Received: 4 September 2022 | Revised: 4 November 2022 | Accepted: 17 November 2022

DOI: 10.1111/1541-4337.13087

COMPREHENSIVE REVIEWS IN FOOD SCIENCE AND FOOD SAFETY

Wool keratin as a novel alternative protein: A comprehensive review of extraction, purification, nutrition, safety, and food applications

Stephen G. Giteru[1] | **Derek H. Ramsey**[1] | **Yakun Hou**[2] | **Lei Cong**[3] | **Anand Mohan**[1] | **Alaa El-Din Ahmed Bekhit**[4]

[1]Alliance Group Limited, Invercargill, New Zealand

[2]College of Food Science and Technology, Hebei Agricultural University, Baoding, China

[3]Department of Agribusiness and Markets, Lincoln University, Lincoln, New Zealand

[4]Department of Food Science, University of Otago, Dunedin, New Zealand

Correspondence
Alaa El-Din Ahmed Bekhit, Department of Food Science, University of Otago, PO Box 56, Dunedin 9054, New Zealand.
Email: aladin.bekhit@otago.ac.nz

Stephen G. Giteru, Alliance Group Limited, P. O. Box 845, Invercargill, 9810, New Zealand.
Email: stephen.giteru@alliance.co.nz

Abstract

The growing global population and lifestyle changes have increased the demand for specialized diets that require protein and other essential nutrients for humans. Recent technological advances have enabled the use of food bioresources treated as waste as additional sources of alternative proteins. Sheep wool is an inexpensive and readily available bioresource containing 95%–98% protein, making it an outstanding potential source of protein for food and biotechnological applications. The strong structure of wool and its indigestibility are the main hurdles to achieving its potential as an edible protein. Although various methods have been investigated for the hydrolysis of wool into keratin, only a few of these, such as sulfitolysis, oxidation, and enzymatic processes, have the potential to generate edible keratin. In vitro and in vivo cytotoxicity studies reported no cytotoxicity effects of extracted keratin, suggesting its potential for use as a high-value protein ingredient that supports normal body functions. Keratin has a high cysteine content that can support healthy epithelia, glutathione synthesis, antioxidant functions, and skeletal muscle functions. With the recent spike in new keratin extraction methods, extensive long-term investigations that examine prolonged exposure of keratin generated from these techniques in animal and human subjects are required to ascertain its safety. Food applications of wool could improve the ecological footprint of sheep farming and unlock the potential of a sustainable protein source that meets demands for ethical production of animal protein.

KEYWORDS
alternative protein source, cysteine, food formulation, glutathione, keratin, protein supply, sheep wool, sulfitolysis

1 | INTRODUCTION

The ever-increasing demand for protein caused by global population growth, rising consumer awareness of the health aspects of proteins in the diet and the rise in per capita income have continued to strain the already scarce supply of available resources (FAO, 2018). As a result, the global protein ingredients market, estimated at USD 40 billion, is expected to grow at a compounded annual growth rate of 10.5% from 2021 to 2028 (Grandview Research, 2021). This demand can be met by improving the efficiency of underperforming crops and livestock production systems, partly by improving management practices and implementing innovative technologies. In addition, low-value secondary bioresource streams generated from food processing in large volumes can undergo valorization to provide novel alternative proteins (FAO, 2018).

Animal-derived proteins contribute the largest share of the global protein market (70.0%; Grandview Research, 2021) due to high worldwide acceptance, favorable regulations, uniformity in large-scale production and the nutritional benefits of essential amino acids balance and a high digestibility (Smith et al., 2022). However, the meat industry has continued to generate large volumes of byproducts of little or no economic use (Toldra et al., 2016, 2021). Accordingly, considerable efforts have been continuing to boost the gross monetary benefit of byproducts that represent 8%−11% of the revenue per animal (Adhikari et al., 2018; Jayathilakan et al., 2012; Shirsath & Henchion, 2021; Vidal et al., 2022) by retrieving proteins, vitamins, and minerals.

The global estimate for the sheep population stands at 1.27 billion heads, producing about 1.9 million metric tons of wool (IWTO, 2022). About 60% of the wool is used in woolen fabric apparel production (Woods, 2012), which has continued to experience a falling demand. The low demand in the textile industry and low commodity prices of wool, estimated at US$ 1.8/kg (Wools of New Zealand, 2022), have led to the disposal of large volumes of low-grade slipe wool (Ossai et al., 2022). Thus, wool is a protein-rich (95%–98% w/w of wool) bioresource already available in large volumes and at affordable prices. However, despite the abundance of protein in wool (80%–85% keratin w/w of total protein), keratin is indigestible owing to the strong disulfide (S–S) bonds that make it insoluble in water, weak acids or alkali and most of the food-grade proteases (Zhang et al., 2018).

Keratin is a cysteine-rich (approximately 10% w/w of protein) structural protein (Bhavsar et al., 2016), one of the two sulfur-containing amino acids, the other being methionine. Cysteine is a semi-essential amino acid that acts with glycine and glutamic acid to facilitate the de novo biosynthesis of glutathione (GSH) and taurine (Kozich & Stabler, 2020; Seidel et al., 2019). Taurine and GSH are significant factors in cell metabolism, redox balance, and general health improvement (Atkuri et al., 2007; McPherson & Hardy, 2011).

Several publications have reviewed keratin extraction methods, focusing on physicochemical, structure, and functional properties of the extracted keratins (Lazarus et al., 2021; Shavandi, Silva, et al., 2017; Sinkiewicz et al., 2016; Tantamacharik et al., 2021; Wang, Yang, et al., 2016). Due to its low cost, biophysical stability in physiological environments, biodegradability, and low immunogenicity, previous studies focused on keratin production for biomaterials, agriculture and textile applications (Shavandi et al., 2016; Goyal et al., 2022; Zoccola et al., 2015). Notwithstanding, some recent investigations explored the functional benefits of keratin using in vitro, in vivo animal models and clinical studies based on humans (Beer et al., 2013b). Although keratin is deficient in essential amino acids such as lysine, methionine, histidine, and tryptophan (Mothé et al., 2017), supplementation with free amino acids can improve the overall protein quality (Grazziotin et al., 2008). Furthermore, in vitro and in vivo cytotoxicity assays reported no adverse effects from keratin supplementation (Dias et al., 2022; Shavandi et al., 2016; Sierpinski et al., 2008; Zhang et al., 2013)

This review is the first comprehensive account of the potential use of keratin in human food. First, the article examines the different wool hydrolysis techniques to generate keratin peptides and amino acids, elucidating the potential for application in edible products. The authors then highlight the nutritional, functional, structural, safety, and toxicological aspects of products generated from the various production methods.

2 | TYPICAL CHARACTERISTICS OF WOOL

2.1 | Wool structure

Wool fibers consist of 95%−98% proteins (about 80%−85% keratin), lipids (0.1%), and minerals (0.5%; Ranjit et al., 2021; Zhang et al., 2018). Its cross-section depicts a three-dimensional hierarchical structure with a covalently attached outer layer of fatty acids lining the exterior cuticular surfaces and β-layers of the cell membrane complex (Figure 1). Wool fibers are made up of three structural units. The first is the hydrophobic exterior lipid of 18-methyleicosanoic acid (18-MEA) (CH_3-$CH_2CH(CH_3)$-$(CH_2)15COOH$). This layer confers surface hydrophobicity to the wool fiber, acting as a natural water-repellent coating

Comprehensive REVIEWS in Food Science and Food Safety

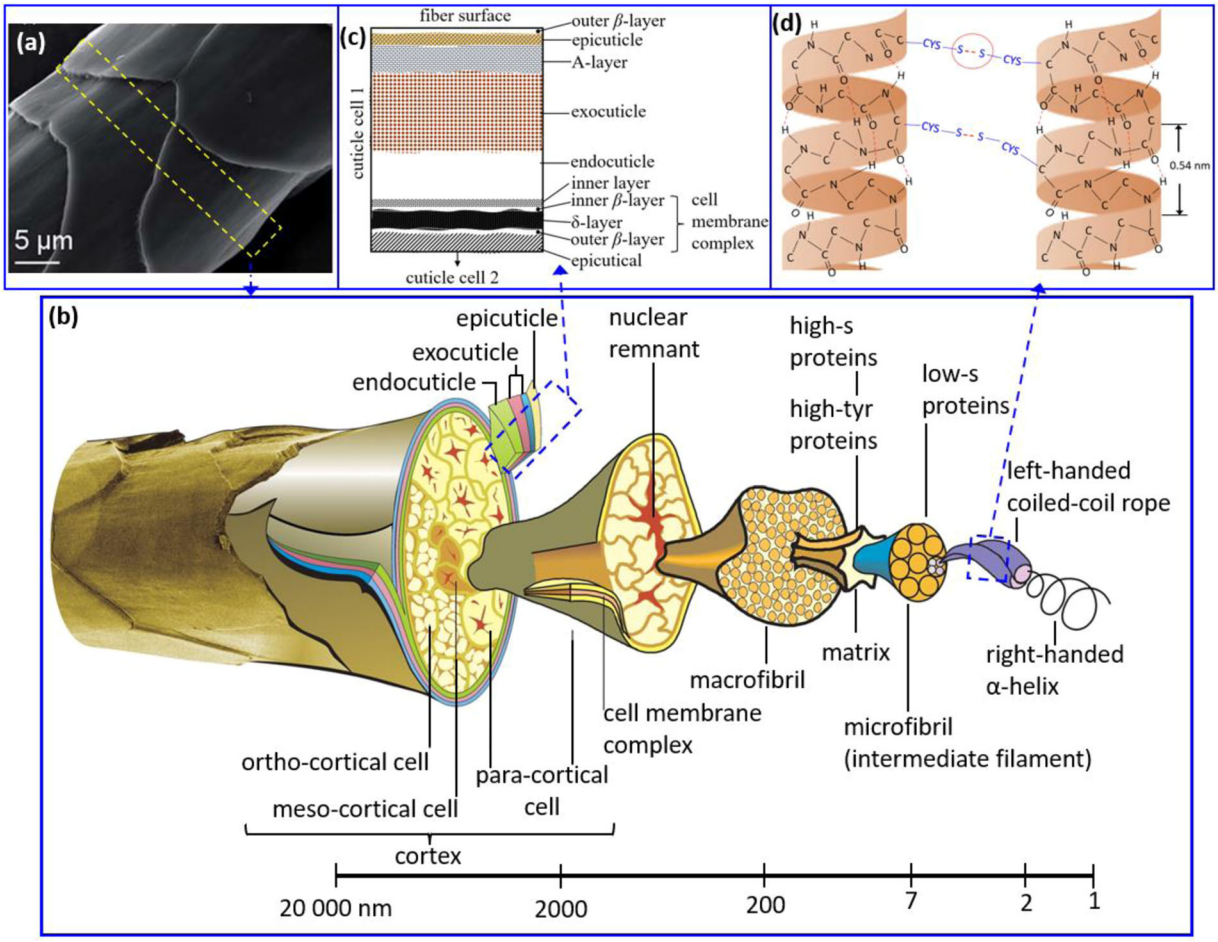

FIGURE 1 (a) Scanning electron microscope image of wool. (b) Schematic diagram of the internal structure of a wool fiber and the corresponding size. Adapted from CSRIO (https://www.scienceimage.csiro.au/library/textile/i/7663/the-structure-of-a-merino-wool-%EE%84%83bre/). (c) An illustration of the transverse section of laminated subcomponents of the first cuticle in mammalian keratin fiber. The cell membrane complex separates the sheet-like layers of the cuticle (Swift & Smith, 2001). (d) Alpha keratin, showing disulfide linkages between α-helices accountable for the intensified strength and stability of keratin (Shavandi, Silva, et al., 2017).

(De Oliveira Martinez et al., 2020). The second structure is a layer of cuticular cells (approximately 0.5 mm thick) oriented toward the hair tip such that there is no resistance when rubbing the fiber in the direction of the follicle toward the tip (Figure 1a). The third component of the wool fibers (mainly in coarse fibers with diameters >35 μm) is the medulla, a central core of cells arranged either continuously or intermittently along the fiber axis and surrounded by a cortex (Moore et al., 2016). Intercellular spaces between medullary cells are air-filled, which confers the fibers with a high thermal insulation ability.

The cuticle layer (epicuticle, exocuticle, and endocuticle), found within the second structural unit, encases a cortex containing highly oriented cortical cells and makes up about 85% of the wool fiber. Generally, wool has three protein classes, (a) the intermediate filament proteins (IFPs), which are low in sulfur; (b) high-sulfur proteins; and (c) high-tyrosine proteins (Ferrareze et al., 2016; Ranjit et al., 2021). The cortical cells are organized into high sulfur macro- and low sulfur microfibrils (IFP), with the latter making up to 50%–60% w/w of the cortex (Figure 1b). The microfibrils comprise three keratin macromolecules in α-helical conformational arrangement, also called protofibrils (De Oliveira Martinez et al., 2020; Fakhfakh et al., 2013).

The α-helical conformation of keratin is the principal constituent of wool and other mammalian features, such as hair, quills, fingernails, horns, hooves, and the epidermal layer of skin (Figure 1b, d). However, other keratin configurations have also been reported, including the β- and γ-keratins occurring in different animal species. β-Keratin represents an extended keratin configuration, a structural protein mainly occurring in the epidermis of reptiles and feathers. γ-Keratin is an amorphous (nonstructural) keratin constituent in avian (beaks and claws) and reptilian (claws and scales) species (Wang, Yang, et al., 2016).

The cell membrane complex on the cuticle layer is the only continuous phase in wool which makes up ~17% w/w of the wool protein that is low cysteine non-keratinous material (Figure 1c). It separates the cuticle cells from the underlying cortex and individual cortex cells. Individual cuticle and cortical cells are surrounded by a proteinaceous membrane, constituting ~1.5% of the total fiber and characterized by having high levels of ε-amino(γ-glutamyl)lysine (Rippon, 2013). This membrane plays a significant role during the degradation of wool fibers because of its hydrophobicity and resistance to chemical effects, making it the most degradation-resistant part of the wool fiber during hydrolysis under various procedures (Swift & Smith, 2001).

2.2 | Keratin structure and functionality

Wool keratins exhibit two protein fractions of molecular weight (Mw) distribution between 45–60 kDa and 11–28 kDa, representing the low sulfur keratin of the IFPs (α-helix) and the high sulfur protein of the matrix embedding the intermediate filament from the fiber cuticle, respectively (Tonin et al., 2006). The α-Keratin is a heterogeneous fibrous protein differentiated from other structural proteins, such as myofibrillar, collagen and elastin, by its high cystine levels (measured as cysteine or ½ cysteine; Zoccola et al., 2009).

Wool keratin has a characteristic high abundance of cystine residues (7%–20% of the total amino acid residues), making the fibers strong and stiff. This is due to the numerous intra- and intermolecular disulfide (S–S) bonds, including sulfhydryl–disulfide (SH–SS; Figure 1d), hydrogen bonding, and Coulombic linkages that facilitate the formation of cysteine by disulfide bridges (Shavandi et al., 2021; Swift & Smith, 2001). Keratin can be considered a natural source of cysteine, which is present as the dimer cystine, including the linked S–S bonds that readily hydrolyze to liberate the monomer cysteine (McPherson & Hardy, 2011). Cysteine is one of the four sulfur-containing amino acids, the others being methionine, homocysteine, and taurine. However, only cysteine and methionine are incorporated into proteins (Brosnan & Brosnan, 2006). Unlike methionine, cysteine residues on the backbone of keratin polypeptides have a highly chemically reactive thiol (–SH) functional group at physiological pH (pH ~7.0), forming disulfide bridges (Cys-S–S-Cys) in its oxidized form (Figure 1d). The S–S interactions play a crucial role in the protein structure, protein folding pathways and stabilization of the three-dimensional keratins subunits (Eslahi et al., 2014), thereby bestowing exceptional resistance to thermal, chemical, and enzymatic reactions of the protein (Vineis et al., 2019). These phenomena contribute to the low utilization of wool in applications requiring soluble keratin, such as food, pharmaceutical, and medical industries (Shavandi, Silva, et al., 2017). Therefore, understanding the seldom explored physical and chemical characteristics of keratin is crucial to improving its extraction and use. For instance, posttranslational modifications can be used for structural alteration to yield keratins of different Mw with improved bioaccessibility for enzymatic activity (Bragulla & Homberger, 2009; Lange et al., 2016).

3 | PRODUCTION OF KERATIN FROM WOOL

As keratin applications continue to emerge, simple, efficient, and environmentally friendly wool hydrolysis techniques are required to promote the adoption and large-scale use of the widely available raw material (Swift & Smith, 2001). Keratin proteins are extracted and purified from wool via the cleavage of disulfide bonds. The breakage of the covalent linkages between the molecules makes the keratin material easily soluble, allowing purification. The initial step of keratin extraction involves the removal of a lipid layer in a pretreatment step involving repeated washing of the wool in tap water containing a detergent at 40°C with agitation or sonication to remove grass, dirt, and loosely attached lipid layers (Deb-Choudhury et al., 2018; Seghir et al., 2020). Another reported technique is the application of 0.5% sodium hypochlorite (NaClO), which can also be helpful in disinfection and stain removal (Xu et al., 2003). In addition, the wool may be washed in a 5% v/v solvent of acetone/ethanol mixture (1:1) and incubated at ambient temperature for 2 h (Seghir et al., 2020) or acetone only at 72°C for 12 h to complete the process of fat removal (Zhang et al., 2018). To reduce potentially hazardous solvents and residual chemicals in the extract, degreasing procedures using lipolytic enzymes, for example, Lipex™ 100 T (Novozymes, Denmark), have been proposed (Mokrejs et al., 2011). A mixture of wool (1% w/w of dry wool) and water at a ratio of 1:50 (wool:water), pH 8.0, is incubated at 40°C for 24 h, followed by filtration and rinsing in water. Finally, washed and dried wool is prepared for further processing by shredding. Apart from the milling, which aims to develop usable wool powder, the next sections describes different keratin solubilization and extraction methods and the potential of producing keratin for human and food applications (Figure 2).

3.1 | Milling

Milling is a chemical-free technique that reduces the wool into a fine powder while maintaining most keratin in

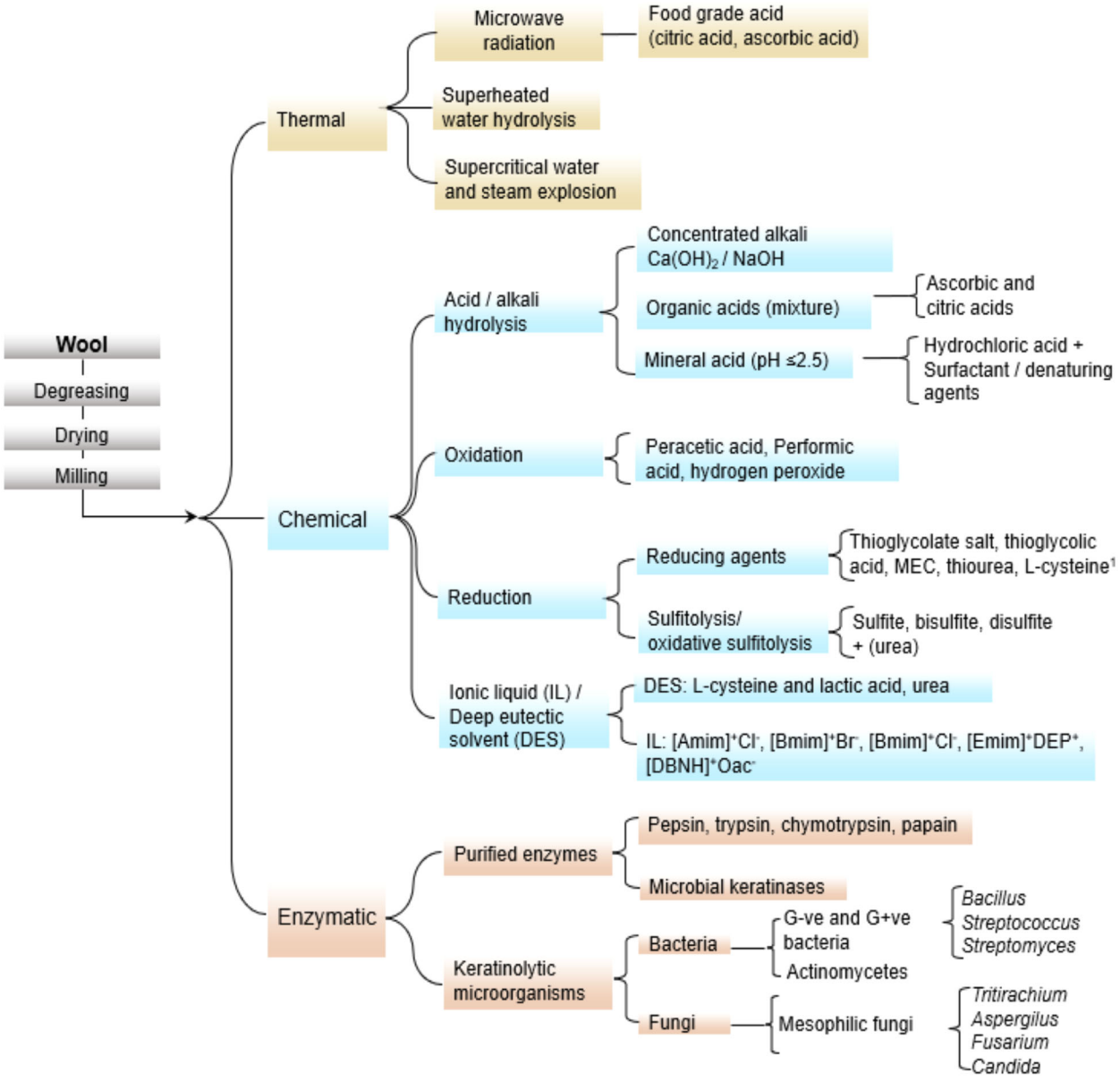

FIGURE 2 Classification of methods used in keratin extraction from wool. $[Amim]^+Cl^-$, 1-allyl-3-methylimidazolium chloride; $[Bmim]^+Br^-$, 1-butyl-3-methylimidazolium bromide; $[Bmim]^+Cl^-$, 1-butyl-3-methylimidazolium chloride; $[DBNH]^+Oac^-$, 1,5-diazabicyclo [4.3.0]non-5-enium acetate; $[Emim]^+DEP^-$, 1-ethyl-3-methylimidazolium diethyl phosphate; G-ve, Gram-negative; G+ve, Gram-positive; MEC, 2-β-mercapto-ethanol. [1]Used with added surfactant and buffering agents, for example, urea, tris-HCl, EDTA, SDS, DTT

the original physicochemical and functional properties (Xu et al., 2003). As a result, pulverized wool acquires increased reactivity and absorbency, enabling use in sorption applications (Naik et al., 2010; Wen et al., 2010). Size reduction by milling is an old practice used to disentangle dry materials using drawing, torsion, and shear actions to induce fracture along the lines of weakness. However, albeit depending on the ultimate size of particles, reducing the size of wool by grinding can lead to cysteine oxidation, lowering its food value. Conventional wool milling involves grinding using a ball mill and sieving (Rajkhowa et al., 2012). The attained particle size and level of keratin degradation are subject to the equipment design, grinding time and milling parameters. For example, cooling the system to regulate the temperature at a minimum can reduce the impact of the heat-induced breakdown of keratin (Rajkhowa et al., 2012; Xu et al., 2003). Xu et al. (2003) demonstrated a multi-stage milling procedure for producing ultrafine wool powder with minimal keratin degradation. First, the authors chopped the wool into 1–3 mm particle lengths using a rotary blade and mixed it with 0.5% NaClO to remove lipid layers for high milling efficiency. After draining the excess liquid, the wool was ground under wet milling conditions in a

water-cooled chamber at ~18°C. Depending on the milling duration, the attained particle size was 2 μm in diameter and 5–10 μm in length (Xu et al., 2003). Other adaptable milling conditions include the rotor speed, grid/screen pore size, and the addition of shearing/impact-enhancing materials, such as yttrium-doped zirconium oxide balls or freeze milling (Hassabo et al., 2014, 2015; Rajkhowa et al., 2012). Rajkhowa et al. (2012) showed that sequential wet and air jet milling could produce small particles (1.5–4.0 μm) of wool fibers. Powdering of wool increases the accessibility to enzyme/chemicals and metal ion binding properties, making it helpful in developing biomedical materials and cosmetic products (Naik et al., 2010). Moreover, washing in NaClO and size reduction have emerged as essential pretreatment steps before chemical and enzymatic hydrolysis of wool to maximize solubilization and recovery of keratin peptides (Zhang et al., 2020). However, information is still limited on the wool powders' suitability for edible food products and its techno-functionalities that could affect digestibility, assimilation, and biological availability of the protein.

