly milky white, except for the ionic liquid and alkali extracts which were yellow [68].

The alkali method led to a disruption of the protein's backbone structure and lower molecular weight (10 kDa), making it unsuitable for preserving wool keratin structure and its harsh effects on keratin proteins limits its application and commercial use [68]. The oxidation method produced high molecular weight keratin extracts and had the highest content of cysteine-S-sulfonated residues, but it had the lowest extraction yield [68]. The viscosity of the polymer solution is an important factor for the technicality of processing for biomedical application, it was highest for extracts obtained from

reduction and oxidation methods, while the alkali extraction showed the lowest viscosity [68].

Table 3. Comparison results for some keratin extraction methods.

Extraction methods	Yield (%)	Protein content (%)	Structural characteristics	Color	Viscosity	Reference
Reduction	54	86	Flake and fibrous	Milky white	High	[68]
Ionic liquid	51	97	Highly granular	Milky white	High	
Alkaline hydrolysis	25	63	Plate-like	Milky white	Low	
Oxidation	5	93	Porous and granular	Yellow	High	

3.2.9. Keratin extraction method purpose, advantages, and disadvantages

There are so many keratin extraction methods, but not all of them have been compared and evaluated in experiments. Information was gathered from previous research and experiments to present a comparison of different keratin extraction methods. Table 4 provides an overview of these methods, highlighting their purpose, advantages, disadvantages, and application fields. The comparison aims to identify the most suitable extraction method for the desired outcome and facilitate decision making.

Table 4. Keratin extraction method purpose, advantages, and disadvantages.

Extraction method	Purpose	Advantages	Disadvantages	Applications
Redox processes	Reduction of fiber from hair and wool [37].	Effective extraction, functional groups oxidation [63].	Toxic chemical agents, low extraction yield, prolonged processing times [64].	Textile and cosmetic industry [37].
Chemical hydrolysis	Wool solubilization and breakdown of keratin [41].	High efficiency [37].	Loss of amino acids, high temperatures and amounts of chemicals [38].	Biomedical applications, textile industry [41].
Enzymatic and microbial treatment	Degradation and hydrolyzation into peptides [41].	Milder treatment conditions [38]. Low energy consumption [37].	Require reducing agents and degradation of protein [37].	Biomedical applications, food industry, animal feed, fertilizers [41].
Ionic liquid dissolution	When there is limitation for other solvents due to evaporation [41].	Safe, recyclable, and non-volatile solvent [37].	Destruction of alpha-helix structure [38].	Polymer production, catalysts, ion-conductive media [41].
Microwave irradiation	Uniform and rapid extraction and reduction of activation energy [37].	Uniform heating, short extraction time [41].	Degradation of amino acid sidechains [41].	Biomedical applications, textile industry [37].
Steam explosion	Fast hydrothermal treatment for keratinous biomass [37].	Quick processing time, low environment impact and low cost [41].	Quality of by-product may be compromised [38].	Bio-based material production [41].
Thermal hydrolysis	Conversion of keratin into oligopeptides [37].	High keratin recovery yield [67].	Requires heat and pressure [37].	Protein hydrolysates, biofuel, and animal feed production [67].

4 Applications

After protein's extraction, keratin and collagen can be used in a wide range of applications in various fields, as they have a significant commercial value due to their unique properties and versatility. A graphical summary of the applications can be seen below (Figure 10).

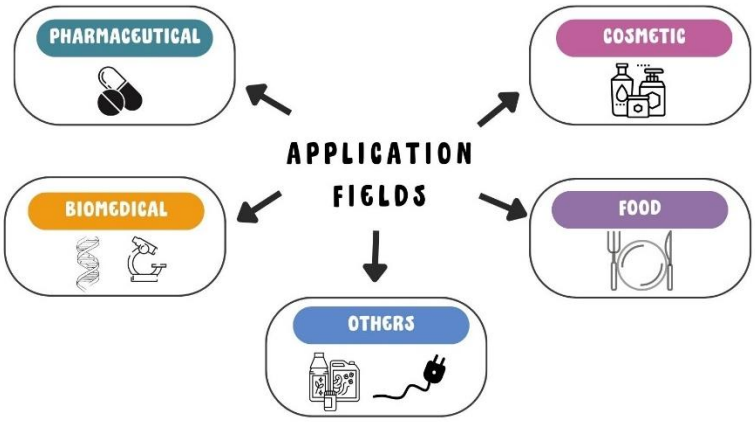


Figure 10. Graphical summary of collagen and keratin applications in the industry.

4.1. Collagen applications

Collagen has distinct physicochemical properties and a diversified nature which makes it suitable for various industries, including the pharmaceutical, biomedical, food, and cosmetics. As a result, its use has increased globally in recent decades.

4.1.1. Pharmaceutical Industry

Collagen is a valuable component in pharmaceutical applications like injectable dispersions, micro-particles, and drug delivery systems due to its ability to attach to cells, weak antigenicity, biocompatibility, and biodegradability [50]. Additionally, oral collagen supplements are popularly used as anti-aging products and are easily accessible in the market [56].

4.1.2. Biomedical industry

Collagen is commonly used in medical procedures as a hemostatic agent and surgical suture, due to its shorter healing time compared to other materials [62]. Collagen has a variety of applications in various medical fields such as tissue engineering, bone substitutes, eye implants, drug, gene, and protein delivery, and as a biomaterial for

forming organoids or neo-organs in gene therapy [62]. In addition, it is also applied in skin replacement, augmentation of soft tissues, artificial skin dermis, heart valve replacement, wound repairing, bone and cartilage reconstruction, tendon and ligament repair, cornea and contact lens grafting, and vascular system grafting and replacement [44].

4.1.3. Food industry

Collagen is used as a functional ingredient and dietary supplement in a variety of food products, as well as in confectionery and beverages to enhance their nutritional and functional characteristics [56]. Collagen is commonly used as a nutraceutical food supplement to combat skin aging and dryness, maintaining bone health, treating brittle nails, and alleviating osteoarthritis pain [69]. In acidic products, collagen is used as a food additive and acts as an emulsifier by improving the texture of meat products [70]. Hydrolyzed collagen can be used as a fat substitute in processed meat products like sausages [50]. Collagen is also employed as a food coating agent, which enhances the juiciness and extends the shelf life of food [70]. In addition, food-grade collagens are used widely as sausage casings and are applied to various meat and fish products such as hamburgers, boneless hams, netted roasts, and fish fillets [50].

4.1.4. Cosmetic industry

Collagen products are used in the cosmetic field to moisturize and soften dry skin and maintain a healthy skin [44]. Additionally, collagen properties make it suitable for the development of highly moisturizing creams and gels that protect against UV radiation, reduce aging and wrinkling, and help in wound healing [59]. In comparison to synthetic chemicals, collagen hydrolysates are often combined with natural oils in cosmetic formulations to improve the antimicrobial effects [44].

4.2. Keratin applications

The chemical, physical, and biological properties of keratin have led to many applications across various industries. Keratin-based materials are known for their remarkable mechanical durability, outstanding biocompatibility, and easy biodegradability, making them highly valuable, practical, and versatile [38].

4.2.1. Biomedical industry

Keratin has various biomedical applications due to its inherent capability to simplify cell adhesion, proliferation, and tissue regeneration [37]. It is used to facilitate bone regeneration, nerve repair, hemostasis, and wound healing [37]. Keratin-based biomaterials are used for regrowth and regeneration of dead or damaged tissue, nerve repair, dental tissue engineering, ocular surface reconstruction, and drug delivery [71]. Keratin hydrogels, polymers, and films are used for their effectiveness in reducing

burn progression and producing biodegradable and biocompatible materials that aid in tissue engineering and wound repair as they promote skin regeneration [71].

4.2.2. Cosmetic industry

Keratin hydrolysates are widely used in cosmetic applications for both hair and skin care. These peptides have a hydrating effect on the skin, reinforce the skin barrier function, and minimize the moisture loss through the skin's layers [37]. Due to the high cysteine content found in hydrolyzed keratins, they are used in a wide range of cosmetic products such as mascaras, bath soaps, detergents, hair growth supplements, and hair products [2]. Keratin is a crucial ingredient in many hair care products, including shampoos, conditioners, and hair loss concealing products due to its ability to strengthen and protect the hair [40]. Furthermore, it is often added to hair toners and hair shading sprays to enhance uniform color retention [40].

4.2.3. Other industries

In the biomaterials field, keratin-rich waste is used as an organic fertilizer due to its abundance of carbon and nitrogen [40]. Keratin extracted from hair can be used in creating construction materials and wall plaster for homes [37]. Moreover, keratin-based biodegradable materials are used in the making of electronic components like resistors, capacitors, and inductors [72]. Additionally, keratin is incorporated in textiles, like wool and silk, to enhance their durability and elasticity [38].

5 Regeneration of recovered proteins: electrospinning

To regenerate the recovered proteins into new fibers, one of the most frequently used technique is called electrospinning. It involves creating fibrous structures by elongating electrified jets using an external electric field [73]. Electrospinning has emerged as a promising technology for generating micro and nanofibers, offering benefits like large specific surface area, high porosity, and uniform pore size [74]. This is a technique with successful applications in tissue engineering and pharmaceutical industry, as it enhances the physicochemical properties, such as solubility, dissolution rate, bioavailability, and stability [73].

To carry out the electrospinning process, a polymer solution is compacted in a syringe and ejected through a needle using a syringe pump [75]. When an electric field is applied to the polymer solution, it forms what is called as Taylor's cone at the end of the needle [75]. Consequently, to propel the resulting flow towards the collector an electric current, typically ranging from 5 to 25 kV, is applied directly to the collector and needle, which are usually placed at a distance of 10 to 20 cm from each other [75]. This leads to the creation of fibrous materials that mimic the ultrastructure of the native extracellular matrix of tissues [76]. The setup configuration of electrospinning can be seen in Figure 11.

a) b)

Figure 11. Electrospinning setup configuration: whole instrumental equipment (a); zoom showing the syringe and collector (b).

5.1. Collagen electrospinning

Electrospinning has a great potential in replicating the native collagen fibrils present in living organisms [77]. The selection of an appropriate solvent is crucial for collagen electrospinning, as it should facilitate fiber formation without compromising the integrity of collagen [78]. Common choices include highly volatile fluorinated solvents, such as HFP (1,1,1,3,3,3-hexafluoro-2-propanol), TFE (2,2,2-trifluoroethanol), acetic acid, and PBS/EtOH (phosphate-buffered saline/ethanol), with HFP being the most popular choice [77].

However, electrospun collagen nanofibers exhibit weak mechanical properties and rapid water solubility, making them less ideal for tissue engineering applications due to rapid degradation [78]. To address these challenges and improve their performance, collagen nanofibers are often cross-linked or blended with synthetic polymers or inorganic substances [78]. Cross-linking techniques can be employed using chemical (glutaraldehyde, genipin, carbodiimides), enzymatic (transglutaminase, tyrosinase, laccase), or physical methods (UV radiation, gamma radiation, hydrothermal treatment) [78].

5.2. Collagen electrospinning comparative chart

The following table provides different methods and applications for collagen's waste recovery and electrospinning techniques.

Table 5. Collagen's electrospinning techniques and applications.

Protein waste source	Recovery technique conditions	Electrospinning conditions	Morphology and fiber diameter	Properties of resulting fiber	Application	Reference
fish scales	Salt solubilization	35 kV, 10 cm, 0.9 mL/h (crosslink: glutaraldehyde)	smooth and uniform 53 nm-259 nm	good moisture content, good interfacial interactions, easy release of the drug molecules	Drug delivery	[79]
Tilapia skin	Salt and acid solubilization	20 kV, 20 cm, 1.8 mL/h	long thick uniform 1470 nm-3820 nm	high hydrogen bonding potential, high collagen reduction	Drug delivery and tissue engineering	[80]
Crust leather	Hydrolysis	20 kV, 20 cm, 1 mL/h	smooth and uniform 104 nm	high thermal stability, good tensile strength, high sound absorption, good thermal stability	Sound absorbance	[81]
tanned leather shavings	Hydrolysis	25 kV, 20 cm, 1.8 mL/h	smooth and uniform 200 nm-700 nm	High antibacterial ability, high electrical output, good stretchability, and excellent mechanical properties	Power supply and mechanical energy	[82]
eggshell	None, purchased	20 kV, 15 cm, 2 mL/h	uniformed, oriented, smooth, and bead-free 111 nm-156 nm	optimal biocompatibility, no cytotoxicity, antibacterial efficacy, and good mechanical properties	Wound healing	[83]
rabbit skin	Heating and drying	20 kV, 10 cm, 3 mL/h (crosslink: glutaraldehyde and ethanol)	uniform with roughness surface 72 nm-181 nm	good biocompatibility, low cell proliferation, good cytocompatibility, good antibacterial activities	Wound healing	[84]
Fish bone	Dialyzed	20 kV, 15 cm, 0.5 mL/h	smooth, uniform 305 nm	high proliferation rate, good biocompatibility, good cell viability, good mechanical properties, nontoxicity, and rapid wound healing	Wound healing	[85]

The analysis of collagen electrospinning reveals a limited number of findings. In *Table 5*, each analysis corresponds to a distinct protein waste source, indicating a lack of correlation. Notably, hydrolysis for leather and salt solubilization for fish skin were

employed more than one time, suggesting a potential relationship between these resources. Furthermore, certain protein wastes were purchased with unknown recovery methods, while manual recovery was applied for others.

However, the conditions and properties of the extracted materials varied significantly across all cases, lacking correlation. Table 2 illustrates that the methods employed aimed either to preserve collagen's stability and functionality through solubilization or to optimize yield and purity via hydrolysis.