3.2 | Thermal extraction

Thermal techniques have recently attracted attention as alternative processes for acquiring high-quality keratin, free of chemical residues and unwanted secondary byproducts. For example, superheated water, steam explosion and microwave-assisted methods have recently been investigated for the thermal hydrolysis of wool (Table 1).

3.2.1 | Superheated water hydrolysis

The superheated water hydrolysis technique is a 'green extraction' method investigated to extract keratin from wool (Zoccola et al., 2015). The method involves boiling wool in alkali media and reducing the peptide length of keratin, which allow the potential for tailoring the process to achieve the desired hydrolysis level. Although the method results in the sterilization of the product, it can produce significant undegraded wool residues (~50%) and low yield of soluble keratin. Bhavsar et al. (2017) showed that superheated water treatment (140°C–170°C for 1 h, wool:liquid ratio of 1:3) could completely hydrolyze wool to low Mw water-soluble peptides and amino acids (Table 1). The hydrolyzed wool was characterized by low sulfur (67–43 kDa) and high sulfur (28–11 kDa) keratin with some cystine loss occasioned by its oxidation to lanthionine (LAN) and lysinoalanine (LAL) in a treatment duration-dependent manner. Optimum treatment conditions under superheated water were reported as 140°C for 60 min, where minimal losses of cystine and phenylalanine occurred (Table 2). However, harsh treatment conditions, such as 170°C for 1 h, could significantly degrade cystine, resulting in poor keratin quality (Bhavsar et al., 2017; Rajabinejad et al., 2017). Bhavsar et al. (2017) reported a twofold increase in LAN residues on shifting the temperature from 140°C to 170°C. Interestingly, the coupling of elevated temperatures with alkali treatment (e.g., 5% KOH and CaO) resulted in a ~2.5-fold increase in LAN formation, affirming the destructive effect of the alkali (Bhavsar et al., 2017). Compared with chemical methods (e.g., sulfitolysis, oxidation, and reduction) superheated water treatment can give similar extraction yield (31% w/w), suggesting higher adoption feasibility due to its low cost and environmentally friendly nature (Rajabinejad et al., 2017).

3.2.2 | Steam explosion extraction

The steam explosion keratin extraction involves exposure of wet wool to high-temperature steam over a short duration, followed by explosive decompression. For instance, keratin extraction is done by soaking 100 g of wool in 300 g water and then applying steam (220°C) for 10 min (Tonin et al., 2006). The resulting slurry is filtered to yield two discrete phases, a wet solid phase and a liquid phase, at yield rates of ~60% and ~20% of the raw materials, respectively. Both phases contain modified wool, with significant conformational changes in the α-helix through the cleaving of disulfide bonds, suggesting cystine degradation. The solid fraction may include some impurities of nondegraded and partially degraded wool and has most amino acids with hydrophilic side groups.

In contrast, the liquid phase holds primarily hydrophilic amino acids. Therefore, to obtain a complete profile of the amino acids in the hydrolysate, it may be necessary to further purify the solid fraction before drying both phases (e.g., at 105°C or freeze-drying) and then recombining them (Table 2). The steam explosion technique is advantageous because hazardous chemicals are not used (Zoccola et al., 2012). However, the processes may be limited by the resulting large particle sizes (diameter of 0.5–3.0 μm) for most (62.4%) of the resulting solid matrix (Tonin et al., 2006). Despite the extensive loss of cysteine, lysine, and other water-soluble amino acids, the water-based high-temperature methods are more economically feasible, environmentally friendly, and produce potentially edible protein hydrolysate.

3.2.3 | Microwave extraction

Microwave extraction of keratin is an extension of the superheated method where extraction is performed in

TABLE 1 Characteristics of some of the keratin extraction conditions from sheep wool

Optimum conditions	Protein yield (%)	Properties of the hydrolysate	Reference
Thermal extraction			
Superheated water at 140°C or 170°C, (final pH 7.9 and 5.16, respectively) material to liquor ratio of 1:3, 1 h processing time	N.R.	- Process capable of degrading wool fibers - Hydrolysates had low molecular weight proteins and amino acids - Extracted polypeptides were dominated by β-sheets and random coil structure - High (170°C) temperatures showed higher formation of lanthionine - More keratin dissolution occurred at 170°C within the processing time	Bhavsar et al. (2017)
Superheated water at 170°C (pressure 7.0 bar), 90 min	N.R.	- Severely damaged structure of the fibers (the optimum level at 60 min). - Decrease in ½ cystine from 9.42 mol% to 0.5 mol% after 60–90 min. - Low molecular weight (Mw) proteins disappeared after 30-min treatment, the product showed Mw between 25 and 3 kDa	Bhavsar et al. (2016)
40 g wool in 150 ml superheated water, 170°C for 30 min	31 (extraction yield w/w)	- Superheated water resulted in similar extraction yield with oxidation, reduction, and sulfitolysis - Higher amount of cysteine lost compared to other methods	Rajabinejad et al. (2017)
Steam explosion, 0.2–0.8 MPa	N.R.	- Explosion destroyed scales, crystals, and crosslinks of macromolecular chains in the fiber. - Regained mechanical properties and the dissolving ability in caustic. - The solution decreased as the explosion pressure increased	Xu et al. (2006)
Steam explosion, 100 g wool soaked in 300 g water at 220°C (10 min)	60	- Fiber keratin was converted into a dark-yellow sludge - The decrease of disulfide bonds, 62.36% of the dry solid, 18.66% of proteins dissolved in the supernatant, 1.12% of sediment, the presence of the wool structure in the treated sample	Tonin et al. (2006)
Wool:liquid ratio = 1:18, food-grade acids citric acid (90 mM) and ascorbic acid (6 mM), pH 2.3 Microwave heating at 20 kW, 2450 MHz, 106°C, 30 min, pressure 220 psi	86	- Amino acid cysteine (8.8 g/100 g) and element selenium (0.29 μg/g). - Keratin was noncytotoxic in vitro at ≤ 2 mg/ml concentration - Keratin inclusion in the diet at 50% (50% casein) did not affect the growth of rats	Dias et al. (2022)
Wool fibers (mass of fiber:mass of water, 1:5). Microwave energy = 150–570 W, temperature = 180°C, 5–7 min to reach temperature set point, total treatment time 1 h	74	- Increase in the extraction yield when decreasing the liquor ratio mass of fiber/mass of water - Loss of cystine, amount of ½ cystine decreased from 11 mole% of the original wool to about 0.1 mol% of the freeze-dried powder - The hydrophilic and hydrophobic amino acids ratio decreased from 1.7 to 1.2.	Zoccola et al. (2012)
40 g wool, 150 ml super-heated water in a microwave oven, 30 min, 180°C	31	- ½ cystine decreased from 11.3 mol% in the original wool to 0.7 mol% in the hydrolyzed sample. - Lanthionine developed in the product (0.9 mol%) - Mw fall in the range of 11–28 kDa	Bertini et al. (2013)

(Continues)

TABLE 1 (Continued)

Optimum conditions	Protein yield (%)	Properties of the hydrolysate	Reference
Alkali and acid hydrolysis			
HCl pH 3.22 and pH 5.55. 60 g wool/400 ml of 4 M HCl, 95°C for 24 h.	N.R.	- Two kinds of keratin polypeptides were collected at pH 3.22 and pH 5.55. Both had smooth, round, and uniform surfaces. - Degree of hydrolysis 33% - Keratin showed adverse effects on cell growth	Zhang et al. (2013)
11 N HCl vapor for 4 h. Neutralized with sodium hydroxide (NaOH), then freeze-dried	86	- Elevated level of hydrophobic peptides in the hydrolysate - No unwanted modifications of cysteines to form lanthionine (LAN) and lysinoalanine (LAL)	Deb-Choudhury et al. (2018)
Alkali mixture (10% CaO or 5% NaOH or 5% KOH), 12 high with agitation, 8 h without agitation, 80°C	KOH = 16 Cao = 36 NaOH = 44	- Hydrolysates contained 11.6%–15.0% nitrogen with the potential for use as fertilizer - CaO resulted in 13 kDa keratin, NaOH gave 35.8 kDa	Berechet et al. (2018)
100 g dried wool, 150 ml of 6 N acidic or alkaline solution (HCl, H_2SO_4 or H_3PO_4) and neutralization by 10 N KOH and 10 N Mg $(OH)_2$ Or 2.5 N KOH, NaOH or $Mg(OH)_2$ and neutralization by 6 N H_3PO_4 in an oven at 100°C	KOH = 71 H_2SO_4 = 65 $Mg(OH)_2$ = 68	- Six types of peptones based on the acid/alkali used - Hydrolysates using HCl, H2SO4, NaOH or KOH were suitable for the preparation of peptone	Arslan & Aydogan (2020)
0.15 M KOH and 0.05 M NaOH at 120°C and pressure 2.03 atm, 20 min	79	- Hydrolysates contained 75%−80% water-soluble materials and partly degraded insoluble keratin	Gousterova et al. (2003)
11 N HCl for 4 h, 11 N NaOH for neutralization	N.R.	- 2% WH and Novagel gave short-chain fatty acids and fecal microbiota compositions - WH increased the apparent cysteine digestibility - WH supplementation was not detrimental to palatability, health, and feed intake	Deb-Choudhury et al. (2018)
2.5%–7.5% (w/v) wool in KOH (0.15 M), NaOH (0.05 M), microwave heating (800 W; 98°C, 1 h) pH 11.0–13.0	85%–100% solubilized wool	- An increasing percentage of solubilized wool with decreasing raw material concentration - Minimal occurrence of undesired degradation	Gousterova et al. (2005)
Oxidation			
5 g wool in 330 ml 24% peracetic acid, 2 days followed by keratin extraction with 100 mM Tris solution	57% water soluble keratin	- High content of cysteine-S-sulfonated residues on keratin extracts - Extracts contained proteins of Mw 40−60 kDa	Shavandi, Carne, et al. (2017)
Cu(II)–wool complex, liquor:wool ratio 100:1, (Cu(II) = 4.5 mM copper (II) nitrate solution adjusted to pH 5.9 using an acetate buffer solution). Mixed with 50 ml of 0.1 M hydrogen peroxide, 55°C		- Oxidation converted cystine into cysteic acid - Elevated levels of aspartic and glutamic acids observed in the residues and the filtrates - Phenylalanine and tyrosine not detectable in the filtrate	Fukatsu (1997)

(Continues)

TABLE 1 (Continued)

Optimum conditions	Protein yield (%)	Properties of the hydrolysate	Reference
150 g of detipped and scoured wool was bleached in 0–12 g/L hydrogen peroxide for 5 min at 40°C. 3L bleaching solution having 0.5 g/L Kieralon OL, pH 4 (adjusted using formic acid)	–	- Peroxide treatment had only a minor effect on the two-dimensional electrophoretic separation of the intermediate filament protein - Peroxide treatment resulted in the disappearance of major high-sulfur protein	Plowman et al. (2003)
Reduction: reducing agents			
2 g wool, L-cysteine 2% (m/vol%), pH 11–13, 0.001–0.1 M NaOH, room temperature, 24 h	30–69	- 42–94 dissolution ratio. Mw: 100 kDa (keratin dimer), 45–65 kDa (type I and II keratins, 10–38 kDa (keratin-associated proteins). - L-cysteine at pH 12 dissolved 67.8% wool fiber.	Zhang et al. (2022)
2.0–4.3 g wool, 5–20 ml solvent (80% total keratin (37.3 g $CaCl_2 \cdot H_2O$, 27.4 g water 23.4 g ethanol, 4 ml thioglycolate), pH 7.0, room temperature	70	- N.R.	Fitz-Binder et al. (2019)
10 g wool, 180 ml urea (7 M), MEC 15 ml (5% v/v), surfactant, water, 6 g SDS (w/v), 50°C, 24 h	48	- Protein had Mw 52 and 66–69 kDa, low Mw band 14–30 kDa - cysteine content 8–9 mol% - The insoluble sediment was attributed to the cuticle	Yamauchi et al. (1996)
10 g wool, 180 ml urea (7 M), MEC 15 ml (5% v/v), surfactant, water, MEC (5% v/v) No SDS, 50°C, 24 h	45	- Aqueous keratin solution had a shelf-life of 1 year when stored in a glass bottle at an ambient temperature.	Yamauchi et al. (1996)
Wool 0.5 mol/L, LiBr, 0.1 mol/L, SDS (0.02 mol/L), 90°C for 4 h, pH = 12	94% wool dissolution rate 50% keratin extraction yield	- Keratin dominated by α-helical conformation	Zeng & Lu (2014)
Reduction: sulfitolysis			
15 g wool, 300 ml solution (Urea 8 M, sodium metabisulfite 0.5 M), pH 6.5, 2-h shaking, 65°C	N.R	- N.R.	Ramirez et al. (2017)
3 g wool in 100 ml, urea (8 M), sodium metabisulfite (0.5 M). Shaking for 2 h at 65°C, pH = 6.5	30	- SDS reduced the aggregation of keratin without effect on yield - Keratin was not degraded at 65°C compared to 100°C - Tw Mw bands 67–45 kDa and 28–11 kDa—preserved under mild conditions (65°C)	Tonin et al. (2007)
Ionic liquid (IL) and deep eutectic solvents (DES)			
DES—L-cysteine ratio in DES (1.6 g L-cysteine in 20 ml of lactic acid) 0.5 g of wool, 105°C, 8 h	94	- L-cysteine and lactic acid mixture an almost complete wool dissolution for enhanced keratin recovery	Okoro et al. (2022)
DES—0.4 g wool, 2 g L-cysteine, and 20 ml lactic acid (90% solution in water), 3,5 h, 95°C	N.R.	- 90% dissolution rate of wool was dissolved per 1 g of the DES - α-helix keratin decreased while its β content increased. - Keratin was produced without losing the long peptide chains	Shavandi et al. (2021)

(Continues)

TABLE 1 (Continued)

Optimum conditions	Protein yield (%)	Properties of the hydrolysate	Reference
DES—choline chloride-urea, 130°C in 5 h	N.R.	- 35.1 mg wool completely dissolved in 1 g DES - DES dissolved the wool cortex layer - Regenerated wool keratin contained fragments of low Mw polypeptide chains - α-helix decreased, while the content of β-sheet and disordered structure increased - Cysteine decreased from 3.4 g/100 g to 0.108 g/100 g in the final product	Jiang et al. (2018)
DES- Choline chloride (ChCl):oxalic acid ($OA{\cdot}2H_2O$), molar ratio 1:2, 80°C, 30 min	33	- 60.2% wool dissolution rate and 32.7 keratin extraction rate - Keratin product showed reduced α-helix structure and crystallinity	Liu et al. (2021)
IL- $[Bmim]^+{\cdot}Cl^-$, 1% wool (w/w of ionic liquid), 130°C, 10 h	11	- The temperature has a strong effect on the solubility - The product showed a β-sheet structure and loss of α-helix - Chloride anion ionic liquids showed better solubility for wool fibers than ionic liquids involving bromide anion	Xie et al. (2005)
IL- $[Amim]^+{\cdot}Cl^-$, 130°C, 640 min	21	- $[Amim]^+{\cdot}Cl^-$, = gave superior wool solubilization compared to $[Bmim]^+{\cdot}Cl^-$, (15% yield). Wool hydrolyzed by breaking down disulfide bonds - Exhibited a β-sheet structure and the disappearance of the α-helix structure.	Li & Wang (2013)
IL- $[Amim]^+$ $[dca]^-$, 130 min. Wool solubility is enhanced by the addition of a reducing agent, mercaptoethanol.	23	- Enhanced dissolution of wool (475 mg wool/g of solvent) Wool solubility enhanced by - Wool keratin kept the protein backbone, increased β-sheets and random coils compared to starting material. - Low Mw peptides	Idris et al. (2014)
IL- $[Emim]^+DMP^-$, wool:IL ratio = 1:10 (w/w), 130°C	100% keratin dissolution	- The sulfhydryl group and disulfide bond content of the regenerated keratin decreased with an increase in water content in the water:IL ratio - Decomposition temperatures of the regenerated keratin were 236°C–240°C. 1 g keratin dissolved in 10 g IL.	Zhang et al. (2017)

Note: N.R., not reported; The protein yield (%) was rounded off to zero decimal places.Abbreviations: $[Amim]^+Cl^-$, 1-allyl-3-methylimidazolium chloride; $[Amim]^+{\cdot}[dca]$-, 1-allyl-3-methylimidazolium dicyanamide; $[Bmim]^+Cl^-$, 1-butyl-3-methyllimidazolium chloride; $[Emim]^+DMP^-$, 1-ethyl-3-methylimidazolium dimethylphosphate; MEC, 2-β-mercaptoethanol.

the presence of acid or alkali in a heated reaction vessel at 170°C–220°C for 60 min. Recent studies have investigated microwave-assisted technology as an environmentally friendly option for keratin extraction in a chemical-free, chemical or enzyme hydrolysis procedure (Bertini et al., 2013; Dias et al., 2019, 2022; Gousterova et al., 2005; Jou et al., 1999; Lee et al., 2016; Zoccola et al., 2012). Using microwaves, the deconstruction of wool produces a mixture of insoluble wool fraction and a soluble protein-rich liquor that has free amino acids, peptides, and low Mw proteins. The mechanism of action during microwave hydrolysis can be explained by the ability of electromagnetic radiation to penetrate deep into the curled α-keratin structure, destabilizing disulfide bonds in cysteine, thus promoting forward hydrolysis (Zoccola et al., 2012). In addition, the supplied electromagnetic energy in heat and pressure induces perturbations owing to the high dielectric constant of α-keratin, causing rapid reorientation of the protein that results in physical damage (Dias et al., 2019; Jou et al., 1999). Regulated microwave energy can help attain the desired free amino acids, peptides and oligopeptide chain length.

A typical microwave treatment involves mixing wool with water/alkali/acid at proportions of 1 part wool to 30—40 parts liquid, then applying the microwave energy (e.g., 150–570 W) at 140°C–200°C for ~30–60 min (Bertini et al., 2013; Zoccola et al., 2012). The time necessary to reach the set temperature depends on the power applied, for instance, 5—7 min at a microwave power of 150–570 W (Zoccola et al., 2012). First, the obtained material is filtered (e.g., 125 μm pore size) to separate into solid (plug) and liquid (20%–30% solids) fractions. The liquid fraction

TABLE 2 Amino acid composition of keratin obtained using various extraction methods

Amino acid	Wool (initial material)	Super-heated water (140°C, 60 min)	Steam explosion (200°C 10 min)[a]	Microwave (150°C, 60 min)	Microwave/food-grade acid (160°C, 30 min)	Alkaline hydrolysis[b]	Acid hydrolysis[c]	Acid hydrolysis[d]	Reduction (8 M urea)[e]	Reduction (L-cysteine)[f]	Esperase enzyme hydrolysis[g]	Esperasae + L-cysteine[h]
Arginine	6.9–10.4	7.3	6.5	7.4	7.4	5.2	10.4	11.1	9.3	11.8	7.6	8.7
Cysteine[i]	11.3–11.7	8.2	3.1	3.4	8.9	–	10.3	5.1	8.0[j]	1.2[j]	8.3[j]	8.4[j]
Histidine	0.9	1.0	0.9	0.9	0.6	0.8	1.0	1.1	1.0	0.8	0.9	0.9
Isoleucine	3.1–3.8	3.6	3.3	3.2	3.0	4.8	3.5	3.8	3.5	3.3	3.3	3.3
Leucine	6.8–8.4	7.8	7.6	7.1	6.5	11.2	8.0	8.5	7.6	7.0	7.1	7.1
Lysine	3.5–3.8	3.2	4.0	3.1	2.2	3.0	3.3	2.4	2.8	3.1	2.5	3.0
Methionine	0.4–0.6	0.4	0.3	0.4	0.4	0.84	0.5	0.6	0.7	0.7	0.5	0.6
Phenylalanine	2.1–3.5	2.7	2.4	2.5	3.4	2.8	3.6	3.6	3.5	2.7	3.2	3.3
Threonine	5.8–6.4	5.3	6.2	6.1	4.8	2.47	5.5	6.2	5.7	4.3	5.9	5.1
Valine	5.3–5.8	6.9	6.0	6.1	5.1	7.6	5.4	6.3	5.6	5.1	4.7	5.2
Alanine	4.1–5.2	5.8	6.3	5.3	2.9	7.9	4.1	4.0	3.9	1.8	3.7	3.6
Aspartic acid	7.0–7.3	7.5	8.7	7.9	2.4	11.3	7.0	7.0	7.0	5.9	6.7	6.6
Glutamic acid	12.8–16.0	13.9	14.5	12.9	10.3	20.8	15.7	14.9	15.3	13.3	15.3	14.3
Glycine	4.1–8.0	7.8	10.0	9.4	4.4	7.0	5.2	5.1	5.0	3.8	3.1	4.7
Proline	6.4	6.7	6.5	6.8	5.8	6.5	6.2	7.1	3.2	6.3	6.1	3.7
Serine	7.7–10.8	9.1	10.4	10.9	5.8	4.0	7.9	7.9	7.2	5.5	5.1	7.0
Tryptophan	–	–	–	–	0.7	–	–	–	–	–	–	–
Tyrosine	3.4–4.0	1.9	3.0	3.8	4.9	3.81	2.8	5.2	4.2	2.5	4.1	4.2
References	Shorland and Gray (1970)	Bhavsar et al. (2017)	Tonin et al. (2006)	Zoccola et al. (2012)	Dias et al. (2022)	Cardamone (2010)	Deb-Choudhury et al. (2018)		Zhang et al. (2018)	Zhang et al. (2018)	Zhang et al. (2018)	Zhang et al. (2018)

Note: All values are in percentage.

[a]Represents amino acid composition of the solid phase.

[b]Alkaline hydrolysis (liquor:wool ratio 20:1, 0.5 N NaOH (pH 13.9), 62°C–65°C, 3 h, dialyzed, lyophilized).

[c]Wool hydrolyzed for 4 h 11 N HCl vapor followed by 24 h HCl hydrolysis, neutralized using equimolar NaOH.

[d]Wool hydrolyzed for 24 h using HCl, neutralized using equimolar NaOH.

[e]8 M urea.

[f]0.165 M L-cysteine.

[g]1 g chopped wool incubated with 400 μL esperase.

[h]0.165 M L-cysteine + 400 μL esperase in 0.02 M 50 ml borax NaOH buffer (pH 10.15). [e,f,g,h]Samples processed at 50°C for 15 h.

[i]Measurements include ½ cysteine.

[j]Cystine content.

contains low Mw proteins, peptides, and free amino acids, including small amounts of cystine and LAN. Then centrifugation is applied to the liquid fraction (e.g., 1200 g for 10 min) to separate the supernatant and precipitate (Bertini et al., 2013; Dias et al., 2022; Feroz & Dias, 2021; Lee et al., 2016; Zoccola et al., 2012). Instead of centrifugation, further filtration of the liquid fraction using a pore size of 0.65 μm in a tangential flow filter can also be applied (Zoccola et al., 2012). The clarified supernatant can be discarded to eliminate water-soluble chemicals and lower the costs associated with moisture removal (Dias et al., 2022). A widespread practice is to air-dry the solid fraction, freeze-dry the precipitate or the whole supernatant fraction, and then mill and recombine them to yield a uniform particle-size keratin product. As much as 85% yield of dry keratin, 87% protein (total nitrogen determination by Kjeldahl method, conversion factor 6.25) has been reported (Dias et al., 2022). The extracted protein is characterized by low Mw (3–8 kDa), showing extensive degradation as evidenced by a lack of α-helices and dominant random/coil conformation (Zoccola et al., 2012).

Compared with the steam explosion technique at 220°C for 10 min, chemical-free microwave-assisted treatment (150°C, 60 min) did not bring about a further increase in solubilized wool (Tonin et al., 2006; Zoccola et al., 2012). Instead, Tonin et al. (2006) reported a yield of ~62% solid phase, ~18% liquid phase and losses of ~18% of the initial dry wool from the steam explosion process, showing high similarity to the 80% solid and 10% liquid phase recovered from the microwave-assisted procedure (Zoccola et al., 2012). Moreover, due to the high temperature and pressure in a wet environment, both techniques resulted in significant cysteine loss (70%) with subsequent LAN formation and hydrogen sulfide release in a time- and temperature-dependent manner (Zoccola et al., 2012). Notwithstanding, higher cysteine recovery and low LAN formation have been reported in a low temperature (140°C) chemical-free superheated water method (Bhavsar et al., 2017). These findings suggest higher disruption of S–S bonds, cysteine degradation and loss of water-soluble amino acids coupled with a high proportion of insolubilized wool (~70%–80% insoluble material) under the microwave process, hence low overall protein yield.

Researchers have attempted to use the synergy between microwave activity and alkali/acid hydrolysis to increase keratin yield by solubilizing more wool (Dias et al., 2019). Compared with other thermal techniques, microwave heating can significantly lower the activation energy, reducing the temperature and time needed for keratin extraction (Chen et al., 2015; Zoccola et al., 2012). Reagent mixtures investigated in conjunction with the microwave process include (1) 6–10 mM ascorbic acid + 90 mM citric acid at pH 2.3 (Dias et al., 2022, 2019), (2) 0.15 M KOH + 0.05 M NaOH (Gousterova et al., 2005). Gousterova et al. (2005) showed that microwave-assisted alkaline hydrolysis (microwave power 800 W, 98°C for 60 min, pH 12.5) of wool could attain 100% wool solubilization, despite that the process resulted in significant losses of cysteine (100% loss) and other essential amino acids (threonine, lysine, leucine). Like other high-temperature techniques, the loss of cysteine through the conversion to LAN is inevitable (Table 2). However, recent investigations by Dias et al. (2022) reported the potential of acquiring high total protein (80%–86% w/w protein) and cysteine (8%–13% of total protein) recovery with negligible formation of LAN. The authors combined a high-pressure microwave process (160°C, 30 min) at a 220-psi pressure vessel and mild organic acids (90 mM citric acid + 6 mM ascorbic acid), known to be food antioxidants. These findings provide a valuable direction for future research to adopt strategies to improve the safety and nutritional value of wool keratin extraction.

3.3 | Chemical methods

3.3.1 | Alkali and acid hydrolysis

Alkaline compounds such as hydroxides of potassium, sodium or calcium 15% (or 4–6 M) and calcium oxide (Table 1) can be used at high temperatures (e.g., 120°C–170°C) for up to 20 h to completely hydrolyze wool into its constituents (Berechet et al., 2018; Bhavsar et al., 2017). Keratin peptides and free amino acids can also be produced by hydrolysis of wool using inorganic acids such as sulfuric or hydrochloric acid (e.g., 6–11 N for up to 6–24 h; Deb-Choudhury et al., 2018; Taskin & Kurbanoglu, 2011). Organic acids such as citric (90 mM) or ascorbic (10 mM) can also be used when accompanied by a physical method, for example, heat, high pressure or microwave treatment (Dias et al., 2019; Savige, 2016). The alkali/acid hydrolysis mechanism involves dissociating hydrogen from sulfate and carboxylic groups at elevated temperatures, resulting in a soluble keratin fraction in which cystine residues are converted to cystic acid. Lower temperatures, such as 65°C–80°C and shorter hydrolysis 2–8 h, are often desirable for generating keratin peptides with minimum degradation (Berechet et al., 2018). Cardamone et al. (2009) showed that 68%−82% of keratin could be extracted from wool using an alkali (pH 12−13) at 65°C for 2–5 h.

Alkaline hydrolysis of wool produces 75%−80% water-soluble materials, including amino acids and peptides, that is, intermediate filaments and microfibrillar components, proteins of the fractured residual cuticle and cortical cells. Other components found in the hydrolyzed wool are salts and lipids, while the rest consists of partly degraded

insoluble keratin (Cardamone et al., 2009). The swelling of wool fibers occurring due to the deprotonation of the carboxylic group and amino acids on exposure to high pH >7.0 impart a negative charge on the protein residues (Bhavsar et al., 2017; Queiroga et al., 2012). Therefore, alkali treatment can be used as a pretreatment step in the enzyme hydrolysis process due to the enhanced accessibility of the wool fibers to the proteolytic enzyme due to swelling, leading to a higher hydrolysis index.

The acid hydrolysis process involves the cleavage of disulfide bonds (S–S) and splitting of some peptide bonds of keratin in wool fibers, resulting in a sulfur (cysteine) and glutamine/glutamic acid-rich keratin product (Rajabinejad et al., 2017). Typical acid hydrolysis of wool involves exposure to acid (e.g., 4–11 N hydrochloric acid) or acid vapor for 4–24 h at 22°C–95°C, resulting in water-soluble hydrolysates (Deb-Choudhury et al., 2018; Zhang et al., 2013). Reactions occurring during acid hydrolysis of wool are depicted in **Equations** (**1**) and (**2**). The resulting hydrolysates are readily water-soluble keratin peptides and amino acids that may also possess microscopic traces of deconstructed wool strands in the form of insoluble fibrous remnants.

$$\mathrm{CysS3/4SCys + R\cdot \ \rightarrow R3/4SCys + \ \cdot SCys,} \qquad (1)$$

$$\mathrm{SCys + RH \rightarrow \ R\cdot \ + HSCys.} \qquad (2)$$

Additional clarification steps such as sedimentation (Deb-Choudhury et al., 2018) or isoelectric precipitation at selected pH (pH 3.22 to pH 5.55; Koleva & Zheleva, 2022; Li et al., 2013; Zhang et al., 2013) are employed after acid/alkali hydrolysis to aid in the purification of the extract. This involves neutralizing the acid/base to precipitate the protein, then freeze-drying to obtain a protein powder. Another benefit of isoelectric precipitation is its use in classifying keratin proteins according to their isoelectric point resulting in different proteins with distinct physicochemical properties (Zhang et al., 2013).

Acid-hydrolyzed wool contains 85%–95% protein (w/w) and comprises all essential amino acids (Table 2) and significantly high cysteine levels than the alkali-based technique (Deb-Choudhury et al., 2018). In addition, while low threonine, serine, and arginine yields have been reported under alkaline hydrolysis, the process yields higher methionine, lysine, and glutamic acid. At usual operation temperatures of 80°C–140°C, alkali hydrolysis of wool is much more destructive to the peptide linkages than the acid process, which lowers the nutritional value of keratin (Friedman, 1999; von Holstein et al., 2014). Cystine, cysteine, and serine residues can rapidly hydrolyze at high pH and undergo β-elimination reactions to yield DAL residues. Subsequently, DAL residues react with cysteine and lysine residues to form the highly stable LAN and LAL, respectively (Steenken & Zahn, 2016). Both LAN and LAL are biologically unavailable and potentially harmful (Bhavsar et al., 2017). In addition, the conditions can also cause racemization and decomposition of amino acids, for example, degradation of cysteine to oxalic and pyruvic acids (Bellagamba et al., 2016). The acid hydrolysis method can also cause the conversion of asparagine to aspartate and glutamine to glutamate (Pasupuleti et al., 2008).

The extensive hydrolysis of the protein leading to elevated levels of low Mw products can result in low protein yields, for example, due to the formation of significant amounts of nonprecipitable material. Thus, harsh alkali treatment has been associated with low protein yields, low cysteine levels, and high protein degradation at elevated temperatures (Bhavsar et al., 2017). Indeed, Shavandi et al. (2016) showed that extensive hydrolysis of the protein peptides and degradation of amino acids under the alkali method resulted in low extraction (~25%) and protein (63%) yields compared with sulfitolysis (54% overall yield) and ionic liquid (IL; 95% protein yield).

Other concerns in alkali/acid methods are the residual sodium salts or extreme hydrolysis of the protein into amino acids, low Mw short-chain peptides (e.g., < 10 kDa) and soluble sulfides (Shavandi et al., 2016). Alkaline sulfides are undesirable due to the intense sulfur odor, while the high oligopeptides and amino acids content, instead of the desired selective opening of the disulfide bonds to obtain soluble polypeptides, is undesirable due to their unwanted flavors, such as bitterness (Su et al., 2020; Toldra et al., 2016). Therefore, the taste of hydrolyzed wool products, such as those containing elevated levels of small peptides and amino acids (particularly those with abundant hydrophobic residues), has been a subject of recent investigations. The remedy proposed is to cleave the hydrophobic amino acids from the peptides to make the product more appealing for food applications (McCarthy et al., 2013; Nchienzia et al., 2010). This can be achieved by (i) extending the hydrolysis periods to remove basic and aliphatic amino acids from the C- and N-terminals of the polypeptides (Hou et al., 2017); (ii) post-hydrolysis treatment using exopeptidases (e.g., keratinase, an isolate from *Bacillus licheniformis*) to cleave the basic and aliphatic functional groups (Abd El-Salam & El-Shibiny, 2017; McCarthy et al., 2013; Toldra et al., 2016; Verma et al., 2017), (iii) flavor modification using functional ingredients such as monosodium glutamate, glycine or inosine to overcome the odd flavors (Hou et al., 2017).

Other drawbacks of the acid/alkaline hydrolysis processes include high residual reagents and the potential formation of salts following neutralization. In addition, processing aids, such as copper salts added to catalyze

cystine oxidation to cystic acid, causes the formation of protein–copper complexes. The complexes make removing the copper residues in subsequent dialysis procedures challenging, making the hydrolysate unusable in food applications (Nagai & Nishikawa, 2014). The phenomenon was demonstrated by Nagai & Nishikawa (2014) using chicken feathers. The authors reported strong complexes of protein and copper on solubilizing keratin in Schweitzer's reagent (an ammonia solution of cupric oxide produced by precipitating copper(II) hydroxide from an aqueous solution of copper sulfate using sodium hydroxide [NaOH], then dissolving the residue in ammonia solution).

Overall, acid hydrolysis, for example, HCl, is more industrially feasible than the alkali method because of the low cost, effectiveness at low concentrations and minimal modification of cysteine to LAL and LAN. However, the application of harsh acidic conditions (e.g., 11 N HCl for 6 h at room temperature) can lower the yield of cysteine and other essential amino acids (e.g., tryptophan and methionine; Deb-Choudhury et al., 2018). On the contrary, the alkaline method, which is destructive to the protein, may not be feasible for commercial application in converting wool to keratin.

3.3.2 | Oxidation

The weakening of numerous inter-and intra-molecular cystine cross-links in wool by reacting with oxidizing agents (Table 1) allows the separation of wool fibers into constituent elements, generating low Mw proteins and peptides. Oxidizing agents such as hydrogen peroxide (Fukatsu, 1997), peracetic acid (PA), or performic acid (PFA; Shavandi, Carne, et al., 2017; Zoccola et al., 2015) can permeate through the wool fiber cortex, converting cystine to cysteic acid residues. The mechanism of action proposed by Fukatsu (1997) involves the formation of complexes between wool and transition metals, for instance, copper ions (Cu (II)–wool complex). Next, the wool-transition metal complex is degraded by reactive species, such as the hydrogen peroxide released by the oxidizing agent. As a result, the process modifies the protein chemically by converting disulfide bonds to sulfonic acid—a non-crosslinked soluble form, which causes significant changes in the physicochemical properties of keratin compared with other methods. For example, PA solubilizes the α-keratin portion of wool, leaving the β-keratin in its insoluble form (Shavandi, Carne, et al., 2017). The process allows protein separation into α-, β-, and γ-keratose, based on their solubility and reaction at different pH.

Investigations have shown that keratin can be extracted by reacting wool (wool:liquid ratio 1:65) with 24% PA and stirring for 24 h at room temperature, followed by filtration (125 μm pore size) to retrieve the oxidized wool. Afterward, the oxidized wool is rinsed with deionized water then treated with a denaturing solvent such as Tris (e.g., 100 mM) or 1 M sodium dodecyl sulfate (SDS) solutions for 2 h at 37°C with shaking. Finally, the extract containing soluble keratin is filtered (125 μm pore size) then dialyzed (12−14 kDa cutoff membrane) against distilled water for 3 days with a regular water change (de Guzman et al., 2011; Rajabinejad et al., 2017; Shavandi, Carne, et al., 2017). Soluble keratin yields from the oxidation process depend upon the type and concentration of the chemical agent, pH, temperature and incubation period. For instance, 57% water-soluble keratin was realized using 24% PA (Shavandi, Carne, et al., 2017) compared with 30% yield using 2% aqueous PA (36%–40% v/v PA in acetic acid; Rajabinejad et al., 2017) and 5% extract using 2% (w/v) PA (for 12 h and 25°C; Shavandi et al., 2016). The pH of the solutions, which is related to the concentration of the oxidizing agent, plays a vital role in the process, where higher yields are obtainable at a low pH (<4.0). The soluble and insoluble keratin portions are characterized by high contents of cysteine-S-sulfonated residues of Mw in the range of 40−60 kDa, representing low sulfur proteins (Bhavsar et al., 2016; Shavandi, Carne, et al., 2017). These observations are related to the oxidation of the naturally occurring disulfides linkages of keratin by PA to form hydrophilic sulfonic group residues on the side chains of cysteine.

Although the oxidation process has historically extracted keratin from keratinous materials, most oxidizing agents are toxic, making it challenging to upscale the process for industrial application (Zhang et al., 2020). Other drawbacks of the technique include the low extraction yield, long processing times, irreversible oxidation of amino acids and loss of protein integrity. For example, keratin oxidation by PA leads to the formation of keratose, a product containing sulfonic acid groups and cysteic acid instead of the covalent crosslinks of disulfide bonds observed in kerateine (de Guzman et al., 2011). Nevertheless, keratin products from the oxidation method are recommended for regenerative medicine applications such as wound care products (de Guzman et al., 2011; Shavandi et al., 2016).

3.3.3 | Reduction

Reducing agents

Reducing agents such as thioglycolic acid, L-cysteine, thiosulfates, sulfites, and 2-β-mercaptoethanol (MEC; Table 1) are the most direct disulfide bonds dissociation methods to obtain keratin polypeptides (Shavandi et al., 2021). The reduction process often requires the presence of protein-denaturing agents such as urea, SDS,

ethylenediaminetetraacetic acid (EDTA) or Tris-HCl, which breaks the hydrogen bonds (Zoccola et al., 2009). The reducing chemicals target the covalent disulfide bonds (R–S–S–R) of inter-/intramolecular cystine chains, cleaving them to generate free thiols (R–SH) of cystine (R is the peptide backbone). The process follows two reversible nucleophilic displacement reactions with the formation of two free thiols attached to the protein chains, which can be reformed back into disulfide crosslinks (Poole et al., 2009).

Urea is often used as a swelling agent or protein denaturant that aids the reduction of keratin by loosening the cortical cells through the hydrogen bonds degradation effect, depolymerizing the inter- and intra-molecular S–S bonds (to –SH groups). However, the resulting cysteine-containing derivatives, also known as "kerateines," can re-oxidize to form disulfide linkages, restoring the original state of keratin, for example, the resistance to enzyme hydrolysis (He et al., 2020). Therefore, the reduced keratin is diffused in a surfactant-water mixture such as SDS, iodoacetic acid, or EDTA, forming large micelle-like structures that prevent re-oxidation and SH groups reformation (Sinkiewicz et al., 2016; Wang et al., 2020; Yamauchi et al., 1996). Cardamone (2010) extracted keratin using 6 M urea, 3 mM EDTA, 1.4 M MEC (at pH 9.1, 62°C–65°C, 4 h). Extraction of keratin using the reduction process resulted in higher retention of native microstructural units, the Amides I, II and the secondary structure than alkaline hydrolysis using 0.5 M NaOH, pH 13.9 (62°C–65°C, 3 h). Compared with the α-helix/β-sheet/disordered units of wool (58.2%/37.9%/3.9%), the reduction method showed high preservation of the original conformation (36.7%/50.2%/13.1%) at Mw 60–40 kDa. However, the alkaline hydrolysis caused excessive fragmentation of keratin peptides into smaller units (8–6 kDa), resulting in a majority of β-sheets and disordered regions (51.8% and 22.5%, respectively) with less of the α-helical units (25.7%; Cardamone, 2010). Generally, reducing agents cannot be recycled, harm the environment, and may have toxic effects, thus being unsuitable for producing edible keratin (Khumalo et al., 2020; Pourjavaheri et al., 2019). Therefore, this method requires carefully selecting chemicals to produce edible keratin.

Recent studies have proposed techniques that could eliminate the use of harmful chemical agents in the reduction hydrolysis of keratinous materials (Kui et al., 2016; Pourjavaheri et al., 2019; Xu et al., 2014). For example, Kui et al. (2016) and Xu et al. (2014) demonstrated the potential of extracting keratin from wool using 5% (w/v) in a solution of 10% L-cysteine (w/w based on wool) in 8 M urea. Compared with the alkaline hydrolysis method, the L-cysteine/urea technique preserved most native microstructural conformation in the extracted keratin, giving 63%–72% pure keratin yield of Mw ~40–50 kDa, with the majority at ~45 kDa (Kui et al., 2016; Xu et al., 2014).