Regarding electrospinning conditions, variability can be seen. Although a preferred voltage of 20 kV is evident, voltages of 25 kV and 35 kV are also observed. Collector distances primarily fall within the 15 cm to 20 cm range, and flow rates range from 0.5 mL/h to 3 mL/h. Cross-linking is applied in only two cases, predominantly using glutaraldehyde. Due to the limited data, it is challenging to establish correlations, given the diverse protein waste sources and recovery techniques.

Morphologically, the nanofibers exhibit a predominantly smooth and uniform structure in most cases, with some noting orientation and the absence of beads. Nanofiber diameters vary considerably, ranging from 104 nm to 405 nm. The reported properties exhibit significant divergence, based on specific applications and processing parameters.

The primary applications focus on pharmaceutical and biomedical purposes, specifically wound healing, and tissue regeneration. The selection of collagen sources and electrospinning conditions depends on the intended application and the desired outcomes. Further research and optimization are essential for having a full potential of collagen-based materials in various fields.

5.3. Keratin electrospinning

Challenges arise when attempting keratin electrospinning due to its low molecular weight [86]. To address this issue, blending keratin with suitable polymers has been explored as an alternative solution [76]. Some of the polymers that are commonly used are polyvinyl alcohol (PVA), poly (3-hydroxybutyric acid-co-3-hydroxyvaleric acid) (PHBV), polycaprolactone (PCL), polyacrylonitrile (PAN), and polyethylene oxide (PEO) as they enhance the spinnability of keratin making it stable and successful at creating nanofiber biomaterials [76]. Furthermore, natural keratin possesses relatively poor viscoelastic properties and lack of flexibility, making them challenging for electrospinning [87]. For this reason, controlling the viscosity of keratin solution is crucial for successful electrospinning results. If the viscosity is too low, the spinning strength decreases, leading to spinning failure, while high viscosity increases resistance of spinning, also resulting in failure [88].

In the electrospinning process, the keratin solution is sprayed under a strong electric field to form keratin filaments, which are then collected and solidified to create nanofibrous films [88]. These keratin-based nanofibrous films offer a large specific area, high aspect ratio, high porosity, uniformity, and easy surface treatment [88]. These films exhibit attractive attributes such as good antibacterial properties, high biocompatibility, excellent wound-healing capabilities, good filtration performance, and low trans-epidermal water loss [88].

5.4. Keratin electrospinning comparative chart

The table below provides various techniques and applications of keratin waste recovery and electrospinning. It can be clearly seen that the field of keratin electrospinning offers more relevant information compared to collagen electrospinning.

Table 6. Keratin's electrospinning techniques and applications.

Protein waste source	Recovery technique conditions	Electrospinning conditions	Morphology and fiber diameter	Properties of resulting fiber	Application	Reference
Chicken feathers	Chemical hydrolysis	12 kV, 15 cm, 0.1 mL/h	smooth 596 nm	imperfect crystals, low thermal stabilities, low thermomechanical properties, high antibacterial activity	Wound healing packaging	[87]
Chicken feathers	Chemical hydrolysis	20 kV, 10 cm, 0.5 mL/h	smooth and uniform 212 nm-280 nm	high porosity, good mechanical properties, high antibacterial activity	Wound healing	[89]
Chicken feathers	Chemical hydrolysis	18 kV, 15 cm, 0.5 mL/h (crosslink: glutaraldehyde)	bead-free, smooth, and uniform 417 nm	good mechanical strength, good water resistance, good water absorption, good hydrophilicity, good thermal degradation behavior, strong swelling, no cytotoxicity	Wound healing	[90]
Chicken feathers	Chemical hydrolysis	18 kV, 12 cm, 0.05 mL/h (crosslink: citric acid vapor)	smooth and uniform 250.83 nm-338.79 nm	excellent compatibility of components, excellent thermal stability, excellent water resistance, and excellent mechanical properties	Wound healing	[86]
Chicken feathers	Chemical hydrolysis Enzymatic treatment	25 kV, 10 cm, 1 mL/h	highly porous, uniform 140 nm-400 nm	good relative activity, good thermal stability, high degree of degradation	Bioactive peptides in food and pharmaceutical	[91]
Chicken feathers	Redox process	30 kV, 20 cm, 3 mL/h	Smooth, evenly distributed, cylindrical 170 nm- 234 nm	good hydrophilicity, high degradation rate, good stability	Tissue regeneration	[92]
Wool	Redox process	16 kV, 15 cm, 0.5 mL/h (crosslink: thermal, oxygen, UV)	smooth 359.4 nm- 459.7 nm	good thermal stability, good mechanical properties, excellent biocompatibility, good cell adhesion and proliferation, nontoxicity	Tissue engineering	[76]
Wool	Redox process	18 kV, 20 cm, 0.6 mL/h (crosslink: thermal)	uniform and monodispersed 167 nm- 289 nm	good thermal stability, good mechanical properties, good cytocompatibility	Tissue regeneration	[93]

Table 6 (continued). Keratin's electrospinning techniques and applications.

Protein waste source	Recovery technique conditions	Electrospinning conditions	Morphology and fiber diameter	Properties of resulting fiber	Application	Reference
Wool	Redox process	20 kV, 20 cm, 0.5 mL/h	smooth and uniform 309 nm-543 nm	low molecular weight, good biocompatibility, good antibacterial activity, high chlorine content, good hydrophobicity	Wound healing	[94]
Wool	Redox process	20 kV, 15 cm, 1.8 mL/h	defects-free, smooth, and cylindrical 290 nm	high swelling behavior, high permeation, good adhesion properties, high swelling ratio, high porosity, good mechanical properties	Drug delivery and wound dressing	[95]
Wool	Redox process	20 kV, 25 cm, 0.1 mL/h	uniform, randomly orientated, and wide 155 nm-168 nm	excellent flexibility, excellent permeability, low filtration resistance, excellent water–vapor transmission rate, strong antibacterial activity, excellent bacterial filtration efficiency	Antibacterial membrane	[96]
Wool	Ionic liquid	12.05 kV, 15 cm, 0.8 mL/h	uniform and bead free 180 nm	excellent wetting property, high moisture permeability, good water transport performance, good antibacterial activity	Protein separation, neural tissue application	[97]
Goat hair	Redox process	20 kV, 22 cm, 0.1 mL/h	fine, randomly oriented, bead free 55 nm	good thermal stability, poor mechanical properties, excellent dye removal efficiency	Filtration	[98]
Goat hair	Manual	12 kV, 12 cm, 0.8 mL/h	Uniform and bead free 100 nm	good wettability, good hydrophilicity, strong antifouling properties, good dye removal efficiency, good mechanical properties	Filtration	[99]
Pig nails	Redox process	25 kV, 15 cm, 0.1 mL/h	smooth and uniform 430 nm-940 nm	good mechanical properties, high thermal stability, good adsorption	Metal adsorption	[100]

Table 6 reveals that the majority of keratin extraction and electrospinning efforts are centered around chicken feathers and wool. Notably, a correlation exists between the recovery technique and the source of protein waste. For instance, chemical hydrolysis is predominantly employed for chicken feathers, while redox process is employed for wool. These findings align with those presented in Table 4, showing a preferred method for these types of analyses. However, it's essential to acknowledge that each experiment was conducted under distinct conditions, leading to different results. Despite the acknowledged superior yield resulting from ionic liquids, they are not commonly applied due to their high costs and complexity.