Furthermore, an aqueous solution of GSH (0.06–0.1 M) containing 8 M urea (pH 10.0) can also extract keratin from wool at 65°C–85°C for 9 h (Su et al., 2020). First, wool is hydrolyzed by treatment at 70°C–75°C, pH 10.5 for 24 h. Next, the solubilized keratin is recovered from the hydrolyzed mix by centrifugation, for example, at 15,000 × *g* for 15–20 min, followed by isoelectric precipitation at pH 4.0 or membrane filtration (pore size 8–14 kDa). Next, the precipitate is washed repeatedly using distilled water to remove the remaining salts. Finally, the obtained concentrate/residue can be oven-dried (50°C) or freeze-dried, then pulverized to uniform particle size (Su et al., 2020). GSH breaks down disulfide bonds of wool fibers by reduction, extracting ~65% of keratin-containing peptides with Mw of 26–66 kDa. The GSH technique is environmentally safe because it does not apply harmful chemicals (Su et al., 2020). However, the prohibitive cost of GSH and L-cysteine might hinder the large-scale application of the methods.

Sulfitolysis and oxidative sulfitolysis

Sulfites can react with cysteine residues in a reversible reaction converting them to cysteine thiols and S-sulfonate anion (Bunte salts, $-S-SO_3^-$). The sulfite source can be microbes or sodium salts of sulfite (SO_3^{2-}), bisulfite (HSO_3^-), and disulfite ($S_2O_5^{2-}$) assisted by NaOH (Shavandi, Silva, et al., 2017). The hydrolysis process relies on the ability of the sulfite agents to reduce and dissolve the S–S bonds, which occurs rapidly at pH > 9.0 and at increasing temperatures (>60°C), sulfite concentration and reaction times (Table 1). The role of NaOH in the solvent mixture is to adjust the pH and degrade the wool outer layers to give the sulfites access to the disulfide bonds. Sulfitolysis of cysteine occurs reversibly, particularly at pH >9.0, with bisulfite or disulfite acting as the active species (Poole et al., 2009). However, with urea and SDS, the reaction is boosted toward forming cysteine thiol and cysteine-S-sulfonate. In addition, SDS protects against the re-oxidation of cysteine-S-sulfonate by air. During sulfitolysis, sodium sulfite reacts with water to form hydrosulfite and hydroxyl ion (OH^-). Next, the OH^- breaks the disulfide bonds to form dehydroalanine (DAL) and perthiocysteine, further dissociating into cysteine and sulfur. Dehydroalanine is a highly reactive product that can crosslink with cysteine and lysine to form LAN and LAL.

Wang et al. (2020) reported a time-and temperature-dependent keratin dissolution using sodium sulfite (30 g/L), NaOH (6 g/L), and SDS (10 g/L) at 90°C and wool:solvent ratio of 1:10. One hour reaction time resulted in a 69% (w/w of keratin in raw wool) keratin dissolution rate, with significant increases to 99% when incubated

for 3 h. In addition, the process resulted in a 60% yield of hydrolyzed keratin, comprising high levels of sulfur-containing amino acids (10–20 kDa) and tyrosine levels (6–9 kDa; Wang et al., 2020). Sulfitolysis of wool to produce keratin can also occur at lower extraction temperatures but a prolonged incubation period. For example, 38%−41% of keratin extraction yield is obtainable by reacting wool with urea (8 M) and sodium metabisulfite (10 g or 0.5 M) for 10 h at 60°C (Aluigi et al., 2014; Shavandi et al., 2016). Although shorter extraction durations (~2–2.5 h) may be applied for moderate keratin yields (32%; Aluigi et al., 2014; Rajabinejad et al., 2017).

Differences in extraction protocols and separation techniques among various groups suggest that parameter optimization is necessary to reduce harmful chemicals and maximize the keratin dissolution rate and yield. For instance, the temperature needs to be above 60°C because the tough scales on wool fibers can withstand lower temperatures (Wang et al., 2020). In addition, elevated temperatures aid in substrate swelling and increase thermal disruption of macromolecular chains, resulting in higher keratin dissolution rates. Nonetheless, caution should be applied during prolonged treatment at elevated temperatures (70°C, pH ≥ 11.0) due to the potential degradation of the protein into artifacts (e.g., LAN and LAL). Moreover, extensively degraded peptides and amino acids can be lost during dialysis, for instance, the 12−14 kDa cutoff membrane (Shavandi et al., 2016).

Sulfitolysis products include S-sulfonated keratin intermediate, high sulfur S-sulfonated keratin and keratin peptides. A significant drawback of sulfitolysis is the conversion of cystine (and cysteine) to cysteic acid and further to keratoses (Rajabinejad et al., 2017). Keratoses are highly heat-stable, water-soluble, nonreactive oxidation products formed after the cleavage of disulfide bonds by sulfitolysis (Sinkiewicz et al., 2018). Compared with keratin, the disulfide bonds in keratoses are substituted by sulfonic acid groups and cysteic acid. Furthermore, keratoses are characterized by low sulfur (60–45 kDa), high Mw, and stronger ionic interaction than keratin. Their presence signals the loss of cysteine through oxidative sulfitolysis (Van Dyke, 2016; Yamauchi et al., 1996). Oxidative sulfitolysis proceeds irreversibly, converting disulfide into two S-sulfonate (Equation 3; Poole et al., 2009).

$$R-CH_2-S-S-CH_2-R+2SO_3^{2-}+H_2O \xrightarrow{[O]} 2R-CH_2-S-SO_3^- + 2OH^-. \quad (3)$$

Sulfitolysis is helpful due to the ease of manipulation for partial hydrolysis to obtain desired keratin characteristics under controlled conditions. Keratin from the sulfitolysis method has been proposed for applications in the biomedical field, for example, in scaffold production or filtration application, where the proteins' mechanical properties are crucial. Moreover, sulfitolysis agents (sulfites, bisulfite, metabisulfite) are permitted in foods, albeit with considerations for allergenicity (Fernandes et al., 2021; Mizani et al., 2016), hence proposed for scaled-up production of food-grade keratin (Zoccola et al., 2009).

3.3.4 | Ionic liquids and deep eutectic solvents

Ionic liquids (ILs) are salts that solely consist of organic cations (for instance, imidazolium, pyrrolidinium, or tetraalkylphosphonium) and organic and inorganic anions (such as acetate or chloride) with a melting point of 100°C (Table 1; Tomlinson et al., 2014). They are nonflammable, nonvolatile, recyclable, and can dissolve biological materials, including wool keratin (Ghosh et al., 2014; Idris et al., 2014; Li & Wang, 2013; Zheng et al., 2015). Ghosh et al. (2014) dissolved wool in hot IL, 1-butyl-3-methylimidazolium chloride (1B3MIMC) at 120°C–180°C, which regenerated keratins after coagulation with water. The keratin product had reduced cysteine content due to the elevated temperatures used. The generated keratin is functional in biomedical applications, where high thermal stability and the ability to stand compression molding are essential characteristics. The performance of ILs can be improved using the synergy with other techniques such as heating, ultrasonication or chemical hydrolysis. Ding et al. (2019) showed that 8 M HCl containing 1B3MIMC as a cosolvent combined with ultrasonication (225 W power at 80°C for 1.4 h) and heating (110°C for 8.3 h) could improve solubilization of the protein from keratinous materials. Compared with HCl only hydrolysis method, the ultrasonic-IL (UIL) process presents shorter reaction times and a higher yield (83.1%) of hydrolyzed keratin and cuts the need for corrosive acids. The obtained keratin from IL-assisted methods is water-soluble, but the ILs, for example, hydrophobic IL, 1-hydroxyethyl-3methylimidazolium bis(trifluoromethanesulfonyl) amide ([HOEMIm][NTf2]), are insoluble (Wang & Cao, 2012). Thus, keratin is purified by removing the IL, followed by dialysis, then precipitation using ethanol, which facilitates the recovery of ILs (Wang & Cao, 2012). Due to the potential toxicity of ILs (Docherty & Kulpa, 2005; Garcia et al., 2005), keratin obtained using this method has not been investigated in human-edible products. However, no information confirms or overrules such contention, and future research is encouraged to explore this knowledge gap.

Deep eutectic solvents (DES) such as choline-chloride (ChCl)-glycerol, ChCl-oxalic acid, ChCl-citric acid, ChCl-lactic acid are potential alternatives to conventional alkali,

acid, and IL solvents (Contreras et al., 2019; Idris et al., 2014; Jiang et al., 2018; Ozel & Elibol, 2021; Smith et al., 2014; Xu et al., 2015). These compounds are considered nontoxic, requiring only water and food-grade chemicals such as lactic acid and cysteine, and replace the need to use conventional chemicals such as urea, sodium sulfite, and NaOH. DES is also renewable, reusable, biodegradable and easily adaptable in industrial processing due to low vapor pressure, flammability, and high dissolution ability (Ozel & Elibol, 2021). Recently investigated applications of DES in keratin production include lactic acid and L-cysteine (Okoro et al., 2022; Shavandi et al., 2021), ChCl-oxalic acid (Wang & Tang, 2018), ChCl-urea (Jiang et al., 2018), L-cysteine-urea (Wang, Li, et al., 2016) and L-cysteine, hydrochloride and sodium sulfite (ethanol-water mixed system; He et al., 2020).

Recently, Shavandi et al. (2021) proposed a new lactic acid and L-cysteine DES to regenerate keratin from wool. The lactic acid and L-cysteine DES (2 g L-cysteine and 20 ml L(+)-lactic acid as a solution in water) can dissolve 90% of wool within 3.5 h at 95°C by mixing 22 mg of wool in 1 g of the solvent. However, prolonged time (8 h) and higher temperatures (105°C) might be required to dissolve a similar proportion of wool at a lower L-cysteine ratio in DES (1.6 g L-cysteine in 20 ml of lactic acid using 0.5 g of wool; Okoro et al., 2022). The mechanism of action in lactic acid/L-cysteine keratin degradation was suggested as the initial degradation of hydrogen bonds by lactic acid, followed by L-cysteine cleavage of disulfide bonds (Okoro et al., 2022). Wang, Li, et al. (2016) obtained 72% solubilization of wool keratin by dissolving 5 g of wool in 100 ml of L-cysteine (0.165 M)-urea (8 M), pH 10.5 at 75°C for 5 h. DES products show increased unfolding of the α-helix and higher β-sheet conformation as observed in x-ray diffraction (XRD), and Fourier transform infrared (FTIR) spectroscopy analysis (Shavandi et al., 2021; Wang, Li, et al., 2016). The hydrolyzed wool products were recovered by dialysis, followed by lyophilization, yielding a mix of hydrolyzed keratin peptides with Mw ranging from 38 to 49 kDa, 14 to 28 kDa, and 3 to 6 kDa (Shavandi et al., 2021). Nonetheless, the DES process has the potential to produce edible keratin, owing to the involvement of non-hazardous solvents, including water, L-cysteine and lactic acid. For instance, lactic acid is made from the fermentation of carbohydrates, for example, lactose, glucose, or sucrose, is nontoxic and requires low concentrations to dissolve wool.

3.3.5 | Enzymatic

Biotechnology has emerged as an alternative method for transforming keratinous material into soluble proteins and amino acids, owing to the high preservation of essential nutrients and less nonnutritive LAN and LAL formation (Gupta & Ramnani, 2006). Wool can be hydrolyzed by keratinases, a special class of proteolytic enzymes—keratin degrading enzymes produced by naturally occurring microbes (Table 3). Keratinases are serine endopeptidases that catalyze the hydrolytic cleavage of the rigid and strongly crosslinked keratin polypeptide chains in wool (Hassan et al., 2020). A more detailed overview of the science behind the structure and catalytic activity of the enzymes has been provided in recent reviews (De Oliveira Martinez et al., 2020; Hassan et al., 2020; Qiu et al., 2020; Verma et al., 2017). The catalytic effectiveness of microbial keratinases has also been used to produce keratin composites, biohydrogen, biopeptides, biodegradable films and the breakdown of prions, among other uses (Balint et al., 2005; Gupta & Ramnani, 2006). The proposed mechanisms during microbial keratinolytic activity include sulfitolysis, proteolysis and deamination (Kornillowicz-Kowalska & Bohacz, 2011). Sulfitolysis breaks down the disulfide bonds to cysteine and S-sulfocysteine by the action of sulfite material released by microbes, and proteolysis cleaves the peptide linkages. In addition, microbial enzymes cause deamidation by attacking the insoluble keratinous materials resulting in proteolysis and the release of free ammonium, which is detected as a rise in pH (Daroit & Brandelli, 2014). The efficiency of enzymatic hydrolysis depends on the substrate concentration, enzyme type and loading, reaction time and temperature (Eslahi et al., 2013a).

The known enzyme producers are bacteria, actinomycetes, and fungi, with the enzymes from *B. licheniformis* PWD-1 (Versazyme, Valkerase, and Prionzyme) and yeasts *Tritirachium album* proteinase-K recombinant being the only ones available commercially in the purified form (Hassan et al., 2020; Kshetri et al., 2020; Qiu et al., 2020). Purified enzymes are expensive, prompting the direct use of microbes on the substrate as biocatalysts to give the desired keratinolytic activity (Table 3). The optimal growth of microbes and enzyme production conditions occur in neutral to alkaline environments, at low temperatures (30°C–60°C) and under reducing conditions, which are favorable for preserving the physical and chemical quality of the hydrolyzed keratin (Fakhfakh et al., 2013; Tuysuz et al., 2021). The degradation of keratinous materials by microbes relies on the synergistic activity of several enzymes. For instance, endoprotease (S8), oligopeptidase/metalloprotease (M3), and an exoprotease (M28) have been identified in a nonpathogenic fungus *Onygena corvina* (Lange et al., 2016).

Keratinolytic bacteria such as *B. licheniformis and Bacillus subtilis* have optimal growth conditions at pH 6.0–11.0 and 30°C−55°C for up to ~7 days (Kshetri et al., 2020; Tuysuz et al., 2021). For instance, *B. subtilis* DB 100 can utilize sheep wool as a sole carbon and nitrogen

TABLE 3 Some important proteases and bacterial keratinases used with/without chemicals for hydrolysis of wool

Bacterial/enzyme	Optimum degradation conditions	Process and product characteristics	Reference
Streptococcus zooepidemicus	Chemical hydrolysis to facilitate the growth of the bacteria Supplemented with molasses Sheep wool peptone (70.6% protein)	- Hyaluronic acid (HA) for food, cosmetic and biomedical industries was produced at 3.54 g/L HA - Sheep wool peptone is rich in amino acids (especially cysteine, lysine, and arginine)	Arslan and Aydogan (2020)
Bacillus pumilus A1 (alkaline keratinases)	50 g/L wool, 0.5 g/L KH_2PO_4, 0.5 g/L K_2HPO_4, 2.0 g/L NaCl, 0.1 g/L KCl, 0.1 g/L $MgSO_4$·7H2), pH6. First autoclaved at 121°C for 20 min, inoculated with 100 ml 6% culture medium, 48 h fermentation at 45°C, pH 10	- 50 g/L wool gave the highest enzyme activity, 587 U - Hydrolysate showed high in vitro digestibility (97%) compared with 3% in untreated wool - Amino acid production—39.7 g/L. - WPH showed radical scavenging activity - WPH showed chelating and reducing power of F^{3+} - Fe^{2+}	Fakhfakh et al. (2013)
Bacillus subtilis/licheniformis *B. subtilis* DB 100		- Bacteria grow on and hydrolyze native and milled wool - Increased level of soluble proteins - Potential increase in the nutritional profile of the broth	Queiroga et al. (2012)
	30°C, pH 7 with agitation Basal medium[a]	- Accessibility to enzyme hydrolysis increased by double autoclaving and alkali treatment of un-defatted chopped sheep wool. - Release of soluble proteins	Zaghloul et al. (2011)
	Basal medium + alkali	- Wool became more accessible to keratinolytic alkaline protease enzyme via disruption of the S–S bonds - 2.6-fold increase in the net levels of released soluble proteins	
Thermoactinomycetes strains	Sheepskin and wool (6 g/L) mixture in a mineral salt medium (g/L) NaCl, 5; $CaCO_3$,5;K_2HPO_4:$3H_2O$, 3.5 (pH 7.2). Incubation at 55°C, 120 h with stirring	- Microbial degradation of wool was shown by low Mw peptides and amino acids - Increase in free amino acids such as asparagine, glycine, proline and lysine	Gousterova et al. (2005)
	1 g/L sodium dodecyl sulfate, 2.6% (v/v) protease (Savinase), along with 8.6 g/L sodium hydrogen sulfite, liquor to fiber ratio of 25 ml/g for 4hr.	- Enzyme activity was dependent on the presence of surfactant. Adsorption of enzyme was required prior to hydrolysis - Enzyme Savinase degrades the intercellular cement preferentially, penetrating quickly into the fiber cortex. - Wool hydrolysis resulted in high-sulfur matrix protein (within 11–28 kDa)	Eslahi et al. (2013a)
Alkaline serine endoprotease, Savinase 16.0 LEX (EC.3.4.21.14)	5 g/L wool, 3.3% enzyme, 214 h hydrolysis time and sonication (80% amplitude, 30 min) Resulting particle size of 215 nm	- Wool nanoparticles - Increased solubility in caustic solution (2.5% NaOH at 80°C)	Eslahi et al. (2013b)

(Continues)

TABLE 3 (Continued)

Bacterial/enzyme	Optimum degradation conditions	Process and product characteristics	Reference
Alcalase (endopeptidase of *Bacillus licheniformis*) or Protamex (endo and exopeptidase from Bacillus spp.)	Alkaline and enzymatic hydrolysis 300% (w/w) water at 80°C, 12% NaOH, 4 h agitation, pH 8, then enzyme hydrolysis 1% Alcalase (2.4 AU-A/g) or 0.5% Protamex (1.786 AU-N/g) followed by 3-h agitation	- Alcalase resulted in 67.2%–81.1% protein content - Cystine 9.6%–19.5% and cysteine sulfur 9.2%–19.2% - Product for use as a nitrogen source in fertilizers	Gaidau et al. (2019)
L-cysteine + esperase + Urea	50 μL esperase, 50 ml of borax, sodium hydroxide buffer (20 mM, pH 10.15, 0.165 M L-cysteine, 8 M urea at 50°C for 15 h. Precipitate dried at 50°C to obtain wool powder	- 90% hydrolysis - The crystallinity of isolated cortical cells was 40% - Hydrolysate obtained using urea contained amino acids and peptides with Mw < 3 kDa	Zhang et al. (2018)
Alcalase (2.4 L)	Alkaline hydrolysis + enzyme Alcalase (1%) 80°C, alkali mixture (10% CaO or 5% NaOH or 5% KOH), 12 h with agitation then 8 h without agitation. Enzyme addition at pH 8.5, 61°C, 4 h stirring.	- Low Mw peptides obtained 13.8 kDa (aimed at availing nitrogen for fertilizer application)	Berechet et al. (2018)
Esperase 6.0 T + alkaline hydrolysis	Degreasing using enzyme Lipex 100 T (Novozymes Denmark), water 1:50, pH 8, 1% enzyme (w/w of dry wool). Stage 1—alkaline hydrolysis at ($Ca(OH)_2$), pH 11.5 at 80°C Stage 2—(protease activity) using 5% enzyme esperase 6.0 T at 60°C, pH 9.0, 24 h.	- Wool decomposition increased with enzyme concentration - Attained 55% degraded wool - Advantageous due to lower temperature use ~80°C	Mokrejs et al. (2011)
Bacillus thuringiensis AD-12 keratinolytic proteinase	Bacteria isolated from soil. Optimal at 30°C pH 7.0. Mn^{2+}, and Li^{+} stimulate activity	- Keratin was degraded into small peptides of Mw < 15 kDa	Gegeckas et al. (2015), Gegeckas et al. (2014)

[a]Basal medium II (0.5 g NH_4Cl, 0.5 g NaCl, 0.3 g K_2HPO_4, 0.4 g KH_2PO_4, 0.1 g $MgCl_2$ and 0.1 g yeast extract per liter).

source, causing degradation of the wool on incubation at 30°C, pH 7.0, for 5 days (Zaghloul et al., 2011). Zaghloul et al. (2011). Suggested supplementation of the growth medium with a modified basal medium II (0.5 g NaCl, 0.3 g K_2HPO_4, 0.4 g KH_2PO_4, and 0.1 g $MgCl_2$ per liter, without ammonium chloride and yeast extract) for enhanced bacterial growth and keratinases production. Actinomycetes, such as *Actinomadura* sp., and *Streptomyces* sp., produce keratinase at an optimum pH of 8.0–11.0 and 40°C−70°C. Fungal keratinases are obtained from *Trichophyton* sp., *Aspergillus* sp., *Microsporum* sp., *Fusarium* sp., and *Candida* sp. at a pH of 5.5–9.0 and optimum temperature of 25°C−50°C (Santos et al., 1996; Taskin et al., 2016). Keratinolytic proteases from fungi show optimum performance in acidic and alkaline conditions (pH 3.0–9.0) and low temperatures 15°C−60°C. For instance, keratin-degrading enzymes from *Aspergillus niger* are characterized as serine and aspartic proteases and show optimum performance at pH 4.0–6.0 and maximum activity at 48–96 h (Lopes et al., 2011). *Aspergillus fumigatu*, which grows at an optimum pH of 9.0 and 45°C, can also produce a keratin-hydrolyzing enzyme. Compared with thermal or chemical methods, enzymatic hydrolysis using keratinases is considered an eco-friendly process that can generate keratin products with desirable physicochemical characteristics (Damps et al., 2017; Queiroga et al., 2012; Zaghloul et al., 2011).

Generally, owing to the multiple strong peptides (S–S) linkages in keratin that do not favor keratinase activity, pretreatment steps to degrade the disulfide bonds before the enzymatic process have been proposed. Partial degradation of disulfide bonds is attained using reducing agents (e.g., L-cysteine hydrochloride, disulfide reductases or dithiothreitol [DTT]), sulfitolysis, alkaline treatment or two times autoclaving (Eslahi et al., 2013b; Qiu et al., 2020). Degraded disulfide bonds expose the peptide linkages to microbial activity, thus enhancing the performance of keratinases (Suh & Lee, 2001). For instance, surfactants (such as nonionic Irgasol or anionic SDS) can increase the susceptibility of wool to enzymatic hydrolysis owing to the blocking of hydrophobic associations among proteins, thus weakening the substrate structure (Eslahi et al., 2013a). Using a protease (Savinase), the conditions for maximum keratin extracted were reported as 2.6% (v/v) protease, 1 g/L SDS with 8.6 g/L sodium hydrogen sulfite (a reducing agent) at liquor to fiber ratio of 25 ml/g for 4hr (Eslahi et al., 2013a).

In another study, a reducing agent, 5.0 mM of DTT, exerted 1.6 times enhancement of the catalytic activity of keratinolytic enzymes obtained from *B. subtilis* KS-1 (Suh & Lee, 2001). A pre-hydrolysis step using L-cysteine increased the catalytic activity of esperase, resulting in 90%−99% hydrolysis compared with 25% hydrolysis when using the enzyme only (Zhang et al., 2018). Albeit relying on the synergy between multiple proteases, some keratinolytic enzymes, such as the alkaline proteases from *Bacillus* sp., AH-101 and KS-1, and S8 serine protease from *O. corvina*, have been investigated for hydrolysis of keratin biomass without prior reduction of S–S bonds (Huang et al., 2015). The microbes can grow exclusively on keratinous materials and are highly thermostable, allowing usage at 70°C–80°C, and pH 12−13.

In further attempts to improve the quality of extracted keratin, Zaghloul et al. (2011) showed that autoclaving (120°C for 30 min) chopped wool in NaOH and HCl mixture at pH ~7.0 followed by fermentation by keratinolytic bacteria (e.g., *Bacillus* sp.) can help to increase the yield and lower the incubation period. The improved hydrolysis after thermal alkali/acid pretreatment was attributable to enhanced protease accessibility to the wool structure, allowing fermentation at 30°C, pH 7.0 for a maximum of 3 days, compared with 5−6 days noted in other microbial hydrolysis procedures. Furthermore, alkali pretreatment contributes to additional partial degradation of the S–S linkages leading to faster hydrolysis and creating a favorable environment for alkaline protease production and hydrolytic activity (Eslahi et al., 2013b; Mokrejs et al., 2011). On the other hand, an acidic medium could inhibit keratinolytic proteases by (1) altering the surface charges of substrate residues hindering enzyme attachment and (2) possible conversion of melanin to secondary compounds that inhibit bacterial growth (Bressollier et al., 1999). Microbial proteases contain numerous metal ion binding sites and are often stimulated by calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions (Qiu et al., 2020). However, transition and heavy metals like copper, silver, mercury, cadmium, and lead can inhibit their activity (Brandelli et al., 2010).

In addition to pretreatment, post-hydrolysis treatment of products from the enzymatic process has also been investigated for improved degradation of wool fibers. For example, alkaline keratin hydrolysates obtained by treatment of wool (1:300, wool:water) using 12% NaOH at 80°C during 4 h agitation were further exposed to enzymatic hydrolysis using Alcalase 2.4 L and Protamex (Novozymes Ltd.; Gaidau et al., 2019). Enzyme Alcalase 2.4 L, an endopeptidase of *B. licheniformis*, was applied at a 1% rate (w/w of wool), while Protamex, a mixture of endo and exopeptidase, was used at a rate of 0.5% (w/w of wool) with agitation for 3 h at 60°C. Indeed, the further hydrolysis of alkaline keratin hydrolysates in an enzymatic process was confirmed by lower Mw (6–2 kDa) and reduced protein content (68%) compared with 11−6.8 kDa at 80% protein before the extra processing. Furthermore, Alcalase 2.4 L was reported to give higher retention of cysteine and cystine sulfur contents, 19.5% and 19.2%, respectively, compared with the alkali method. Eslahi et al. (2013b) combined enzymatic wool hydrolysis with ultrasonic treatment for 30 min at 80% amplitude using

Heilscher Ultrasonics (UP200S, 200 W, 24 kHz). Ultrasonic energy modifies macromolecules by creating cavitation, where microscopic bubbles collapse on solid material surfaces, creating shockwaves that cause structural damage. As this occurs, localized heat generated by the process causes further dissociation of intermolecular linkages breaking up the wool into cortical cells and macrofibrils (Fan & Yu, 2009). Compared with untreated hydrolysates, ultrasonic processing resulted in higher fragmentation, evidenced by decreased particle size from 492 to 282 nm after 8 h hydrolysis (Eslahi et al., 2013b). One drawback in using enzymes (such as trypsin, pepsin, savinase, and esperase) is their inability to penetrate and degrade the wool structure beyond the cell membrane complex (Eslahi et al., 2013a). As demonstrated by Damps et al. (2017), preactivation of wool in alkaline media (e.g., NaOH for 1 h) may be sufficient to boost the yield of keratin peptides to a maximum of 85% in a pepsin-assisted extraction process (24 h at pH 2.0 and 21°C).

Enzymatic hydrolysis of wool to keratin results in low Mw peptides (10–44 kDa) that are highly soluble in water (Zaghloul et al., 2011; Zhang et al., 2018). The protein contains elevated phenylalanine, leucine, lysine, tyrosine, valine, proline, isoleucine, and aspartic glutamic acid. Moreover, microbial/enzyme-based hydrolysates provide better alternatives due to improved digestibility and nutrient bioavailability (Fakhfakh et al., 2013; Kumar et al., 2012; Maciel et al., 2016). For instance, (Fakhfakh et al., 2013) reported total keratin degradation when wool (50 g/L w/w wool) was exposed to microbial/enzymatic hydrolysis using keratinolytic bacteria, *Bacillus pumilus* A1 in a mixture of salt solution in a 2-day fermentation at pH 10.0 and 45°C. Zhang et al. (2018) reported >90% solubility rates of keratin using L-cysteine-assisted esperase hydrolysis in the presence of urea. Besides, the processing at low temperatures (50°C) and a pH of 10.15 allowed 100% cysteine preservation (Table 2), suggesting negligible LAN and LAL formation (Zhang et al., 2018). Furthermore, enzyme-based keratin extraction methods are potentially environmentally friendly and can yield food-grade products (Damps et al., 2017). Thus, the microbial/enzymatic process can address the challenges associated with harsh conditions and toxic chemicals in keratin extraction. However, the enzyme production cycles and durations required to hydrolyze the wool are long, posing a scale-up challenge (Okoro et al., 2022).

4 | NUTRITIONAL AND SAFETY CONSIDERATIONS OF KERATIN

In its natural form, keratin cannot be enzymatically degraded by humans following ingestion through the diets, thus requiring modification into its hydrolyzed form. During the extraction process, the indigestible cystine is transformed into cysteine, a mix of small and large peptides and free amino acids. The potential for generating keratin from wool using various extraction methods was shown in the earlier sections. The current section discusses the crucial nutritional aspects of the extracted protein, the essential amino acids content, and its digestibility, bioavailability and toxicity. The nutritional profile of keratin varies according to the extraction method (Table 2), with the overall quality often presented in diverse perspectives, including the proximate and amino acid analysis. The protein requirements for humans are usually determined using the amino acid demand, which considers individual amino acid proportions to fulfill the requirement (Ertl et al., 2016; Gervasi et al., 2020). Sulfur-containing amino acids (methionine and cysteine) and lysine are significant indices of keratin quality because they are limiting elements in food proteins (Friedman, 1999). Generally, keratin is low in methionine, histidine, lysine and tryptophan, which have thus been recommended for supplementation in keratin-based food formulations (Dias et al., 2022; Shorland & Gray, 1970).

The digestion of the native wool keratin by mammalian proteolytic enzymes is limited because of the strong disulfide bonds that make it insoluble in water, weak acids, alkali solutions, and organic acids (Dias et al., 2022; Shavandi, Silva, et al., 2017). The dissociation of disulfide bonds in keratin by chemicals or keratinases converts it into smaller, more soluble peptides and free amino acids. However, due to the strong structural formation of keratin, most hydrolysis procedures, such as heat, acid and alkali, are potentially destructive to the protein, as earlier demonstrated in this review. The protein damage includes destroying essential/semi-essential amino acids, reducing their digestibility and assimilation. For example, typical hydrolysis of keratin at high pH (>11.0) or temperatures (>70°C) results in significant irreversible degradation of cystine to the amino acid derivatives LAN and LAL (Bhavsar et al., 2017; Zhang et al., 2018; Zoccola et al., 2012). LAN and LAL are potentially toxic and nonnutritive byproducts of wool hydrolysis that cannot be converted back to cysteine or lysine by mammals (Friedman, 1999). Consequently, LAN and LAL levels have frequently served as indices of nutritional damage of protein due to extraction conditions (Deb-Choudhury et al., 2018). LAN and LAL are undetectable after 4 h acid (11 N HCl) hydrolysis of wool (Deb-Choudhury et al., 2018) or at pH ≤10.0 for approximately 8 h and temperatures of ≤50°C (Zhang et al., 2018). Extractions under mild conditions result in a higher recovery of cysteine, with a significant yield increase in enzyme-assisted processes.

It is important to note that the extraction methods such as reduction, oxidation, sulfitolysis, and IL can lead to a

retention of the chemical residues, such as surfactants, in the final product, making it unsuitable for food applications. For example, Yamauchi et al. (1996) reported the presence of SDS (an anionic surfactant) residues at 5%−17% w/w of the protein content, which was closely associated with keratin even after repeated dialysis following a reduction extraction process.

Consuming a cysteine-rich diet has proven to enhance biological functions, including the significant antioxidant role of GSH (Crum et al., 2018; McPherson & Hardy, 2011). Certain food-grade extraction methods can recover more than 70% (w/w) of cysteine from wool (Table 2), with cysteine content ranging from 12% to 16% (w/w) of the total protein (Gaidau et al., 2019). These techniques include superheated water (140°C for 60 min; Bhavsar et al., 2017), high-pressure microwave-assisted food-grade acid method (Dias et al., 2022), acid hydrolysis (Deb-Choudhury et al., 2018), enzyme esperase and 8 M urea (Zhang et al., 2018). In addition, these techniques can lead to high recovery of threonine, arginine, and leucine, potentially allowing the generation of keratin for food applications. At the same time, however, harsh conditions such as high pH (pH > 11.0) or high temperature and time settings (150°C for 60 min) lead to the degradation of nutrients such as cysteine (Zoccola et al., 2012). For instance, (Deb-Choudhury et al., 2018) observed 88% cysteine recovery following acid (11 N HCl) hydrolysis of wool for 24 h. However, predigestion of wool in the vapors of 11 N HCl for 4 h and further hydrolysis by HCl exposure for 24 h resulted in cysteine loss, giving only 43% recovery. Applications of keratin as an ingredient in specialized diets is an area of growing interest hence the need to determine its digestibility and nutritional benefits.

4.1 | Digestibility and bioavailability

Protein digestibility is a significant quality factor determining its postprandial net utilization (Valenzuela et al., 2019). However, limited studies have reported the apparent and true protein digestibility and the biological value of keratin generated from wool. Some research groups have performed in vitro digestibility studies evaluating proteolytic activity in peptic or tryptic enzymatic digestion alone or in combination with the pancreatic system (Dias et al., 2022; Fakhfakh et al., 2013; Yamauchi et al., 1996). The in vitro digestibility values in the studies represented the protein released upon the digestion of 1 g of the sample in relation to the total protein in 1 g of a predigestion sample (Fakhfakh et al., 2013). The values estimate the transformation of the strong disulfide linkages in keratin into labile low Mw peptides and amino acids capable of absorption through the gut wall. The in vitro digestibility of keratin depends upon the extraction method, hence the extent of protein hydrolysis; assay enzymes used; and the technique applied in the assay, such as the stage of simulated gastrointestinal digestion. Notably, as mentioned above, keratin-rich dietary sources are recommended to attain the health benefits of cysteine. This is because individual amino acids are poorly absorbed and may be toxic when used as nutritional supplements (Baker, 2006; Wolber et al., 2016).

The hydrolysis of wool causes a noticeable increase in protein availability for gastrointestinal enzyme activity, boosting the nutritional value of extracted keratin. A recent study by Dias et al. (2022) used an in vitro pepsin digestion setup (45°C for 16 h) to investigate the hydrolysis of keratin (87% w/w of total protein) generated via a high-pressure microwave extraction (160°C, 30 min). The results showed remarkable differences in the pepsin digestibility of the soluble and insoluble keratin fractions at 87% and 53%, respectively. These findings agreed with Yamauchi et al. (1996), showing that keratin extracted via a reduction process (7 M urea, 3% SDS, and 1% MEC) could be degraded by enzyme trypsin at 37°C, albeit requiring prolonged exposure (2 weeks) to attain 50%−60% degradation. Yamauchi et al. (1996) attributed the difficulty in the tryptic degradation of keratin to the formation of a highly cross-linked three-dimensional film matrix and the potential formation of keratin-surfactant (MEC) complexes during the extraction process. Moreover, these studies indicate that the extent of gastrointestinal enzyme degradation of keratin depends on prevailing S–S bonds, implying that adequate wool exposure to the extraction parameters can maximize the yield of digestible material.

The microbial or enzymatic degradation of wool is a preferred alternative to improve the nutritional value of keratin because the breakdown of the strong S–S linkages by enzymes increases the susceptibility of keratin to an attack by the digestive enzyme(s). For example, wool hydrolysis using the keratinolytic bacterium *B. pumilus* A1 at pH 10 and 45°C for 2 days not only resulted in maximum amino acids and peptides production (39.7 g/L) but also led to remarkably high in vitro peptic-pancreatic digestibility (97%) of the extracted keratin (Fakhfakh et al., 2013). Furthermore, according to the study by Fakhfakh et al. (2013), a simulated sequential in vitro digestibility assay, for example, using pepsin (gastric phase) at pH 2.0, 37°C for 2 h, followed by pancreatin (intestinal phase) at pH 8.0, 37°C for 16 h, can result in total hydrolysis of keratin into amino acids and peptides, potentially increasing the intestinal absorption (Fakhfakh et al., 2013; Minekus et al., 2014). Generally, the hydrolysis of wool using microbial enzymes caters to low-cost and mild reaction conditions, preserving essential amino acids that may be prone to

degradation in harsh conditions, such as high temperatures and alkali (Zhang et al., 2018).

However, despite the technical, financial, and ethical constraints in applying in vitro assays, the data obtained do not accurately predict the digestibility under in vivo conditions. For instance, the single-stage assays using pepsin, trypsin or pancreatin may not accurately portray the in vivo digestion process (Sarwar, 1997). This is because of the potential neglect and lack of a complete understanding of the synergy between the sequential activity of gastrointestinal enzymes and the role of intestinal microbes in further degrading the protein. Moreover, the single digestive enzyme selected may not be ideal for keratin as the substrate (Yamauchi et al., 1996). Therefore, in vivo studies investigating the growth performance of animal and human models have been used to evaluate the digestibility and certain bio-functionalities of keratin peptides, and more studies are warranted.

The digestibility and health improvement potential of keratin hydrolysates have been tested on rodents, both rats and mice (Dias et al., 2022; Nahed et al., 2012; Oluba et al., 2019), cats (Deb-Choudhury et al., 2018), chicken (Alabi et al., 2021), and humans (Crum et al., 2018). Wool keratin supplementation in feeds can positively influence the growth performance parameters (fed conversion efficiency, weight gain, weight of liver, kidney, and heart) of various animal models. Dias et al. (2022) investigated the effect of 50% dietary supplementation using keratin compared to a casein-based diet in a growing rat model ($n = 10$ per group) for 95 days. Compared with the pure casein diets, keratin extracts obtained via a high-pressure microwave method could likewise support the growth performance of rats, demonstrating the potential for use as a protein source in mammalian diets. The findings of sustained growth performance in wool keratin diets were also replicated in keratin peptides from other sources. These included rats ($n = 6$ per group) supplemented with enzyme-extracted feather keratin hydrolysate (FKH) (20% w/w of feed; Grazziotin et al., 2008), fish ($n = 15$ per group) on acid (HCL)-extracted FKH (12% w/w; Zhang et al., 2014), and broiler chickens ($n = 45$ per group) on acid-extracted FKH (2% w/w; Alabi et al., 2021). Animal models have shown that keratin supplemented diets could boost the apparent digestibility of cysteine and cystine compared with other protein additives and nutrients. In the study by Deb-Choudhury et al. (2018), the inclusion of 2% keratin (acid hydrolyzed) in cat diets boosted the apparent cysteine digestibility to 78.7% from the 50%–60% determined in dietary supplementation using conventional fibers (microcrystalline cellulose). These findings corroborated earlier studies by Wolber et al. (2016), showing that FKH (acquired via acid hydrolysis/alkali precipitation) supplementation could boost cysteine and methionine digestibility and gut absorption in rat models. Additionally, replacing conventional ingredients with keratin could result in higher water and meal intakes, as evidenced in the model of rats (Dias et al., 2022) and cats (Deb-Choudhury et al., 2018). The mechanism of higher meal intake has not been elucidated, but it can be postulated as improved palatability of the feed (Deb-Choudhury et al., 2018). On the other hand, increased water intake can be attributed to the stimulation of the thirst mechanism due to the excretion of excess ammonia via urine, a likely phenomenon due to increased free amino acids and peptides (Suzuki et al., 1998) or the high osmolality of keratin (Dias et al., 2022). The normal osmolality for mammalian cells at physiological pH ranges from 275 to 295 mOsm/kg H_2O (Hooper et al., 2015). Keratin increases the osmolality of solutions in a concentration-dependent manner as follows; 295, 310, and 436 mOsm/kg H_2O for suspensions containing 2, 20, and 200 mg/ml protein, respectively (Dias et al., 2022).

Based on the unique amino acid profile, the completeness of protein is determined using its digestibility in combination with the amino acid score (AAS). AAS measures the ability of the absorbed nitrogen to meet the essential amino acid requirement at safe protein intake levels; determined from mg of amino acid in 1 g test protein divided by the mg of amino acid in the requirement pattern (FAO/WHO/UNU, 2007). Therefore, the product, AAS × digestibility, is recommended for determining the protein quality of a food source, giving the protein digestibility corrected AAS (PDCAAS). However, to the authors' knowledge, the crude protein digestibility of wool keratin determined over the digestive tract to establish its PDCAAS has not been reported.

4.2 | Toxicological considerations

4.2.1 | Effect of extraction methods and keratin composition

Degrading the disulfide bonds in keratin could produce potentially toxic hydrolysates unsuitable for human consumption. Safety concerns arise from chemical contamination, co-extracted compounds or products of digestion and metabolism of the protein. Some optimum conditions used to solubilize wool and extract keratin reveal a wide range of extraction catalysts. However, some chemicals are considered toxic and nonfit for human consumption (Okoro et al., 2022). Keratin is a novel protein and has not been widely targeted for human consumption as food. Thus, information on the safety and health effects of keratin in humans is limited. Still, some human supplements in the market containing keratin (e.g., Cynatine) and several animal trials support a high margin of safety. Several attempts to

improve the safety of keratin destined for food, cosmetic or biomedical applications have been reported, including selecting extraction techniques, solvents and extraction aids (Dias et al., 2022). It has been demonstrated that some chemical aids, such as SDS and urea, do not affect the yield but only improve keratin solubility (Tonin et al., 2007; Zhang et al., 2022). The cost and safety of keratin extraction methods can be enhanced by avoiding some chemicals, for example, urea (Zhang et al., 2022).

In addition, determining the loading of potentially toxic elements could help assess the toxicity of residual extraction aids. For example, the levels of potentially toxic heavy metals, including arsenic (As), cadmium (Cd), mercury (Hg), and lead (Pb) in sheep wool following a typical laboratory determination (digestion with 100-fold diluted nitric acid) can reach a maximum of (ppm) As < 1.51, Cd < 0.48, Hg < 0.14, and Pb < 6.94, with a strong influence of diet and location of the animals (Patkowska-Sokoła et al., 2009). Therefore, applying food-grade chemicals during extraction could help maintain these levels within acceptable limits (Bhavsar et al., 2016). For example, the use of citric (90 mM) and ascorbic (6 mM) acids in a high-pressure microwave process resulted in (ppm) As < 3.0, Cd < 0.02, Hg < 1.0, and Pb < 10.0 (Dias et al., 2022). In contrast, superheated water hydrolysis (185°C, 30 min) resulted in Cd, Hg, Pb (ppm) 0.174, < 0.05, and < 1.0 (Bhavsar et al., 2016). Another study showed Pb at 0.29 ppm and undetectable levels of As and Cd following PA extraction of keratin (Shavandi, Carne, et al., 2017), revealing the potential for maintaining the contamination of heavy metals at a minimum. Notably, wool is an excellent absorbent of heavy metals such as Pb, which occasions accumulation potential during the handling process, which has occasioned its use as an aid for the removal of toxic metals from water resources (Ki et al., 2007; Sun et al., 2009). Thus, ascertaining contamination of heavy metals and other residual chemicals in technical grade processing aids such as NaOH and HCl could facilitate edible keratin extraction.

4.2.2 | In vitro toxicology assessment

Numerous in vitro cytotoxicity studies using keratin from various sources reported no adverse effects in cell culture models (Dias et al., 2022; Shavandi et al., 2016; Sierpinski et al., 2008; Zhang et al., 2013). For instance, at 2 mg/ml concentration, keratin extracts can promote mouse fibroblast-like L-929 cell lines (Dias et al., 2022). The hydrolyzed keratin was obtained via a high-pressure (200 psi) microwave process (160°C, 30 min) using organic acids (citric acid (90 mM) and ascorbic acid (6 mM; pH 2.3). Another study reported no cytotoxicity against human fibroblast-like cells (L929) from 2.5 mg/ml alkali extracts of wool obtained using 2% NaOH at 80°C for 3 h (Shavandi et al., 2016). Furthermore, keratin peptides extracted via acid (4 M HCl, ≤pH 5.0, 95°C; Zhang et al., 2013) or alkali (0.5 N NaOH, 2 h, 90°C and precipitated at pH 5.55; Li et al., 2013) dissolution of wool had no adverse effect on the viability of human foreskin fibroblast cell lines (HFF-1) at maximum concentrations of 1 mg/ml or 0.5 mg/ml, respectively. Apart from boosting the potential for application in food formulations, the lack of toxicity in hydrolyzed wool could facilitate its use in biomedical, pharmaceutical and cosmetic fields, for instance, the development of structural biomaterials (Kirsner et al., 2012; Shavandi et al., 2016).