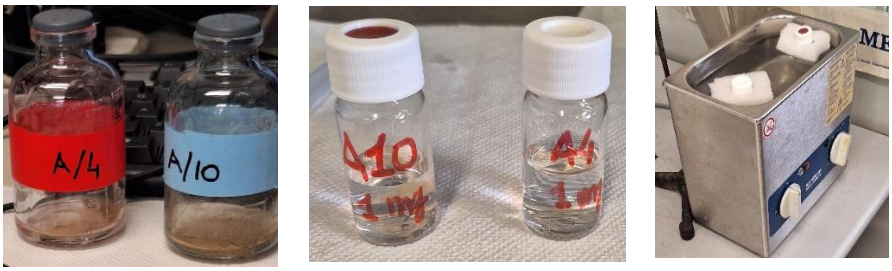
In terms of electrospinning conditions, voltages range from 12 kV to 30 kV, collector distances range from 10 cm to 25 cm oscillating mostly around 20 cm, and flow rates vary from 0.05 mL/h to 3 mL/h. Some analyses incorporate additives to modify keratin fibers, such as PLA, PEG400, PAN, and PVA, potentially influencing the resulting properties. However, establishing a direct correlation between methods is challenging, given the different outcomes for each case and the presence of multiple influencing factors. The conditions applied employ a notable influence on fiber diameter and morphology, making them unique in each instance.

Crosslinking conditions exhibit variability and are not applied in all cases, potentially impacting the properties and stability of the resulting fibers. While morphology results differ across analyses, the majority are characterized as smooth and uniform. Additionally, the properties of the resulting fibers display significant diversity across cases. For this reason, establishing a clear correlation between electrospinning conditions and resulting morphology or fiber diameter becomes challenging.

The predominant applications narrow on the pharmaceutical and biomedical field, primarily for wound healing and tissue regeneration. This emphasizes the adaptability of keratin-based materials in addressing medical challenges. The applications are mostly targeted into the pharmaceutical field, as most of them are used for wound healing and tissue regeneration. Depending on specific goals and reactant availability, different approaches can be taken to obtain distinct results.

6 Real case laboratory analysis

In the frame of an ongoing research project targeted to a circular economy of keratin extracted from feather waste, some experimental analyses were conducted in the laboratory, specifically targeting keratin extraction. The keratin samples (Figure 12a) had previously undergone biochemical and enzymatic recovery, performed by a group of collaborators, for 4 and 10 days respectively, followed by lyophilization. Notably, these samples exhibited a brown color and the possibility of melanin formation due to microbial activity. Same colored results were obtained for the chicken feathers keratin extraction performed by *Khumalo et al.* [92]. Consequently, the resulting mixtures were assumed to be a keratin blend and stored at -20°C.



a) b) c)

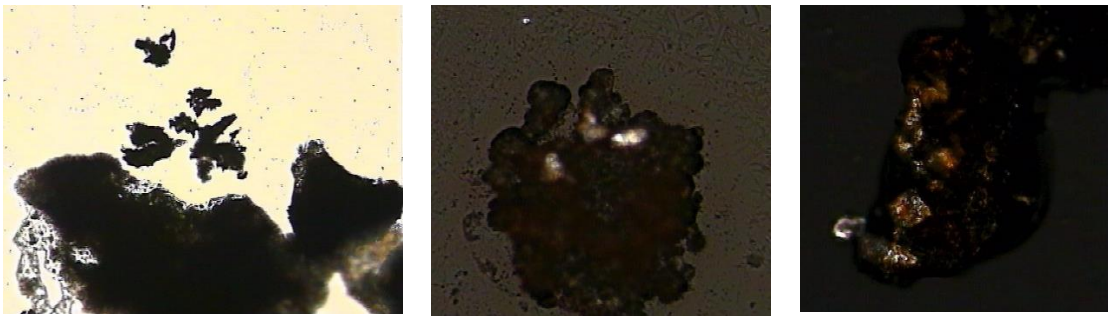
Figure 12. Keratin samples and procedures: a) lyophilized samples after enzymatic hydrolysis; b) samples dissolved in water; c) ultra-sound assisted solubilization.

A 0.1 M solution was prepared for each sample using two times deionized water (Figure 12b). An ultra-sound bath was employed for solubilization purposes (Figure 12c), as keratin is typically insoluble in water. For measuring the charge originating from amino acids, zeta-potential was measured on the solutions with a value of -40 mV to generate an attraction to positive potentials. Multiple peaks were observed, reflecting the presence of variously charged residues. Furthermore, an FT-IR analysis was performed on the solutions, confirming the presence of the expected functional groups, such as amines and carboxylic acids.

Dynamic light scattering analyses were conducted in plastic cuvettes for the solutions, with resulting particle sizes of approximately 420 nm and 66 nm for the 4-day sample, and 458 nm and 70 nm for the 10-day sample. The lack of significant difference in particle size between the two samples suggest either the presence of two diverse keratin populations or alterations due to residues from the recovery processes within the keratin mixture.

The lyophilized solid keratin samples were observed under an optical microscope at 10x magnification (Figure 13a). When polarized light was applied, it was possible to see brighter spots indicative of broader structures exhibiting a higher degree of order, a characteristic often expected for proteins (Figure 13b). To enhance visibility under

polarized light, staining agents were employed (Figure 13c), such as Congo red, typically used for living tissues and ordered structures, and thioflavin, which is more sensitive to fibers. The results displayed intense brightness and straight patterns, indicating a structured powder with higher crystallinity. The presence of crystals could be attributed to inherent sample properties or the lyophilization process.



a) b) c)

Figure 13. Keratin samples under optical microscope and scattering light: a) view under the optimal microscope; b) view under polarized light; c) view under polarized light after applying staining agents.

7 Conclusions

This literature review highlights the significant potential of a circular economy approach in recovering and valorizing food protein waste, with a specific emphasis on collagen and keratin. These two proteins offer unique properties that make them valuable in several industries, including pharmaceuticals, biomedical, food, cosmetics, and more.

Extraction methods for collagen and keratin are discussed, emphasizing the need for the implementation of highly efficient and environmentally conscious techniques to optimize the recovery. A comprehensive analysis of each extraction method is provided, offering insights into protein recovery by mentioning its specific purpose, inherent advantages, associated disadvantages, and overall performance.

Furthermore, the review highlights the application of electrospinning as a promising technology to transform the recovered collagen and keratin into nanofibers, offering benefits such as enhanced physicochemical and mechanical properties. This technique has proven valuable specifically in the biomedical and pharmaceutical fields, especially in wound healing and tissue regeneration.