Recent developments have shown that keratin peptides can express selective inhibition of human tumor cells while supporting the growth of normal cells, raising interest in cancer therapy applications (Damps et al., 2017). For example, at a minimum effective concentration of 0.1%, keratin peptides obtained via enzymatic (pepsin) hydrolysis of wool showed significant suppression of metastatic cutaneous squamous cell carcinoma (SCC-25), with no cytotoxicity effect on healthy adult human epidermal keratinocytes (sHEK; Damps et al., 2017). Nonetheless, the mechanism of the selective antiproliferative impact of keratin peptides, such as the influence of the peptide length and cellular interaction (adsorption and adhesion of cells onto the peptides), is still unknown. Indeed the physical and chemical characteristics of biological macromolecules, for instance, the density and charge of surface residues, can influence their biocompatibility and cytotoxicity (Giteru et al., 2020; Yu et al., 2017; Zhang et al., 2013). Zhang et al. (2013) evaluated the biocompatibility of keratin extracts obtained from the hydrolysis of wool using 4 M HCl (95°C until dissolution) and precipitation at either pH 3.22 or 5.55. Apart from the general revelation of disulfide bond (S–S) degradation in extracted keratin, the FTIR data showed remarkable differences in the unfolding status of the extracts, suggesting differences in the type and bio-functions of the amino acids. For example, at pH 5.55, keratin acquired a more unfolded position (β-sheet) and gave a minimum adverse effect concentration of 1 mg/ml on the fibroblast cell line (HFF-1). However, keratin samples obtained at pH 3.22 showed a more compact structure and a lower inhibition concentration (0.1 mg/ml) against HFF-1. The findings demonstrated the potential of keratin polypeptides and amino acids to promote/inhibit cell adhesion, growth, and proliferation.

The lack of toxicity in the studies mentioned above presents keratin as a potentially safe ingredient for human consumption. Notwithstanding, the fate of ingested keratin peptides, kinetics, clearance, metabolism and immune response (e.g., allergenicity) has not been elucidated. In addition, the previous conclusions on noncytotoxicity were based on in vitro tests using low keratin concentrations

(typically < 2.5 mg/ml) and higher sample concentrations are rarely evaluated. The disparities at higher keratin doses can emanate from co-extracted compounds and subsequent variation in osmolality from the physiological level.

4.2.3 | In vivo toxicology assessment

The safety of keratin peptides has also been evaluated in vivo using animal and human studies (Dias et al., 2022; Li et al., 2013). In vivo studies focus on the potential effects of cysteine (combined with cystine), one of the most abundant amino acids following keratin degradation. Assimilated cysteine is readily oxidized to the insoluble cystine dimer, whereas cysteine and cystine can be toxic (neurotoxic) at elevated levels (Benevenga & Steele, 1984; Colombani et al., 1999; McPherson & Hardy, 2011; Zhou & Freed, 2004). However, free cysteine concentration is subject to regulation by the liver and physiological processes are tuned to limit the absorption of excess cysteine from the dietary supply (Wolber et al., 2016).

Both acute (n=100 per group, keratin dose = 50–1000 mg/kg) and subacute (n = 10 per group, keratin dose = 1–100 mg/kg) 30-day oral toxicity studies using mice showed no adverse effects or clinical signs of toxicity of alkali-extracted keratin (precipitated at pH 5.55) at. More importantly, keratin supplementation at a maximum dose of 1000 mg/kg in the acute oral toxicity study did not affect the feed consumption, body weight and blood composition. Additionally, subacute toxicity using 100 mg/kg keratin did not result in death or toxicity of the mice liver, lungs, kidney, and spleen (Li et al., 2013). Furthermore, no adverse effect on the growth parameters of rodents was observed on supplementing rats feeds with high-pressure-microwave extracted keratin (111 g/kg) at 1:1 ratio with casein (112 g/kg) in diets containing 20% total protein (Dias et al., 2022).

In another study, Houltham et al. (2014) used a blind, randomized, cross-over design to assess the gastrointestinal health of athletes (n = 17 total participants) after consuming a protein bar and drink (cocoa-flavored) formulated using either casein or keratin (Keraplast Technologies Ltd., Christchurch, New Zealand). The study followed a ramped dose protocol attaining 40 g/day target protein by day 5 of two 14-day blocks, interspersed with a 2-week washout period between the formulations. Keratin consumption did not adversely affect the gastrointestinal health of the athletes. However, although the keratin bar (34% keratin, 45% carbohydrate, and 8% fat) and beverage (67% keratin, 19% carbohydrates, and 4% fat) showed high consumer acceptability, they were found to increase the prevalence of flatulence and changes in flatulence odor compared with casein controls (Houltham et al., 2014). These findings suggested the potential for using keratin in improving health and related ergogenic benefits (postexercise recovery) for athletes. The authors attributed the adverse flatulence prevalence to the increased production of sulfur compounds due to the cysteine-rich keratin diet.

In related studies, keratin ingestion in feather keratin-supplemented diets did not adversely affect the growth of rat models (Crum et al., 2018; Wolber et al., 2016). For example, 4-week partial (50%) supplementation of a casein diet with keratin (FKH) resulted in no changes in the food intake, weight gain, bone mineral density, organ weight, white blood cell counts and GSH of rats (Wolber et al., 2016). Another study by Crum et al. (2018) reported no adverse effect on the health of human subjects supplemented with 0.8 g/kg body weight/day FKH (sourced from Keraplast Technologies Ltd., Christchurch, New Zealand) at a 1:1 ratio with casein over 4 weeks. Although the majority of the studies reported no changes in the blood parameters, keratin can boost the blood hematocrit and hemoglobin levels (gram/liter; both ∼5%), as observed in rats on keratin-casein feeds compared with the casein or pea-only diets (Wolber et al., 2016).

Regardless of no adverse effects on the growth performance, liver, and cardiovascular biomarkers of human and rodent models, the reported in vivo studies lack insights on the long-term impact of ingested keratin because of the exposure dose and duration effect. For instance, ∼50 g/day keratin intake (86% w/w protein) has been proposed to meet the Food and Agriculture (FAO) standard daily amino acid requirement for a 70 kg adult male (Dias et al., 2022). Notably, despite some chemicals being unsafe for food formulations, they still return nontoxicity effects using in vitro assays; these include sulfitolysis (8 M urea and ∼7% sodium metabisulfite), oxidation (2% PA, 1 M SDS), and an IL (1-butyl-3-methylimidazolium chloride, $BMIM^{+}Cl^{2}$; Shavandi et al., 2016). Therefore, the lack of toxicity in cell culture models does not imply safe consumption. Thus, safety can be ensured by selecting wool hydrolysis materials and evaluating residual chemicals. Nevertheless, many keratin extracts may still be applicable in nonedible products, including biomaterials production (e.g., wound healing and tissue engineering), drug delivery, and cosmetic fields (Shavandi et al., 2016).

5 | BENEFITS OF DIETARY KERATIN

Hydrolyzed animal proteins are becoming increasingly popular in food applications as flavorings, functional ingredients, or nutritional supplements for amino acid supply (Martínez-Alvarez et al., 2015). The treatment of

wool using one of the methods presented earlier in this review results in amino acid-rich keratin with significantly improved solubility in water and dilute acids that allow potential application in human and animal feed. Cysteine-rich proteins, such as keratin, are emerging as more viable ingredients in food and natural nutritional supplements for amino acid supply and improved antioxidant status in health and disease (Miniaci et al., 2016; Wolber et al., 2016). Some bio-functional properties of keratinaceous products are summarized in Table 4. Besides supplying amino acids for growth support, keratin may possess antioxidant and anti-inflammation (anticancer) properties.

5.1 | Muscle growth and maintenance

Muscle growth is the sum of continuous protein synthesis and loss from protein degradation. Tissue injury (Wan et al., 2020) and intensive exercise (Kumar et al., 2009) results in poor net muscle protein balance several hours following the event, but the protein turnover rate increases for several days after that (Pikosky et al., 2006). Research on strategies to increase skeletal muscle quality and quantity in such situations shows the need for an increased supply of nitrogen to promote protein synthesis over catabolism (Moore, 2019). Increased protein intake, such as supplementation, can enhance tissue repair, promoting recovery after muscle injury and exhaustive exercise. Indeed, research findings have demonstrated the critical role of cysteine in promoting healing during muscle injury and trauma, which occurs by modulation of GSH concentration (Ignowski et al., 2018). Cysteine is the basic structural unit in protein translation and a critical substrate for GSH synthesis that contributes to the multiple cellular defense mechanism.

Degraded wool contains low Mw proteins and amino acids, particularly rich in cysteine and glutamine/glutamic acids. Limited investigations have focused on linking wool-based keratin to muscle development in humans and animals. However, discoveries using keratin from other sources, such as hair and FKH, suggest that keratin (cysteine source) may positively contribute to muscle protein development (Crum et al., 2018; Wolber et al., 2016). Crum et al. (2018) investigated the potential of FKH to increase the lean body mass of active cohorts following a 4-week consumption of keratin (0.8 g of FKH/kg body weight/day) by 15 endurance-trained males. Keratin-based diets showed significantly improved body composition (muscle development), blood, cardiorespiratory variables, and cycling performance than casein protein. Indeed, the FKH-treated group showed an increase in bone-free lean mass in the legs (880 g gain) compared with the casein-treated group (70 g gain; Crum et al., 2018). The study results demonstrated that keratin might be a suitable protein supplement for maximizing increases in lean body mass but not an ergogenic aid for endurance athletes.

5.2 | Keratin peptides as bioactive agents

Materials from natural sources, including keratin in protein hydrolysates and peptides, have been investigated for their role as bioactive agents in modulating body functions. Wool keratin contains approximately 10% cysteine (w/w of total protein), higher than other animal byproducts, including casein and whey (Oosthuyse & Millen, 2016), beef, lamb and poultry meat and offal (Brown, 1989; Taheri et al., 2013), seafood (Brown, 1989; Hansen et al., 2010), legumes, and cereals (Kalman, 2014; Øverland et al., 2009; Rafii et al., 2018; Ulrich & Jakel, 2003). L-cysteine exhibits anti-inflammatory, immune-boosting, and antioxidant properties and several vital physiological roles (e.g., skeletal muscle functions and cellular redox homeostasis; Asano et al., 2018; McPherson & Hardy, 2011; Seidel et al., 2019). Cysteine-rich proteins from animal byproducts have been proposed as nutraceuticals for antioxidant and health-boosting functions in humans and pets (carnivores; McPherson & Hardy, 2011; Tozer et al., 2008). Aside from cysteine supply, keratin possesses bioactive peptides that exhibit antioxidant, antithrombotic, and hypocholesterolemic activities and modulation effects on the cardiovascular system (Fontoura et al., 2019; Sharma et al., 2017). Additionally, keratin peptides can support the body in developing healthier hair follicles and reduce hair fall (Wang et al., 2017). Keratin-based formulations can be made into tablets, capsules, powdered products for external applications, and as edible materials—nutraceuticals. Several nutraceutical products containing wool keratin have been commercialized with claims for health improvement. *K*eraGEN-IV Nutraceutical®, DFK-FLEX®, GLOW® and Kerapro® tablets or beverages (Keraplast Technologies LLC, Lincoln, New Zealand) can boost the production of collagen, hair follicle and nail strength improvement and increase skin compactness (Houltham et al., 2014). Clinical trials also demonstrated that Cynatine® FLX1 (Beer et al., 2013b) and Cynatine® HNS (500 mg/day; Roxlor LLC, Delaware, USA; Beer et al., 2014) could supplement bioactive peptides and amino acids responsible for protecting and rebuilding damaged joints and improving the growth and strength of hair and nails. Other product claims include boosting body protein supply and promoting healing on injury or vigorous activity (Beer et al., 2013a, 2014; Kelly et al., 2007; Kelly & Marsh, 2012). Wool-derived keratin hydrogels can accelerate wound healing by stimulating tissue

TABLE 4 Biofunctional properties of wool keratin

Keratin product	Assay	Observations	Potential physiological benefit	Reference
WKH—Fermentation of wool by keratinolytic bacteria *Bacillus pumilus* A1	In vitro antioxidant assays: 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activity, reducing power and metal (Fe2+) chelating activity.	- DPPH radical scavenging activity with an IC_{50} - WKH could react with free radicals to form stable products	Antioxidant and immunity booster: - Source of protein and antioxidants in diets	Fakhfakh et al. (2013)
Cynatine® FLX and HNSa (prepared from WKH)	Human trials using single center, randomized, parallel group, double-blind, placebo controlled 60-day intervention study with 50 subjects.	- Boosted glutathione and other sulfur-rich proteins and peptides in human trials - Reduced concentration of malondialdehyde, a biological marker for oxidative stress	Antioxidant and anti-inflammatory - Increase the vulnerability of tumor cells - Treatment of HIV patients - Improvement in skin compactness, wrinkle reduction, elasticity - Improvement of skin and nail health in ≤90 days - Relieve symptoms of knee osteoarthritis (reduced pain and stiffness of knees)	Beer et al. (2013b) Beer et al. (2014)
WKH—Acid and alkali hydrolyzed	Tested Cu(II) ions and ascorbate-mediated degradation of hyaluronan in the absence and presence of keratin hydrolysate	- Keratin inhibited HA degradation formation of hydroxyl-, alkoxy- and peroxy-type radicals. - Reduced $ABTS^{\bullet+}$ cation radical in the presence of keratin hydrolysate	- Antioxidant - Antimicrobial - Anti-inflammatory	Matyasovsky et al. (2017)
WKH—Acid hydrolyzed, pepsin digestion	In vitro tests using transformed human keratinocytes SCC-25 and human epidermal keratinocytes HEKa	- Keratin reduced the proliferation of squamous cell carcinoma cells up to 67% of cancer cells	- Anti-inflammatory, anticancer	Damps et al. (2017)
WKH and complexes with chitosan or cellulose	Quantifying the anti-inflammatory influence on macrophage marker CD11b	- Composites acted as an anti-inflammatory agent after the addition of keratin. Reduced the growth of vancomycin-resistant *Enterococcus* (VRE) and *Escherichia coli*. - Keratin showed drug-carrying ability	- Anti-inflammatory properties - Bactericidal - Controlled drug release systems	Rosewald et al. (2014)

Note: Cynatine® FLX and HNS have keratin from sheep wool (https://www.roxlor.com/cynatineflx.php.
Abbreviation: WKH, wool keratin hydrolysates.

regeneration (i.e., new capillaries and collagen synthesis) and hair follicle development (Moore et al., 2016).

5.2.1 | Antioxidant activity

Natural antioxidant compounds, including antioxidant peptides (Fontoura et al., 2019; Zou et al., 2016), have gained interest in food preservation owing to the potential adverse effect of synthetic antioxidants, such as butyl hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Due to the electron donor property of the thiol group, keratin residues can exert antioxidant activity by scavenging reactive oxygen species, preventing hydroxyl-, alkoxy- and peroxy-type radicals (Alahyaribeik & Ullah, 2020; Matyasovsky et al., 2017). Matyasovsky et al. (2017) investigated the antioxidant activity of keratin hydrolysates from wool against the degradation of high-molar-mass hyaluronic acid (HA), a naturally occurring biopolymer (mucopolysaccharide acid) and particularly prone to oxidation. The study results showed that keratin could reduce the oxidation power of 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid; $ABTS^{\bullet+}$) cation radical and exhibited antimicrobial activity effect against some strains of *Escherichia*, *Staphylococcus*, *Pseudomonas*, *Candida*, and *Aspergillus*.

Antioxidant peptides such as GSH may act as free radicals scavengers and metal ion chelators (Fakhfakh et al., 2013; Su et al., 2020) that can help to reduce cell damage (McLeay et al., 2017). The antioxidant activity is also attributable to the presence of DAL—electrophilic residues with antioxidant properties—that also facilitate electron donor activity (sulfur reduction action; Gaidau et al., 2019). DAL is formed by the cleavage of disulfide bonds (S–S) in cystine under alkaline conditions, resulting in more oxidized sulfonated groups (Ebrahimgol et al., 2014). The sulfur-reducing action of DAL from acid/alkali and enzyme extraction procedures is also suggested to facilitate the antimicrobial property of keratin (Fakhfakh et al., 2013; Gaidau et al., 2019; Matyasovsky et al., 2017). Experimental models using *Fusarium spp*., a pathogenic plant fungus, showed that keratin, extracted via alkaline-enzymatic hydrolysis of wool, could provide bioactive activity and biostimulant for crops (Gaidau et al., 2021). Another study by Fakhfakh et al. (2013) reported the significant antioxidant potential of keratin obtained from the hydrolysis of wool using *Bacillus pumilus* A1. Keratin peptides exhibited metal (Fe^{2+}) chelation activity, reducing power and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging activities, which are related to hydrogen atom transfer reactions to peroxyl radicals and its electron transfer ability (Bonifacio et al., 2021).

Food products containing keratin could synergistically acquire antioxidant properties to improve their quality (Fontoura et al., 2019). Cysteine derivatives, such as N-acetylcysteine (NAC), have exhibited anticancer properties, including antimutagenic activity toward various genotoxic agents (Estensen et al., 1999). A double-blind placebo-controlled study using patients with increased risk for colon cancer showed a significant reduction in the proliferative index of colon cancer under NAC treatment. In the study by Estensen et al. (1999), administering 800 mg/day of NAC for 12 weeks resulted in more normal cells in rectal biopsy than in the placebo group. Additionally, keratinous materials derived from wool have potential anticancer properties (Damps et al., 2017). Enzyme-extracted keratin peptides showed a minimum effective concentration of 0.1% (w/v) against the proliferation of squamous carcinoma cells with no effect on healthy keratinocytes (Damps et al., 2017).

The deficiency in dietary cysteine and low hepatic GSH concentrations are linked to an increase in oxidative stress, age-related degenerative processes, growth retardation and numerous diseases (Droge, 2005; McPherson & Hardy, 2011). Keratin, the richest natural source of cysteine, could play an important nutritional role in eliminating such deficiencies and diseases. Further investigations are necessary to identify antioxidant peptides from wool keratin-containing cysteine and determine the mechanism of their action and biological activity in vivo.

5.2.2 | Reduction of muscle atrophy and anti-aging

In addition to their antioxidant property, keratin peptides can promote the health of hair, skin, and nails by boosting the production of collagen for repair (Fernandes et al., 2012; Villa et al., 2013). Hydrolyzed proteins are a source of easily absorbable amino acids on ingestion, providing rapid replenishment of protein when urgently required to repair damaged tissues, such as postsurgery, ulcers, burns, and muscle-damaging exercise (Thomson & Buckley, 2011). Naturally, keratin is produced by the body to maintain tissue integrity, including skin and hair, but the body's ability to produce keratin decreases with age. Therefore, keratin supplements can be beneficial in replenishing keratin balance and supporting hair growth (Beer et al., 2013b, 2014). Beer et al. (2013b) investigated the ability of Cynatine® (Roxlor, La Ciotat, France), a commercial keratin-based supplement, to relieve symptoms associated with knee osteoarthritis. The study involved male and female subjects ($n = 50$), with 25 individuals receiving 500 mg of a placebo (maltodextrin) or Cynatine® FLX per day for 60 days. The study results showed that Cynatine® could alleviate symptoms of osteoarthritis, including pain and stiffness. The keratin-based material was suggested to perform the role by reducing

interleukin-1 (IL-1), which stimulates prostaglandins E2 (PGE2; Kras et al., 2013). PGE2 is attributed to joint inflammation; thus, its suppression is considered a therapy for joint pain (Kras et al., 2013; Li et al., 2010). Cynatine® FLX showed a stimulating effect on cell growth by enhancing the production of enzymes that boost joint polymer synthesis by catalyzing the transfer of sulfur from 3 phosphoadenosine-5-phosphate (PAPS). The anti-inflammatory activity of keratin derivatives is attributed to the role of cysteine in boosting skeletal muscle functions, repression of inflammatory cytokine tumor necrosis factor α, improvement of immune function, potential for hepatoprotective effects and boost to plasma albumin levels (Caulin et al., 2000).

Another study showed improvement in skin and nail health in a randomized 90-day administration of Cynatine® (500 mg keratin/day) to 50 females ($n = 25$ per group) compared with a placebo control group ($n = 25$; Beer et al., 2013a). The oral ingestion of keratin tablets showed improved skin hydration, elasticity, wrinkle reduction and increased compactness. Some of the suggested mechanisms in executing these functionalities by keratin include the role of cysteine in (i) regulation of protein synthesis; (ii) antioxidant protective functions; and (iii) catabolism of taurine, a key component in bile salts (Miniaci et al., 2016; Wolber et al., 2016). Food health formulations and supplements have traditionally incorporated natural products involved in tissue rebuilding to support collagen and ligament biosynthesis and muscle tissue maintenance through cellular repair. For instance, oral supplementation of chondroitin sulfate (CS) and glucosamine (GlcN) is recommended for osteoporosis and osteoarthritis management (Hochberg et al., 2016; Stellavato et al., 2019). Keratin derivatives can play such roles that are warranted by further investigations.

5.3 | Keratin peptides as prebiotics

Prebiotics refers to the unique dietary fiber of plant origin that feeds the health-promoting gut bacteria (probiotics). Animal-derived materials have attracted growing interest as potential alternatives to improve the gut ecosystem for monogastric animals (Azad et al., 2020; Lopez-Santamarina et al., 2020). Increasing evidence has indicated that products of wool hydrolysis, keratin peptides and amino acids can act as microbial substrates affecting the growth of gut microbiota and influencing the nature of metabolites. For example, Deb-Choudhury et al. (2018) documented the potential of keratin to regulate fecal short-chain fatty acids (SCFA) and fecal microbial composition in cats, which is implicated in maintaining intestinal health. The study results demonstrated the significant influence of keratin-based diets on fecal SCFA, including valeric, acetic, n-butyric, isobutyric, lactic, propionic, succinic, and isovaleric. Furthermore, the high levels of SCFA suggested increased growth of SCFA-producing bacteria, including *Bifidobacterium, Megasphaera, Bulleidia*, and *Catenibacterium* (Deb-Choudhury et al., 2018). The benefits of SCFA include (1) promoting the development of acidic conditions that control the establishment of pH-sensitive pathogenic microbes, (2) suppression and regulation of gut inflammation by increasing the production of anti-inflammatory cytokines (e.g., IL-10 and TGFβ), (3) decreasing pro-inflammatory cytokines (e.g., IL-6, IL-8, and TNFα), and (4) activating transcription factor Foxp3 (Minamoto et al., 2019).

Additional in vitro studies have demonstrated the potential of keratin to influence the growth of microbes and consequently the type and quantity of valuable metabolites, for example, the production of single-cell proteins, ethanol, organic acids, polysaccharides, pigments, enzymes, vitamins, and antibiotics (Arslan, 2021; Taskin et al., 2016; Tuysuz et al., 2021). Thriving using keratin as a substrate is possible for those microbes capable of using keratin as a source of nitrogen. Taskin et al. (2016) showed that sheep wool peptone (SWP; 67.8% protein), characterized by elevated levels of glutamic acid and cystine (~8.18% and 5.0% of the total amino acids, respectively), could promote the growth performance and metabolites release from *A. niger* and *Escherichia coli*. Still, the medium can moderately support the growth of *B. subtilis, Penicillium chrysogenum*, and *Saccharomyces cerevisiae*, which have the potential for producing valuable substances, such as organic acids, single-cell proteins and enzymes.

Another study by Arslan & Aydogan (2020) showed that SWP could support the development of *Streptococcus zooepidemicus* ATCC 35246, boosting the production of HA. HA is a high-value metabolite microbial polysaccharide with known biomedical and pharmaceutical benefits. The ability of the keratin-rich medium to support the growth of HA-producing bacteria was related to the high protein and higher amino acid content (arginine, tyrosine, threonine, proline, serine, and cystine) than commercial tryptone peptone and protease peptone.

6 | KERATIN APPLICATIONS IN FOOD MATERIALS

6.1 | Composite materials

Interest in sustainable materials from natural, renewable, and biodegradable polymers continued to grow to meet consumer and regulatory agencies demands for sustainable food production systems. Keratin is a

functional natural biopolymer because its cysteine residues can form intra-/inter-molecular oxidative disulfide (–S–S–; Fernández-d'Arlas, 2019). In addition, cysteine is highly susceptible to crosslink development in a network of hierarchical microstructure, which imparts rigidity and boosts the stability and resistance of formed biomaterials to proteolysis (Zhang et al., 2017). These characteristics can help formulate bio-based materials for packaging and other food applications. Coupled with the ease of modification to increase its solubility, –S–S– linkages in extracted keratin can undergo controlled composite development with different plasticizers (e.g., glycerol), co-polymers, and cross-linkers to form desirable matrices. Thus, keratin is an attractive biomacromolecule in food materials, including bioplastics, biodegradable films, electrospun nanofibers, and nanoparticles (Table 5; Aluigi et al., 2013; Bertini et al., 2013). The keratin-based biomaterials can be used in the delivery and controlled release of nutraceuticals, immobilization of bioactive substances, filtration, biosensors, and reinforcement of composite films. The main advantages of keratin in composite development include its low cost, ease of chemical modification, high availability, high compatibility, biodegradability, and nontoxic nature.

Several mechanisms of composite formation using keratin have been proposed, including the reduction or oxidation of the protein to form sidechains of cystine groups (Fitz-Binder et al., 2019). For instance, functional keratin bioplastics were developed through the splitting of disulfide (–S–S–) bonds using a mild alkaline oxidation process by hydrogen peroxide (H_2O_2; Fernández-d'Arlas, 2019). The cleaving of thiol linkages allowed the self-assembly of keratin into defined α-helical conformation, resulting in three-dimensionally bridged structures that stabilized the composite against degradation. Accordingly, cross-linking in the keratin biocomposites was promoted by retaining high Mw fractions, such as 31, 22, and 13 KDa, under the mild treatment processes (Fernández-d'Arlas, 2019). However, excessive hydrolysis could turn the cysteine and cystine sulfur groups into oxidized groups, which causes the loss of the ability to self-assemble (Gaidau et al., 2019). The influence of the highly abundant cysteine-rich (–S–S– linkages) residues have been demonstrated by high transmittance (99%) of keratin composite films, depicting a high-order structure (Yamauchi et al., 1996). Although the highly cross-linked materials retained their susceptibility to in vitro enzyme degradation, they were shown to degrade slowly in the natural environment. Several compounds can be used to manipulate the biocomposite properties. For example, glycerol, a commonly used plasticizing agent, lowers the thiols links, whereas SDS can increase the hydrophobic character of keratin composite materials (Fernández-d'Arlas, 2019; Yamauchi et al., 1996).

Despite the progress in developing and characterizing composite materials from keratin, limited studies have evaluated the cytotoxicity of food-related products. For instance, sources of toxicity in keratin-based materials may originate from the selection of solvents, secondary biopolymers and interactions with modification agents. The use of harsh and potentially toxic chemicals in keratin extraction could limit the application of the material (Yamauchi et al., 1996). Adopting mild processing and safe procedures, for example, microwave-extraction using food-grade chemicals (e.g., citric acid and ascorbic acid, Dias et al., 2022), DES (L-cysteine-lactic acid; Okoro et al., 2022) or enzymatic methods (Gaidau et al., 2019) can support the adaptation of keratin.

6.2 | Encapsulation agent

Keratin has been investigated as a functional material in the fabrication of drug delivery systems due to its pH-responsiveness (de Guzman & Rabbany, 2016; Li et al., 2018) and mucoadhesive (Cheng et al., 2018) properties that allow targeted and controlled drug release. The amphoteric property and the isoelectric point (pI) of 5.30 assists in the binding of molecules to keratin, allowing electrostatic interaction with other proteins and establishment of a pH-triggered release under alkaline (>pH 7.0) conditions or size (Mw) based-release under acidic conditions (< pH 4.0). For instance, in a hair keratin model by de Guzman and Rabbany (2016), polyethylene glycol immobilized keratin can adsorb to bioactive ingredients and release them at appropriate conditions was investigated. Keratin showed selective electrostatic attraction of test proteins at different PIs, for example, attraction toward lysozyme (at pH 11.0) and hemoglobin (at pH 7.0) and repulsion toward albumin (at pH 5.0). The pH-dependent interaction between keratin and the proteins established a control release profile of albumin > hemoglobin > lysozyme. This property aids in absorbing and releasing bioactive protein molecules, such as vascular endothelial cells growth factor C (de Guzman & Rabbany, 2016). Another study by Cheng et al. (2018) showed dominant electrostatic and hydrogen bonding interactions at pH 4.5 and hydrogen bonding and hydrophobic interactions at pH 7.4 between keratin (reduced keratin) nanoparticles and porcine gastric mucin were crucial in the development of a targeted gastric drug delivery system. While the mentioned interactions dominated the association between keratin and active ingredients or external surfaces, FTIR investigations showed that intramolecular interactions in keratin nanoparticles were dominated by $–SO_2–S–$ and –SO–S– linkages (Ebrahimgol et al., 2014). The authors also showed that keratin was required at a concentration

TABLE 5 Wool-keratin composites and their potential applications in food materials

Keratin extraction method	Keratin—composite mixture	Mechanism of composite development	Critical functions	Potential application in food	References
Superheated water	Isotactic polypropylene, polypropylene and keratin composites prepared by melt-mixing at 190°C for 10 min at 60 rpm	- Maleic anhydride grafted polypropylene used to promote keratin dispersion - Keratin increased the crystallization rate and reduced the thermo-oxidative degradation of polypropylene	- Stable polypropylene matrix using keratin particles.	Polypropylene composite matrix-film material	Bertini et al. (2013)
Oxidation	Keratin concentrate 63 g/L in isopropanol, NaOH, glycerol (50% vol./water solution), SDS (10 wt%), sonication 10 min	- The mild oxidative method splits disulfide (–S–S–) bonds - Glycerol acted as a plasticizer, and SDS increased the hydrophobic character of the films - Cross-linking with formaldehyde could increase the toughness	Absorbed up to 35 wt% water at an ambient of 80% RH. Toughness 19 MJ/m^3 The films could absorb UV light Keratin macromolecules had high thermal stability (up to 200°C)	Bioplastic packaging	Fernández-d'Arlas (2019)
Reduction: reducing agent	Casting 40°C, 24 h	- Keratin polypeptides grafted with lipoic acid (30% lipoic acid into keratin solution at 40°C, 2 h reaction time) - Grafting lipoic acid on thiol groups weakens the intra- and intermolecular forces of keratin, increasing order in macromolecular chains rearrangement	- Modified keratin became uniform and compact, - Materials acquired better thermal and mechanical properties. - Tensile strength (TS) unmodified films = 4.28–11.78 MPa (modified films = 17.88 MPa), elongation at break (EAB) unmodified = 0.87% (modified = 6.08%)	Films packaging	Li et al. (2022)
Reduction: reducing agents	Keratin (0.5% w/w) heat pressed 60°C, 2.3–2.3 bar. Drying 20°C−25°C	- Coagulation of pressed keratin in water - Partial hydrolysis left undissolved wool–wool fibers were targeted to form a fibrous matrix	- Tensile strength increased with increasing keratin content. TS = 15 MPa - Water retention value = 75% w/w of composite Maximum and 100% keratin - Degraded wool provided porosity - The composite had high water absorption and retention capacity	Keratin-based bio-composite	Fitz-Binder et al. (2019)

(Continues)

TABLE 5 (Continued)

Keratin extraction method	Keratin—composite mixture	Mechanism of composite development	Critical functions	Potential application in food	References
Reduction: reducing agents	10 ml reduced keratins, 50 wt.% glycerol (0.08 ml), spread in 40 cm^2 80°C for 15 min	- SDS acted as a surfactant—promoted extraction and stabilized protein solution	- Biodegradable keratin film was developed - Films degraded (50%–60% degradation) in vitro by trypsin at 37°C on incubation for 2 weeks. - Keratin film was degraded subcutaneously in mice - Soluble in organic solvents (DMF and DMSO) - Transparency >99% - Thickness 50 μm - Swelling extent 140% - TS = 0.15 MPa, Youngs Modulus (EM) = 0.88 MPa, - Permeable to glucose, urea, and sodium chloride.	Film packaging/bioactive film	Yamauchi et al. (1996)
Reduction: reducing agents	Keratin (≤15%) in poly (vinyl alcohol; PVA) solution (1:20 w/w in water) fiber matrix	- PVA-keratin hydrogen bonds were established during the spinning process. - Mechanical properties decreased with increasing keratin content- maximum keratin content = 15%	- Fiber of diameter 110 μm with oblate cross-section and rough surface - 5%–15% keratin TS = 5–16 cN, EAB = 43%–78%	Fiber nutraceuticals/drugs delivery	Liu et al. (2018)
Reduction: reducing agents	Keratin sponge dissolved in formic acid (0.3% w/v) at 70°C for 24 h, electro-sprayed	- Reduction did not induce macromolecule rapture - Keratin at >0.5% unsuitable for electro-spraying due to formation of agglomerated particles with irregular and flattened shapes.	- Keratin nanoparticle with size 36–72 nm developed	Electro sprayed nanoparticles bioactive delivery system	Ebrahimgol et al. (2014)
Reduction: reducing agents	Keratin (1%–3%), alginate (2.5%) and pectin (2.5%), glycerin (2.5%), formic acid (5%), and deionized water (84.5%). Electrospinning using keratin-polyacrylonitrile (15% (copolymer with 6% methyl acrylate) complex	- N.R.	- Composite film developed with antibacterial effect	Film packaging/antimicrobial film	Goyal et al. (2022)
Reduction: sulfitolysis	Keratin:poly(butylene succinate; PBS) (1:1blend). Electrospun mats	- The immiscible keratin-PBS mixture could be electrospun into nanofibers	- Blending improved mechanical integrity, thermal stability - E-spun matts nanofiber diameter = 290 nm	Nanofiber mats delivery of nutraceuticals/drugs	Guidotti et al. (2020)

(Continues)

TABLE 5 (Continued)

Keratin extraction method	Keratin—composite mixture	Mechanism of composite development	Critical functions	Potential application in food	References
Reduction: sulfitolysis	Electrospinning solutions of keratin in formic acid at 20 and 15 wt.%	- Thermal treatment decreased solubility in water—induced formation of amide bonds - Water stability was attributed to crosslinks between acid and base groups of some amino acid side chains.	- Nanofibers with mean diameters of about 400 and 250 nm - Water stable after 24-h immersion	Nanofiber -wastewater treatment	Aluigi et al. (2013)
Reduction: sulfitolysis	Poly(ethylene oxide; PEO) 4 (5% w/w) Films of 0.01 mm thickness Cast at 40°C	- SDS prevents protein aggregation - Keratin interaction with PEO suppressed PEO crystallization - PEO interfered with keratin self-assembly—gave stability to keratin β-sheet	- Transparency increased with an increase in keratin content	Films and fiber packaging	Tonin et al. (2007)
Reduction: sulfitolysis	15% w/w keratin, 10% w/w citric acid Cast 20°C, 65% RH	- Citric acid promoted macromolecular arrangement in keratin-rich α-helix	- Transparent film, high elongation, minimal solubility in water - Active packaging (biocidal effect)	Film packaging	Ramirez et al. (2017)
Alkaline and enzymatic hydrolysis	Hydrolyzed keratin (67%–81% protein) dried in Petri dishes at 40°C	- Excessive hydrolysis into low Mw protein (2 kDa) increased the tendency to aggregate, poor matric development on excess hydrolysis	- The colors of the keratin films varied from light yellow and yellow for alkaline hydrolysates to brown for alkaline-enzymatic keratin hydrolysates	Metabolism precursors in cultured systems	Gaidau et al. (2019)
Enzymatic	Keratin nanoparticles produced at enzyme loading: 3.3%, substrate concentration: 5 g/L and hydrolysis time: 214 h, ultrasonic treatment (SDS and sodium bisulfite used)	- Enzymatic degradation of wool fiber and segregation of inner cortical cells. - Cleavage of cysteine linkages by the sulfite increased susceptibility to enzyme degradation—achieved nanoparticle size (122–342 nm)	- Sonication decreased the particle size, increased crystallinity, solubility and thermal stability - Nanoparticles successfully incorporated in chitosan/gelatin composite films - Lower degradation rate of nanoparticles on embedding in the films	Nanoparticle bioactive delivery system	Eslahi et al. (2013b), Eslahi et al. (2014)

Abbreviation: SDS, sodium dodecyl sulfates; NR, not reported.

of 0.5% to develop electro-sprayed keratin nanoparticles, although formic acid was involved in establishing the structure (Ebrahimgol et al., 2014).

Keratin nanoparticles can be formed through ionic gelation (Li et al., 2018) and electrospraying (Ebrahimgol et al., 2014). The high cysteine content allows keratin to be reduction-responsive, facilitating bioconjugation with other biopolymers during the formation of nanomaterials. It has been suggested that keratin nanoparticles can be developed without cross-linkers, surfactants, and organic solvents (Li et al., 2018). Li et al. (2018) showed that keratin-based pH/GSH/enzyme triple stimuli-responsive nanoparticles were highly biocompatible with the subjects and could effectively deliver targeted drugs to stop the proliferation of cancer cells.

The potential of wool keratin interaction with other biological macromolecules to bind and release drugs has also been investigated (Rosewald et al., 2014; Tran & Mututuvari, 2015). Keratin complexes with cellulose and chitosan have been developed using butylmethylimmidazolium chloride ($[BMIm^+Cl^-]$), an IL, and showed a unique ability to encapsulate and release ciprofloxacin (CPX). Incorporating keratin into the composite resulted in a desirable slow release and slower release rate of CPX. The improved binding property of keratin was attributable to the secondary structure that supported keratin with a denser structure (Tran & Mututuvari, 2015). In vitro cytotoxicity assays using 3-(4,5-dimethylthiazol-2-yl)−2,5-diphenyl-2H-tetrazolium bromide (MTT) assay indicated that keratin-based materials were nontoxic (Eslahi et al., 2014; Li et al., 2018; Pongkai et al., 2017; Shavandi et al., 2016; Ye et al., 2020), and have a high potential for use as drug carriers. Notwithstanding, cellular toxicity can be introduced by the presence of residual chemicals in the extract. Therefore, extraction methods should be thoroughly vetted when targeted for food, cosmetics, or biomedical applications.

7 | ENVIRONMENTAL AND COST CONSIDERATIONS IN KERATIN PRODUCTION

Currently, nonspinnable, coarse and short wool fibers are discarded into landfills posing environmental hazards (Fitz-Binder et al., 2019; Zoccola et al., 2015). Currently, disposal methods for low-grade wool are limited to landfill because incineration or burning for energy production is inefficient and leads to greenhouse gas production and burning gases containing NO_x and SO_2 (Gaidau et al., 2019). Generally, wool has a low degree of natural degradation, posing a potential long-term environmental pollution on its accumulation. Even so, wool is a possible alternative protein source to fulfill the global protein demand (Dias et al., 2022). Diverting this wool into other uses could reduce the contribution of the livestock sector to greenhouse gas emissions, enhance bioresource conservation, and reduce landfill-related costs. The industrial importance of keratin to other animal waste products is well documented (Ossai et al., 2022; Shavandi, Silva, et al., 2017). Keratin has potential applications in biomedical and tissue engineering, building and construction, automotive and aerospace, pharmaceuticals, and water and wastewater treatment. Other applications include agriculture and horticulture, electrical and electronic devices, food and beverage, energy, textile and tannery industries (Ossai et al., 2022). Valorization of keratinous wastes promotes the development of a bio-based economy that is economically and environmentally sustainable. Although this can be attained by selecting ecologically friendly chemicals and processes, the success of adoption depends on the ability of the method to eliminate the drawbacks of off-odor and taste and improve digestibility, affordability and safety.

Cost-effective extraction methods can be developed using safe technologies (Dias et al., 2022). In addition, techniques designed to achieve solubilized wool with zero solid waste, such as the high-pressure microwave technology utilizing low concentrations of organic acids (Dias et al., 2022) or alkaline and alkaline-enzymatic methods (Gaidau et al., 2019) appear to be particularly useful from an environmental point of view. However, information on the economic aspects of food-grade keratin extraction at the bench, pilot, and industrial levels is currently limited, making it difficult to accurately predict the extracted materials cost. Techno-economic feasibility of a keratin plant may include analysis of product and process safety, efficiency and yield, cost of chemicals, energy input, postextraction handling steps such as dialysis and moisture removal, time of extraction at industrial scale and potential for upscaling (Brown et al., 2016; Gaidau et al., 2019; Ossai et al., 2022). Brown et al. (2016) and Cassoni et al. (2018) attempted to compare the relative cost of different keratin solubilization methods (Table 6). Economic considerations include the ease of handling bulk chemicals and potentially hazardous waste on an industrial scale. According to the authors, the ease of performing the procedures decreased with the increase in cost. Nevertheless, the sustainability of these techniques can be elucidated by further research on the life cycle assessment of the processes.

8 | CONCLUSIONS AND FUTURE PERSPECTIVES

Wool hydrolysis has historically been optimized for non-food biomaterial, biomedical and cosmetic applications. However, the growing interest in food applications has

TABLE 6 Economic indicators of keratin extraction methods adapted and modified from Brown et al. (2016) and Ossai et al. (2022)

Hydrolysis method	Price[a]	Extraction time[b]	Steps	Temperature[c]	Viability of scale up[d]
Oxidation/PCC	1	1	3	RT	++
Reduction/sulfide	2	2	3	RT	++
Reduction-oxidation/thioglycolate	3	3	5	RT	+
Oxidation/peracetic acid	4	3	5	37	+
Denaturation-reduction/MBS	5	1	1	65	–
Denaturation-reduction/UTM	6	3	3	50	–
Acid or alkaline	3	3	3	–	++
Ionic liquids	3	1	2	–	++
Sulfitolysis	3	1	1	–	+
Reduction	3	1	2	–	+
Hydrothermal/steam explosion	6	2	2	–	++
Microwave irradiation	6	3	1	–	++
Super-heated water	6	2	2	–	++
Enzymatic	3	2	3	–	++
Microbial	2	3[e]	3	–	++
Deep eutectic solvents	3	2	1	–	++

[a]Relative costs are based on chemicals per 1 g of wool. Actual prices are not shown as they will vary over time and will depend on supplier availability.
[b]Relative time required to complete the process (1 means less than 1 h, 2 means 1–9 h, 3 means more than 10 h).
[c]Process temperature.
[d]Ease of scaling up (– means less feasible, ++ most viable). Common postextraction procedures such as dialysis and lyophilization steps were disregarded for ease of comparison.
[e]Represents 24–72 h.

led to the adoption of existing processes with significant alterations to accommodate safety and nutritional quality. The characteristics of extracted keratin, including the Mw, structure and amino acid profile, are tunable by varying extraction procedures. For instance, chemical techniques (acid/alkali, oxidation, sulfitolysis, reduction, ILs, and DESs) efficiently hydrolyze wool by reducing disulfide (S–S) linkages. Keratin quality losses are attributable to the loss of essential amino acids, the destruction of some amino acids (e.g., cysteine, methionine, lysine and threonine), and nonnutritive amino acids (LAN and LAL). The loss is prominent at high pH (>10), elevated temperatures or prolonged exposure. Some extraction agents, such as surfactants (MEC) and reducing compounds (SDS), are likely to yield toxic products unsuitable for food and pharmaceutical applications. In addition, reductive or oxidative agents such as sulfites, thiols, and peroxides are poisonous and difficult to remove in the final product (Yamauchi et al., 1996). However, the processes involving alkali/acid, DESs, temperature and pressure or microbial enzymes can yield nontoxic material for bio-application. Harsh conditions (e.g., elevated temperatures and pressure) or high chemical concentrations are needed to degrade the strong disulfide interactions in keratin into soluble peptides and amino acids. This can potentially degrade keratin into nonnutritive amino acids, increase chemical removal costs (e.g., dialysis) or form keratin–chemical complexes (Ye et al., 2020). Nevertheless, the microwave process under high pressure (e.g., 220 psi, 160°C, 30 min) has a high potential for commercialization due to the uniform heat distribution, reduction in the total processing time, and increased energy efficiency (Dias et al., 2022). Still, the process yields nondetectable LAN and LAL levels, suggesting high preservation of the protein quality.