One important consideration for further laboratory research is the application of electrospinning to the ongoing research project from feather waste to transform recovered keratin into practical applications in different fields. Additionally, the applications of recovered proteins in the food field should be further analyzed, as they haven't been explored much yet. This will be a great opportunity to prevent the loss of valuable properties and reduce food waste aligning with the goals of the circular economy.

References

[1] R. Santagata, M. Ripa, A. Genovese, and S. Ulgiati, "Food waste recovery pathways: Challenges and opportunities for an emerging bio-based circular economy. A systematic review and an assessment," *Journal of Cleaner Production*, vol. 286. 2021. doi: 10.1016/j.jclepro.2020.125490.

[2] M. Peydayesh, M. Bagnani, W. L. Soon, and R. Mezzenga, "Turning Food Protein Waste into Sustainable Technologies," *Chem Rev*, Jun. 2022, doi: 10.1021/acs.chemrev.2c00236.

[3] U. Schweiggert-Weisz, P. Eisner, S. Bader-Mittermaier, and R. Osen, "Food proteins from plants and fungi," *Current Opinion in Food Science*, vol. 32. 2020. doi: 10.1016/j.cofs.2020.08.003.

[4] J. Małecki, S. Muszyński, and B. G. Sołowiej, "Proteins in food systems—bionanomaterials, conventional and unconventional sources, functional properties, and development opportunities," *Polymers*, vol. 13, no. 15. 2021. doi: 10.3390/polym13152506.

[5] M. Henchion, M. Hayes, A. M. Mullen, M. Fenelon, and B. Tiwari, "Future protein supply and demand: Strategies and factors influencing a sustainable equilibrium," *Foods*, vol. 6, no. 7, 2017, doi: 10.3390/foods6070053.

[6] A. N. A. Aryee, D. Agyei, and C. C. Udenigwe, "Impact of processing on the chemistry and functionality of food proteins," in *Proteins in Food Processing, Second Edition*, 2017. doi: 10.1016/B978-0-08-100722-8.00003-6.

[7] F. Zhubi-Bakija *et al.*, "The impact of type of dietary protein, animal versus vegetable, in modifying cardiometabolic risk factors: A position paper from the International Lipid Expert Panel (ILEP)," *Clinical Nutrition*, vol. 40, no. 1, 2021, doi: 10.1016/j.clnu.2020.05.017.

[8] H. Kamal, C. F. Le, A. M. Salter, and A. Ali, "Extraction of protein from food waste: An overview of current status and opportunities," *Comprehensive Reviews in Food Science and Food Safety*, vol. 20, no. 3. 2021. doi: 10.1111/1541-4337.12739.

[9] T. M. Hicks and C. J. R. Verbeek, "Protein-Rich By-Products: Production Statistics, Legislative Restrictions, and Management Options," in *Protein Byproducts: Transformation from Environmental Burden Into Value-Added Products*, 2016. doi: 10.1016/B978-0-12-802391-4.00001-X.

[10] A. P. Shirsath and M. M. Henchion, "Bovine and ovine meat co-products valorisation opportunities: A systematic literature review," *Trends in Food Science and Technology*, vol. 118. 2021. doi: 10.1016/j.tifs.2021.08.015.

[11] T. Ahmad *et al.*, "Treatment and utilization of dairy industrial waste: A review," *Trends in Food Science and Technology*, vol. 88. 2019. doi: 10.1016/j.tifs.2019.04.003.

[12] A. F. Pires, N. G. Marnotes, O. D. Rubio, A. C. Garcia, and C. D. Pereira, "Dairy by-products: A review on the valorization of whey and second cheese whey," *Foods*, vol. 10, no. 5. 2021. doi: 10.3390/foods10051067.

[13] F. Toldrá, M. Reig, and L. Mora, "Management of meat by- and co-products for an improved meat processing sustainability," *Meat Sci*, vol. 181, 2021, doi: 10.1016/j.meatsci.2021.108608.

[14] Mònica Toldrà, S. A. Lynch, R. Couture, and C. Álvarez, "Blood proteins as functional ingredients," in *Sustainable Meat Production and Processing*, 2018. doi: 10.1016/B978-0-12-814874-7.00005-5.

[15] E. ben Slimane and S. Sadok, "Collagen from cartilaginous fish by-products for a potential application in bioactive film composite," *Mar Drugs*, vol. 16, no. 6, 2018, doi: 10.3390/md16060211.

[16] Z. Shea, W. M. Singer, and B. Zhang, "Soybean Production, Versatility, and Improvement," in *Legume Crops [Working Title]*, 2020. doi: 10.5772/intechopen.91778.

[17] Y. Wang and L. Serventi, "Sustainability of dairy and soy processing: A review on wastewater recycling," *Journal of Cleaner Production*, vol. 237. 2019. doi: 10.1016/j.jclepro.2019.117821.

[18] J. Y. Chua and S. Q. Liu, "Soy whey: More than just wastewater from tofu and soy protein isolate industry," *Trends in Food Science and Technology*, vol. 91. 2019. doi: 10.1016/j.tifs.2019.06.016.

[19] M. M. Rahman, K. Mat, G. Ishigaki, and R. Akashi, "A review of okara (soybean curd residue) utilization as animal feed: Nutritive value and animal performance aspects," *Animal Science Journal*, vol. 92, no. 1. 2021. doi: 10.1111/asj.13594.

[20] *Food Outlook – Biannual Report on Global Food Markets*. 2021. doi: 10.4060/cb7491en.

[21] USDA, "Oilseeds: World Markets and Trade," *Foreign Agricultural Service*, 2022.

[22] P. Ancuţa and A. Sonia, "Oil press-cakes and meals valorization through circular economy approaches: A review," *Applied Sciences (Switzerland)*, vol. 10, no. 21. 2020. doi: 10.3390/app10217432.

[23] K. Östbring, C. Tullberg, S. Burri, E. Malmqvist, and M. Rayner, "Protein recovery from rapeseed press cake: Varietal and processing condition effects on yield, emulsifying capacity and antioxidant activity of the protein rich extract," *Foods*, vol. 8, no. 12, 2019, doi: 10.3390/foods8120627.

[24] T. C. Confortin *et al.*, "Oil yields, protein contents, and cost of manufacturing of oil obtained from different hybrids and sowing dates of canola," *J Environ Chem Eng*, vol. 7, no. 2, 2019, doi: 10.1016/j.jece.2019.102972.

[25] B. S. Adeleke and O. O. Babalola, "Oilseed crop sunflower (Helianthus annuus) as a source of food: Nutritional and health benefits," *Food Science and Nutrition*, vol. 8, no. 9. 2020. doi: 10.1002/fsn3.1783.

[26] Z. Mohammadi Shad, C. Venkitasamy, and Z. Wen, "Corn distillers dried grains with solubles: Production, properties, and potential uses," *Cereal Chemistry*, vol. 98, no. 5. 2021. doi: 10.1002/cche.10445.

[27] U. Al-Turki, S. Duffuaa, and M. Bendaya, "Trends in turnaround maintenance planning: literature review," *Journal of Quality in Maintenance Engineering*, vol. 25, no. 2. 2019. doi: 10.1108/JQME-10-2017-0074.

[28] C. M. Galanakis, "Sustainable Applications for the Valorization of Cereal Processing By-Products," *Foods*, vol. 11, no. 2. 2022. doi: 10.3390/foods11020241.

[29] Y. Zheng, N. Gao, J. Wu, and B. Yin, "Rice bran protein: Extraction, nutraceutical properties, and potential applications," in *Rice Bran and Rice Bran Oil: Chemistry, Processing and Utilization*, 2019. doi: 10.1016/B978-0-12-812828-2.00011-1.

[30] M. Spaggiari, C. Dall'asta, G. Galaverna, and M. D. D. C. Bilbao, "Rice bran by-product: From valorization strategies to nutritional perspectives," *Foods*, vol. 10, no. 1. 2021. doi: 10.3390/foods10010085.

[31] M. Irakli, A. Lazaridou, and C. G. Biliaderis, "Comparative evaluation of the nutritional, antinutritional, functional, and bioactivity attributes of rice bran stabilized by different heat treatments," *Foods*, vol. 10, no. 1, 2021, doi: 10.3390/foods10010057.

[32] R. Jayawardena, J. D. Morton, C. S. Brennan, Z. F. Bhat, and A. E. D. A. Bekhit, "Meat co-products," in *Alternative Proteins: Safety and Food Security Considerations*, 2022. doi: 10.1201/9780429299834-11.

[33] M. Meyer, "Processing of collagen based biomaterials and the resulting materials properties," *Biomed Eng Online*, vol. 18, no. 1, 2019, doi: 10.1186/s12938-019-0647-0.

[34] C. Cao, Z. Xiao, C. Ge, and Y. Wu, "Animal by-products collagen and derived peptide, as important components of innovative sustainable food systems—a comprehensive review," *Critical Reviews in Food Science and Nutrition*. 2021. doi: 10.1080/10408398.2021.1931807.

[35] C. Cao *et al.*, "Effects of Extraction Methods on the Characteristics, Physicochemical Properties and Sensory Quality of Collagen from Spent-Hens Bones," *Foods*, vol. 12, no. 1, 2023, doi: 10.3390/foods12010202.

[36] U. G. K. Wegst, H. Bai, E. Saiz, A. P. Tomsia, and R. O. Ritchie, "Bioinspired structural materials," *Nat Mater*, vol. 14, no. 1, 2015, doi: 10.1038/nmat4089.

[37] C. R. Chilakamarry *et al.*, "Extraction and application of keratin from natural resources: a review," *3 Biotech*, vol. 11, no. 5. 2021. doi: 10.1007/s13205-021-02734-7.

[38] S. Feroz, N. Muhammad, J. Ranayake, and G. Dias, "Keratin - Based materials for biomedical applications," *Bioactive Materials*, vol. 5, no. 3. 2020. doi: 10.1016/j.bioactmat.2020.04.007.

[39] Z. Peng, X. Mao, J. Zhang, G. Du, and J. Chen, "Biotransformation of keratin waste to amino acids and active peptides based on cell-free catalysis," *Biotechnol Biofuels*, vol. 13, no. 1, 2020, doi: 10.1186/s13068-020-01700-4.

[40] C. Chaitanya Reddy *et al.*, "Valorization of keratin waste biomass and its potential applications," *Journal of Water Process Engineering*, vol. 40, 2021, doi: 10.1016/j.jwpe.2020.101707.

[41] A. Shavandi, T. H. Silva, A. A. Bekhit, and A. E. D. A. Bekhit, "Keratin: Dissolution, extraction and biomedical application," *Biomaterials Science*, vol. 5, no. 9. 2017. doi: 10.1039/c7bm00411g.

[42] H. Kamal, C. F. Le, A. M. Salter, and A. Ali, "Extraction of protein from food waste: An overview of current status and opportunities," *Comprehensive Reviews in Food Science and Food Safety*, vol. 20, no. 3. 2021. doi: 10.1111/1541-4337.12739.

[43] H. Jafari *et al.*, "Fish collagen: Extraction, characterization, and applications for biomaterials engineering," *Polymers*, vol. 12, no. 10. 2020. doi: 10.3390/polym12102230.

[44] M. Ahmed, A. K. Verma, and R. Patel, "Collagen extraction and recent biological activities of collagen peptides derived from sea-food waste: A review," *Sustainable Chemistry and Pharmacy*, vol. 18. 2020. doi: 10.1016/j.scp.2020.100315.

[45] A. M. E. Matinong, Y. Chisti, K. L. Pickering, and R. G. Haverkamp, "Collagen Extraction from Animal Skin," *Biology*, vol. 11, no. 6. MDPI, Jun. 01, 2022. doi: 10.3390/biology11060905.

[46] M. Yamauchi, Y. Taga, S. Hattori, M. Shiiba, and M. Terajima, "Analysis of collagen and elastin cross-links," in *Methods in Cell Biology*, vol. 143, 2018. doi: 10.1016/bs.mcb.2017.08.006.

[47] Y. Liu, D. Ma, Y. Wang, and W. Qin, "A comparative study of the properties and self-aggregation behavior of collagens from the scales and skin of grass carp (Ctenopharyngodon idella)," *Int J Biol Macromol*, vol. 106, 2018, doi: 10.1016/j.ijbiomac.2017.08.044.

[48] M. G. Gandolfi, P. Taddei, A. Pondrelli, F. Zamparini, C. Prati, and G. Spagnuolo, "Demineralization, collagen modification and remineralization degree of human dentin after EDTA and citric acid treatments," *Materials*, vol. 12, no. 1, 2019, doi: 10.3390/ma12010025.

[49] X. Zhang, S. Xu, L. Shen, and G. Li, "Factors affecting thermal stability of collagen from the aspects of extraction, processing and modification," *Journal of Leather Science and Engineering*, vol. 2, no. 1. 2020. doi: 10.1186/s42825-020-00033-0.

[50] T. R. L. Senadheera, D. Dave, and F. Shahidi, "Sea cucumber derived type i collagen: A comprehensive review," *Marine Drugs*, vol. 18, no. 9. 2020. doi: 10.3390/md18090471.

[51] K. M. Song, S. K. Jung, Y. H. Kim, Y. E. Kim, and N. H. Lee, "Development of industrial ultrasound system for mass production of collagen and biochemical characteristics of extracted collagen," *Food and Bioproducts Processing*, vol. 110, 2018, doi: 10.1016/j.fbp.2018.04.001.

[52] C. Cao, Z. Xiao, C. Ge, and Y. Wu, "Animal by-products collagen and derived peptide, as important components of innovative sustainable food systems—a comprehensive review," *Critical Reviews in Food Science and Nutrition*, vol. 62, no. 31. 2022. doi: 10.1080/10408398.2021.1931807.

[53] R. Ahmed, M. Haq, and B. S. Chun, "Characterization of marine derived collagen extracted from the by-products of bigeye tuna (Thunnus obesus)," *Int J Biol Macromol*, vol. 135, 2019, doi: 10.1016/j.ijbiomac.2019.05.213.

[54] Y. Tan and S. K. C. Chang, "Isolation and characterization of collagen extracted from channel catfish (Ictalurus punctatus) skin," *Food Chem*, vol. 242, 2018, doi: 10.1016/j.foodchem.2017.09.013.

[55] Z. Song *et al.*, "Characterization and comparison of collagen extracted from the skin of the Nile tilapia by fermentation and chemical pretreatment," *Food Chem*, vol. 340, 2021, doi: 10.1016/j.foodchem.2020.128139.

[56] A. León-López, A. Morales-Peñaloza, V. M. Martínez-Juárez, A. Vargas-Torres, D. I. Zeugolis, and G. Aguirre-Álvarez, "Hydrolyzed collagen-sources and applications," *Molecules*, vol. 24, no. 22. 2019. doi: 10.3390/molecules24224031.

[57] U. H. Mohamad Razali, H. Ya'akob, N. M. Sarbon, N. H. Zainan, D. J. Dailin, and D. N. A. Zaidel, "Improving collagen processing towards a greener approach: current progress," *Journal of Chemical Technology and Biotechnology*. 2023. doi: 10.1002/jctb.7332.

[58] M. M. Schmidt *et al.*, "Collagen extraction process," *International Food Research Journal*, vol. 23, no. 3. 2016.

[59] M. I. Avila Rodríguez, L. G. Rodríguez Barroso, and M. L. Sánchez, "Collagen: A review on its sources and potential cosmetic applications," *Journal of Cosmetic Dermatology*, vol. 17, no. 1. 2018. doi: 10.1111/jocd.12450.

[60] A. N. Akram and C. Zhang, "Effect of ultrasonication on the yield, functional and physicochemical characteristics of collagen-II from chicken sternal cartilage," *Food Chem*, vol. 307, 2020, doi: 10.1016/j.foodchem.2019.125544.

[61] Y. Zhang, L. Ma, L. Cai, Y. Liu, and J. Li, "Effect of combined ultrasonic and alkali pretreatment on enzymatic preparation of angiotensin converting enzyme (ACE) inhibitory peptides from native collagenous materials," *Ultrason Sonochem*, vol. 36, 2017, doi: 10.1016/j.ultsonch.2016.11.008.

[62] T. Muthukumar, G. Sreekumar, T. P. Sastry, and M. Chamundeeswari, "Collagen as a potential biomaterial in biomedical applications," *Reviews on Advanced Materials Science*, vol. 53, no. 1, 2018, doi: 10.1515/rams-2018-0002.

[63] S. G. Giteru, D. H. Ramsey, Y. Hou, L. Cong, A. Mohan, and A. E. D. A. Bekhit, "Wool keratin as a novel alternative protein: A comprehensive review of extraction, purification, nutrition, safety, and food applications," *Compr Rev Food Sci Food Saf*, vol. 22, no. 1, 2023, doi: 10.1111/1541-4337.13087.

[64] C. Zhang, L. Xia, J. Zhang, X. Liu, and W. Xu, "Utilization of waste wool fibers for fabrication of wool powders and keratin: a review," *Journal of Leather Science and Engineering*, vol. 2, no. 1. 2020. doi: 10.1186/s42825-020-00030-3.

[65] B. Ben Seghir *et al.*, "Preparation methods keratin and nanoparticles keratin from wool: a review," *Algerian Journal of Chemical Engineering*, vol. 01, pp. 0–000, 2020, doi: http://doi.org/10.5281/zenodo.3930645.

[66] A. A. Elgharbawy, F. A. Riyadi, M. Z. Alam, and M. Moniruzzaman, "Ionic liquids as a potential solvent for lipase-catalysed reactions: A review," *Journal of Molecular Liquids*, vol. 251. 2018. doi: 10.1016/j.molliq.2017.12.050.

[67] K. Tasaki, "A novel thermal hydrolysis process for extraction of keratin from hog hair for commercial applications," *Waste Management*, vol. 104, 2020, doi: 10.1016/j.wasman.2019.12.042.

[68] A. Shavandi, A. E. D. A. Bekhit, A. Carne, and A. Bekhit, "Evaluation of keratin extraction from wool by chemical methods for bio-polymer application," *J Bioact Compat Polym*, vol. 32, no. 2, 2017, doi: 10.1177/0883911516662069.

[69] M. Lupu, G. Gradisteanu Pircalabioru, M. Chifiriuc, R. Albulescu, and C. Tanase, "Beneficial effects of food supplements based on hydrolyzed collagen for skin care (Review)," *Exp Ther Med*, 2019, doi: 10.3892/etm.2019.8342.

[70] P. Hashim, M. S. Mohd Ridzwan, J. Bakar, and D. Mat Hashim, "Collagen in food and beverage industries," *International Food Research Journal*, vol. 22, no. 1. 2015.

[71] R. R. Yan *et al.*, "Preparation and applications of keratin biomaterials from natural keratin wastes," *Applied Microbiology and Biotechnology*, vol. 106, no. 7. 2022. doi: 10.1007/s00253-022-11882-6.

[72] P. Cataldi *et al.*, "Keratin-Graphene Nanocomposite: Transformation of Waste Wool in Electronic Devices," *ACS Sustain Chem Eng*, vol. 7, no. 14, 2019, doi: 10.1021/acssuschemeng.9b02415.

[73] S. M. Tan *et al.*, "Electrospinning and its potential in fabricating pharmaceutical dosage form," *Journal of Drug Delivery Science and Technology*, vol. 76. 2022. doi: 10.1016/j.jddst.2022.103761.

[74] J. Xue, J. Xie, W. Liu, and Y. Xia, "Electrospun Nanofibers: New Concepts, Materials, and Applications," *Acc Chem Res*, vol. 50, no. 8, 2017, doi: 10.1021/acs.accounts.7b00218.

[75] E. Álvarez-Castillo, M. Felix, C. Bengoechea, and A. Guerrero, "Proteins from agri-food industrial biowastes or co-products and their applications as green materials," *Foods*, vol. 10, no. 5. 2021. doi: 10.3390/foods10050981.

[76] J. Yang *et al.*, "Investigation of Keratin/Poly(ethylene oxide) Nanofiber Membrane Prepared with Different Post-crosslinking Method," *Fibers and Polymers*, vol. 24, no. 2, 2023, doi: 10.1007/s12221-023-00078-y.

[77] D. Visser, K. Rogg, E. Fuhrmann, J. Marzi, K. Schenke-Layland, and H. Hartmann, "Electrospinning of collagen: enzymatic and spectroscopic analyses reveal solvent-independent disruption of the triple-helical structure," *J Mater Chem B*, vol. 11, no. 10, 2023, doi: 10.1039/d2tb02602c.

[78] J. X. Law, L. L. Liau, A. Saim, Y. Yang, and R. Idrus, "Electrospun Collagen Nanofibers and Their Applications in Skin Tissue Engineering," *Tissue Engineering and Regenerative Medicine*, vol. 14, no. 6. 2017. doi: 10.1007/s13770-017-0075-9.

[79] A. P. Samanta, M. S. Ali, J. T. Orasugh, S. K. Ghosh, and D. Chattopadhyay, "Crosslinked nanocollagen-cellulose nanofibrils reinforced electrospun polyvinyl alcohol/methylcellulose/polyethylene glycol bionanocomposites: Study of material properties and sustained release of ketorolac tromethamine," *Carbohydrate Polymer Technologies and Applications*, vol. 3, 2022, doi: 10.1016/j.carpta.2022.100195.

[80] C. Bi *et al.*, "Effect of extraction methods on the preparation of electrospun/electrosprayed microstructures of tilapia skin collagen," *J Biosci Bioeng*, vol. 128, no. 2, 2019, doi: 10.1016/j.jbiosc.2019.02.004.

[81] S. Selvaraj, S. Ramalingam, S. Parida, J. R. Rao, and N. F. Nishter, "Chromium containing leather trimmings valorization: Sustainable sound absorber from collagen hydrolysate intercalated electrospun nanofibers," *J Hazard Mater*, vol. 405, 2021, doi: 10.1016/j.jhazmat.2020.124231.

[82] L. Li *et al.*, "Electrospun hydrolyzed collagen from tanned leather shavings for bio-triboelectric nanogenerators," *Mater Adv*, vol. 3, no. 12, 2022, doi: 10.1039/d2ma00457g.

[83] M. Bello *et al.*, "Electrospun poly (ε-caprolactone)-eggshell membrane nanofibrous mat as a potential wound dressing material," *Biochem Eng J*, vol. 187, 2022, doi: 10.1016/j.bej.2022.108563.

[84] E. Matei *et al.*, "Sustainable coated nanostructures based on alginate and electrospun collagen loaded with antimicrobial agents," *Coatings*, vol. 11, no. 2, 2021, doi: 10.3390/coatings11020121.

[85] R. Senthil, R. Berly, T. B. Ram, and N. Gobi, "Electrospun poly(Vinyl) alcohol/collagen nanofibrous scaffold hybridized by graphene oxide for accelerated wound healing," *International Journal of Artificial Organs*, vol. 41, no. 8, 2018, doi: 10.1177/0391398818775949.

[86] J. Ding *et al.*, "Environmentally friendly biological nanofibers based on waste feather keratin by electrospinning with citric acid vapor modification," *J Appl Polym Sci*, vol. 138, no. 18, 2021, doi: 10.1002/app.50348.

[87] D. A. Ertek, N. O. Sanli, Y. Z. Menceloglu, and S. Avaz Seven, "Environmentally friendly, antibacterial materials from recycled keratin incorporated electrospun PLA films with tunable properties," *Eur Polym J*, vol. 185, 2023, doi: 10.1016/j.eurpolymj.2022.111804.

[88] R. Wang and H. Tong, "Preparation Methods and Functional Characteristics of Regenerated Keratin-Based Biofilms," *Polymers*, vol. 14, no. 21. 2022. doi: 10.3390/polym14214723.

[89] E. Serag, A. M. A. El-Aziz, A. El-Maghraby, and N. A. Taha, "Electrospun non-wovens potential wound dressing material based on polyacrylonitrile/chicken feathers keratin nanofiber," *Sci Rep*, vol. 12, no. 1, 2022, doi: 10.1038/s41598-022-19390-3.

[90] W. Chen, Z. Gao, M. He, Y. Dou, G. Yin, and J. Ding, "Vapor-phase glutaraldehyde crosslinked waste protein-based nanofiber nonwovens as an environmentally friendly wound dressing," *React Funct Polym*, vol. 172, 2022, doi: 10.1016/j.reactfunctpolym.2022.105203.

[91] M. Sarathi, N. Doraiswamy, and G. Pennathur, "Enhanced stability of immobilized keratinolytic protease on electrospun nanofibers," *Prep Biochem Biotechnol*, vol. 49, no. 7, 2019, doi: 10.1080/10826068.2019.1605524.

[92] M. Khumalo, B. Sithole, T. Tesfaye, and P. Lekha, "Valorization of Waste Chicken Feathers: Fabrication and Characterization of Novel Keratin Nanofiber Conduits for Potential Application in Peripheral Nerve Regeneration," *J Nanomater*, vol. 2022, 2022, doi: 10.1155/2022/7080278.

[93] G. Suarato *et al.*, "From fabric to tissue: Recovered wool keratin/polyvinylpyrrolidone biocomposite fibers as artificial scaffold platform," *Materials Science and Engineering C*, vol. 116, 2020, doi: 10.1016/j.msec.2020.111151.

[94] Y. Wang, Y. Wang, L. Li, Y. Zhang, and X. Ren, "Preparation of antibacterial biocompatible polycaprolactone/keratin nanofibrous mats by electrospinning," *J Appl Polym Sci*, vol. 138, no. 7, 2021, doi: 10.1002/app.49862.

[95] G. Guidotti *et al.*, "Regenerated wool keratin-polybutylene succinate nanofibrous mats for drug delivery and cells culture," *Polym Degrad Stab*, vol. 179, 2020, doi: 10.1016/j.polymdegradstab.2020.109272.

[96] B. Shen *et al.*, "Preparation of Ag doped keratin/PA6 nanofiber membrane with enhanced air filtration and antimicrobial properties," *Polymers (Basel)*, vol. 11, no. 9, 2019, doi: 10.3390/polym11091511.

[97] X. Zhong, R. Li, Z. Wang, W. Wang, and D. Yu, "Eco-fabrication of antibacterial nanofibrous membrane with high moisture permeability from wasted wool fabrics," *Waste Management*, vol. 102, 2020, doi: 10.1016/j.wasman.2019.11.005.

[98] P. S. David, A. Karunanithi, and N. N. Fathima, "Improved filtration for dye removal using keratin–polyamide blend nanofibrous membranes," *Environmental Science and Pollution Research*, vol. 27, no. 36, 2020, doi: 10.1007/s11356-020-10491-y.

[99] A. Karunanidhi, P. S. David, and N. N. Fathima, "Electrospun Keratin-Polysulfone Blend Membranes for Treatment of Tannery Effluents," *Water Air Soil Pollut*, vol. 231, no. 6, 2020, doi: 10.1007/s11270-020-04682-z.

[100] L. Wei *et al.*, "Extraction of Keratin from Pig Nails and Electrospinning of Keratin/Nylon6 Nanofibers for Copper (II) Adsorption," *Polymers (Basel)*, vol. 15, no. 2, 2023, doi: 10.3390/polym15020467.

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