Compared with the chemical processes, enzymatic degradation of wool using keratinases can result in high extraction yields without harmful chemicals in an environmentally friendly process. Furthermore, deactivation and purification processes allow enzyme removal, yielding potentially edible products. The protein quality yield of the microbial process can be boosted during the pretreatment step by predigestion using less concentrated solvents (e.g., alkali), ultrasonication, microwave, or wool grinding into an ultra-fine powder.

The potential of using keratin-rich wool hydrolysate as a source of nutrients (amino acids) in human diets has been documented. Compared with individual amino acids, the supplementation of human food using keratin peptides has shown faster absorption of cysteine and methionine, which can help provide rapid protein replenishment and balanced protein intake. Keratin-formulated food products have the potential for better nutritional quality (protein content and amino acid availability), diet functionality (regulating gut microbes), and other critical biological

functions, including SCFA production. Furthermore, due to the low levels of lysine, methionine, histidine, and tryptophan, the formulated diets can be fortified with pure amino acids or supplemented with dietary-rich sources.

Finally, the available studies did not show any adverse effects of keratin consumption in vitro and in vivo using human and animal models. However, despite the numerous findings of nontoxic products from keratin extracted using various means, the safety after long-term consumption has not been adequately reported. In addition, due to the multiple substances involved and numerous steps during the hydrolysis process, there is a need to set the maximum allowed levels of chemical residues for these methods. Nonetheless, food applications of wool could improve the ecological footprint of sheep farming and unlock the potential of a sustainable source of high protein content that meets demands for the ethical production of animal protein.

AUTHOR CONTRIBUTIONS

Stephen G. Giteru: Conceptualization; Investigation; Methodology; Formal analysis; Writing – original draft; Visualization; Supervision. **Derek H. Ramsey:** Conceptualization; Funding acquisition; Methodology; Formal analysis; Supervision. **Yakun Hou:** Writing – original draft; Investigation; Conceptualization; Methodology; Project administration. **Lei Cong:** Conceptualization; Validation; Visualization; Project administration. **Anand Mohan:** Writing – original draft; Methodology; Validation; Formal analysis. **Alaa El-Din Ahmed Bekhit:** Conceptualization; Validation; Writing – original draft; Writing—review & editing; Supervision; Formal analysis.

ACKNOWLEDGMENTS

The authors thank the Alliance group for funding this review and their respective organizations for logistical support.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

ORCID

Alaa El-Din Ahmed Bekhit https://orcid.org/0000-0003-2569-8620

REFERENCES

Abd El-Salam, M. H., & El-Shibiny, S. (2017). Preparation, properties, and uses of enzymatic milk protein hydrolysates. *Critical Reviews in Food Science and Nutrition*, *57*(6), 1119–1132. https://doi.org/10.1080/10408398.2014.899200

Adhikari, B. B., Chae, M., & Bressler, D. C. (2018). Utilization of slaughterhouse waste in Value-added applications: Recent advances in the development of wood adhesives. *Polymers (Basel)*, *10*(2). https://doi.org/10.3390/polym10020176

Alabi, O. O., Daodu, M. J., Shoyombo, A. J., Akpor, O. B., Oluba, O. M., Adeyonu, A. G., & Abdulazeez, J. (2021). Growth performance of ross broilers fed dietary inclusion of hydrolyzed chicken feather meal. *Annals of the Romanian Society for Cell Biology*, *25*(7), 138–146. https://www.annalsofrscb.ro/index.php/journal/article/view/9456

Alahyaribeik, S., & Ullah, A. (2020). Methods of keratin extraction from poultry feathers and their effects on antioxidant activity of extracted keratin. *International Journal of Biological Macromolecules*, *148*, 449–456. https://doi.org/10.1016/j.ijbiomac.2020.01.144

Aluigi, A., Corbellini, A., Rombaldoni, F., Zoccola, M., & Canetti, M. (2013). Morphological and structural investigation of wool-derived keratin nanofibres crosslinked by thermal treatment. *International Journal of Biological Macromolecules*, *57*, 30–37. https://doi.org/10.1016/j.ijbiomac.2013.02.013

Aluigi, A., Tonetti, C., Rombaldoni, F., Puglia, D., Fortunati, E., Armentano, I., Santulli, C., Torre, L., & Kenny, J. M. (2014). Keratins extracted from Merino wool and Brown Alpaca fibres as potential fillers for PLLA-based biocomposites. *Journal of Materials Science*, *49*(18), 6257–6269. https://doi.org/10.1007/s10853-014-8350-9

Arslan, N. P. (2021). Use of wool protein hydrolysate as nitrogen source in production of microbial pigments. *Journal of Food Processing and Preservation*, *45*(7). https://doi.org/10.1111/jfpp.15660

Arslan, N. P., & Aydogan, M. N. (2020). Evaluation of sheep wool protein hydrolysate and molasses as Low-cost fermentation substrates for hyaluronic acid production by streptococcus zooepidemicus ATCC 35246. *Waste and Biomass Valorization*, *12*(2), 925–935. https://doi.org/10.1007/s12649-020-01062-w

Asano, K., Suzuki, T., Saito, A., Wei, F. Y., Ikeuchi, Y., Numata, T., Tanaka, R., Yamane, Y., Yamamoto, T., Goto, T., Kishita, Y., Murayama, K., Ohtake, A., Okazaki, Y., Tomizawa, K., Sakaguchi, Y., & Suzuki, T. (2018). Metabolic and chemical regulation of tRNA modification associated with taurine deficiency and human disease. *Nucleic Acids Research*, *46*(4), 1565–1583. https://doi.org/10.1093/nar/gky068

Atkuri, K. R., Mantovani, J. J., Herzenberg, L. A., & Herzenberg, L. A. (2007). N-Acetylcysteine—A safe antidote for cysteine/glutathione deficiency. *Current Opinion in Pharmacology*, *7*(4), 355–359. https://doi.org/10.1016/j.coph.2007.04.005

Azad, M. A. K., Gao, J., Ma, J., Li, T., Tan, B., Huang, X., & Yin, J. (2020). Opportunities of prebiotics for the intestinal health of monogastric animals. *Animal Nutrition*, *6*(4), 379–388. https://doi.org/10.1016/j.aninu.2020.08.001

Baker, D. H. (2006). Comparative species utilization and toxicity of sulfur amino acids. *The Journal of Nutrition*, *136*(6), 1670S–1675S. https://doi.org/10.1093/jn/136.6.1670S

Balint, B., Bagi, Z., Toth, A., Rakhely, G., Perei, K., & Kovacs, K. L. (2005). Utilization of keratin-containing biowaste to produce biohydrogen. *Applied Microbiology and Biotechnology*, *69*(4), 404–410. https://doi.org/10.1007/s00253-005-1993-3

Beer, C., Wood, S., & Veghte, R. H. (2013a). A randomized, double-blind, placebo-controlled clinical trial to investigate the effect of Cynatine((R)) HNS on skin characteristics. *International Journal of Cosmetic Science*, *35*(6), 608–612. https://doi.org/10.1111/ics.12084

Beer, C., Wood, S., & Veghte, R. H. (2013b). A randomized, double-blind, placebo-controlled clinical trial to investigate the effect

of Cynatine(R) FLX on symptoms of osteoarthritis. *Journal of Dietary Supplements*, *10*(3), 184–194. https://doi.org/10.3109/19390211.2013.822449

Beer, C., Wood, S., & Veghte, R. H. (2014). A clinical trial to investigate the effect of Cynatine HNS on hair and nail parameters. *Scientific World Journal*, *2014*, 641723. https://doi.org/10.1155/2014/641723

Bellagamba, F., Caprino, F., Mentasti, T., Vasconi, M., & Moretti, V. M. (2016). The impact of processing on amino acid racemization and protein quality in processed animal proteins of poultry origin. *Italian Journal of Animal Science*, *14*(2), 3770. https://doi.org/10.4081/ijas.2015.3770

Benevenga, N. J., & Steele, R. D. (1984). Adverse effects of excessive consumption of amino acids. *Annual Review of Nutrition*, *4*(1), 157–181. https://doi.org/10.1146/annurev.nu.04.070184.001105

Berechet, M. D., Niculescu, M., Gaidau, C., Ignat, M., & Epure, D. G. (2018). Alkaline-enzymatic hydrolyses of wool waste for different applications. *Revista de Chimie-Bucharest*, *69*(7), 1649–1654. https://doi.org/10.37358/RC.18.7.6388

Bertini, F., Canetti, M., Patrucco, A., & Zoccola, M. (2013). Wool keratin-polypropylene composites: Properties and thermal degradation. *Polymer Degradation and Stability*, *98*(5), 980–987. https://doi.org/10.1016/j.polymdegradstab.2013.02.011

Bhavsar, P., Zoccola, M., Patrucco, A., Montarsolo, A., Mossotti, R., Rovero, G., Giansetti, M., & Tonin, C. (2016). Superheated water hydrolysis of waste wool in a semi-industrial reactor to obtain nitrogen fertilizers. *ACS Sustainable Chemistry & Engineering*, *4*(12), 6722–6731. https://doi.org/10.1021/acssuschemeng.6b01664

Bhavsar, P., Zoccola, M., Patrucco, A., Montarsolo, A., Rovero, G., & Tonin, C. (2017). Comparative study on the effects of superheated water and high temperature alkaline hydrolysis on wool keratin. *Textile Research Journal*, *87*(14), 1696–1705. https://doi.org/10.1177/0040517516658512

Bonifacio, V. D. B., Pereira, S. A., Serpa, J., & Vicente, J. B. (2021). Cysteine metabolic circuitries: Druggable targets in cancer. *British Journal of Cancer*, *124*(5), 862–879. https://doi.org/10.1038/s41416-020-01156-1

Bragulla, H. H., & Homberger, D. G. (2009). Structure and functions of keratin proteins in simple, stratified, keratinized and cornified epithelia. *Journal of Anatomy*, *214*(4), 516–559. https://doi.org/10.1111/j.1469-7580.2009.01066.x

Brandelli, A., Daroit, D. J., & Riffel, A. (2010). Biochemical features of microbial keratinases and their production and applications. *Applied Microbiology and Biotechnology*, *85*(6), 1735–1750. https://doi.org/10.1007/s00253-009-2398-5

Bressollier, P., Letourneau, F., Urdaci, M., & Verneuil, B. (1999). Purification and characterization of a keratinolytic serine proteinase from *Streptomyces albidoflavus*. *Applied and Environmental Microbiology*, *65*(6), 2570–2576. https://doi.org/10.1128/AEM.65.6.2570-2576.1999

Brosnan, J. T., & Brosnan, M. E. (2006). The sulfur-containing amino acids: An overview. *The Journal of Nutrition*, *136*(6 Suppl.), 1636S–1640S. https://doi.org/10.1093/jn/136.6.1636S

Brown, E. M., Pandya, K., Taylor, M. M., & Liu, C. - K. (2016). Comparison of methods for extraction of keratin from waste wool. *Agricultural Sciences*, *07*(10), 670–679. https://doi.org/10.4236/as.2016.710063

Brown, R. G. (1989). Protein in dog food. *Canadian Veterinary Journal*, *30*(6), 528–531. https://www.ncbi.nlm.nih.gov/pubmed/17423363

Cardamone, J. M. (2010). Investigating the microstructure of keratin extracted from wool: Peptide sequence (MALDI-TOF/TOF) and protein conformation (FTIR). *Journal of Molecular Structure*, *969*(1–3), 97–105. https://doi.org/10.1016/j.molstruc.2010.01.048

Cardamone, J. M., Nuñez, A., Garcia, R. A., & Aldema-Ramos, M. (2009). Characterizing wool keratin. *Research Letters in Materials Science*, *2009*, 1–5. https://doi.org/10.1155/2009/147175

Cassoni, A. C., Freixo, R., Pintado, A. I. E., Amorim, M., Pereira, C. D., Madureira, A. R., & Pintado, M. M. E. (2018). Novel Eco-friendly method to extract keratin from hair. *ACS Sustainable Chemistry & Engineering*, *6*(9), 12268–12274. https://doi.org/10.1021/acssuschemeng.8b02680

Caulin, C., Ware, C. F., Magin, T. M., & Oshima, R. G. (2000). Keratin-dependent, epithelial resistance to tumor necrosis factor-induced apoptosis. *Journal of Cell Biology*, *149*(1), 17–22. https://doi.org/10.1083/jcb.149.1.17

Chen, J., Ding, S., Ji, Y., Ding, J., Yang, X., Zou, M., & Li, Z. (2015). Microwave-enhanced hydrolysis of poultry feather to produce amino acid. *Chemical Engineering and Processing: Process Intensification*, *87*, 104–109. https://doi.org/10.1016/j.cep.2014.11.017

Cheng, Z., Chen, X., Zhai, D., Gao, F., Guo, T., Li, W., Hao, S., Ji, J., & Wang, B. (2018). Development of keratin nanoparticles for controlled gastric mucoadhesion and drug release. *Journal of Nanobiotechnology*, *16*(1), 24. https://doi.org/10.1186/s12951-018-0353-2

Colombani, P. C., Bitzi, R., Frey-Rindova, P., Frey, W., Arnold, M., Langhans, W., & Wenk, C. (1999). Chronic arginine aspartate supplementation in runners reduces total plasma amino acid level at rest and during a marathon run. *European Journal of Nutrition*, *38*(6), 263–270. https://doi.org/10.1007/s003940050076

Contreras, M. D. M., Lama-Munoz, A., Manuel Gutierrez-Perez, J., Espinola, F., Moya, M., & Castro, E. (2019). Protein extraction from agri-food residues for integration in biorefinery: Potential techniques and current status. *Bioresource Technology*, *280*, 459–477. https://doi.org/10.1016/j.biortech.2019.02.040

Crum, E. M., McLeay, Y. D., Barnes, M. J., & Stannard, S. R. (2018). The effect of chronic soluble keratin supplementation in physically active individuals on body composition, blood parameters and cycling performance. *Journal of the International Society of Sports Nutrition*, *15*(1), 47. https://doi.org/10.1186/s12970-018-0251-x

Damps, T., Laskowska, A. K., Kowalkowski, T., Prokopowicz, M., Puszko, A. K., Sosnowski, P., Czuwara, J., Konop, M., Różycki, K., Borkowska, J. K., Misicka, A., & Rudnicka, L. (2017). The effect of wool hydrolysates on squamous cell carcinoma cells in vitro. Possible implications for cancer treatment. *PLoS One*, *12*(8), e0184034. https://doi.org/10.1371/journal.pone.0184034

Daroit, D. J., & Brandelli, A. (2014). A current assessment on the production of bacterial keratinases. *Critical Reviews in Biotechnology*, *34*(4), 372–384. https://doi.org/10.3109/07388551.2013.794768

de Guzman, R. C., Merrill, M. R., Richter, J. R., Hamzi, R. I., Greengauz-Roberts, O. K., & Van Dyke, M. E. (2011). Mechanical and biological properties of keratose biomaterials. *Biomaterials*, *32*(32), 8205–8217. https://doi.org/10.1016/j.biomaterials.2011.07.054

de Guzman, R. C., & Rabbany, S. Y. (2016). PEG-immobilized keratin for protein drug sequestration and pH-mediated delivery. *Journal of Drug Delivery*, *2016*, 7843951. https://doi.org/10.1155/2016/7843951

De Oliveira Martinez, J., Cai, G., Nachtschatt, M., Navone, L., Zhang, Z., Robins, K., & Speight, R. (2020). Challenges and opportunities in identifying and characterising keratinases for Value-added peptide production. *Catalysts*, *10*(2). https://doi.org/10.3390/catal10020184

Deb-Choudhury, S., Bermingham, E. N., Young, W., Barnett, M. P. G., Knowles, S. O., Harland, D., Clerens, S., & Dyer, J. M. (2018). The effects of a wool hydrolysate on short-chain fatty acid production and fecal microbial composition in the domestic cat (Felis catus). *Food & Function*, *9*(8), 4107–4121. https://doi.org/10.1039/c7fo02004j

Dias, G. J., Haththotuwa, T. N., Rowlands, D. S., Gram, M., & Bekhit, A. E. A. (2022). Wool keratin—A novel dietary protein source: Nutritional value and toxicological assessment. *Food Chemistry*, *383*, 132436. https://doi.org/10.1016/j.foodchem.2022.132436

Dias, S. N. G. P. J., Bekhit, A. E.-D., Selvanesan, L., & Bernhardt, H. S. (2019). United States Patent No. US20190144494A1.

Ding, S., Sun, Y., Chen, H., Xu, C., & Hu, Y. (2019). An ultrasonic-ionic liquid process for the efficient acid catalyzed hydrolysis of feather keratin. *Chinese Journal of Chemical Engineering*, *27*(3), 660–667. https://doi.org/10.1016/j.cjche.2018.05.008

Docherty, K. M., & Kulpa, J. C. F. (2005). Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. *Green Chemistry*, *7*(4), 185–189. https://doi.org/10.1039/b419172b

Droge, W. (2005). Oxidative stress and ageing: is ageing a cysteine deficiency syndrome? *Philosophical Transactions of the Royal Society B*, *360*(1464), 2355–2372. https://doi.org/10.1098/rstb.2005.1770

Ebrahimgol, F., Tavanai, H., Alihosseini, F., & Khayamian, T. (2014). Electrosprayed recovered wool keratin nanoparticles. *Polymers for Advanced Technologies*, *25*(9), 1001–1007. https://doi.org/10.1002/pat.3342

Ertl, P., Knaus, W., & Zollitsch, W. (2016). An approach to including protein quality when assessing the net contribution of livestock to human food supply. *Animal*, *10*(11), 1883–1889. https://doi.org/10.1017/S1751731116000902

Eslahi, N., Dadashian, F., Hemmati Nejad, N., & Rabiee, M. (2014). Evaluation of wool nanoparticles incorporation in chitosan/gelatin composite films. *Journal of Applied Polymer Science*, *131*(11). https://doi.org/10.1002/app.40294

Eslahi, N., Dadashian, F., & Nejad, N. H. (2013a). An investigation on keratin extraction from wool and feather waste by enzymatic hydrolysis. *Preparative Biochemistry and Biotechnology*, *43*(7), 624–648. https://doi.org/10.1080/10826068.2013.763826

Eslahi, N., Dadashian, F., & Nejad, N. H. (2013b). Optimization of enzymatic hydrolysis of wool fibers for nanoparticles production using response surface methodology. *Advanced Powder Technology*, *24*(1), 416–426. https://doi.org/10.1016/j.apt.2012.09.004

Estensen, R. D., Levy, M., Klopp, S. J., Galbraith, A. R., Mandel, J. S., Blomquist, J. A., & Wattenberg, L. W. (1999). N-Acetylcysteine suppression of the proliferative index in the colon of patients with previous adenomatous colonic polyps. *Cancer Letters*, *147*(1), 109–114. https://doi.org/10.1016/S0304-3835(99)00281-5

Fakhfakh, N., Ktari, N., Siala, R., & Nasri, M. (2013). Wool-waste valorization: Production of protein hydrolysate with high antioxidative potential by fermentation with a new keratinolytic bacterium, *Bacillus pumilus* A1. *Journal of Applied Microbiology*, *115*(2), 424–433. https://doi.org/10.1111/jam.12246

Fan, J., & Yu, W.-D. (2009). Basic parameter study for the separation of wool fibre components by ultrasonic irradiation in formic acid. *Research Journal of Textile and Apparel*, *13*(2), 69–74. https://doi.org/10.1108/RJTA-13-02-2009-B008

FAO. (2018). *The future of food and agriculture—Alternative pathways to 2050*, Rome, Italy. http://www.fao.org/3/CA1553EN/ca1553en.pdf

FAO/WHO/UNU. (2007). *Protein and amino acid requirements in human nutrition*. Report of a joint FAO/WHO/UNU expert consultation, Geneva, Switzerland. http://apps.who.int/iris/bitstream/handle/10665/43411/WHO_TRS_935_eng.pdf;jsessionid=92D4EF87671F9C84317E390E500F9C06?sequence=1

Fernandes, I., Rodrigues, J. A., & Almeida, C. M. M. (2021). Sulfites in fresh meat and meat preparations commercialized in Portugal. *Food Analytical Methods*, *15*(1), 172–184. https://doi.org/10.1007/s12161-021-02115-z

Fernandes, M. M., Lima, C. F., Loureiro, A., Gomes, A. C., & Cavaco-Paulo, A. (2012). Keratin-based peptide: biological evaluation and strengthening properties on relaxed hair. *International Journal of Cosmetic Science*, *34*(4), 338–346. https://doi.org/10.1111/j.1468-2494.2012.00727.x

Fernández-d'Arlas, B. (2019). Tough and functional cross-linked bioplastics from sheep wool keratin. *Scientific Reports*, *9*(1), 14810. https://doi.org/10.1038/s41598-019-51393-5

Feroz, S., & Dias, G. (2021). Hydroxypropylmethyl cellulose (HPMC) crosslinked keratin/hydroxyapatite (HA) scaffold fabrication, characterization and in vitro biocompatibility assessment as a bone graft for alveolar bone regeneration. *Heliyon*, *7*(11), e08294. https://doi.org/10.1016/j.heliyon.2021.e08294

Ferrareze, P. A. G., Correa, A. P. F., & Brandelli, A. (2016). Purification and characterization of a keratinolytic protease produced by probiotic *Bacillus subtilis*. *Biocatalysis and Agricultural Biotechnology*, *7*, 102–109. https://doi.org/10.1016/j.bcab.2016.05.009

Fitz-Binder, C., Pham, T., & Bechtold, T. (2019). A second life for low-grade wool through formation of all-keratin composites in cystine reducing calcium chloride–water–ethanol solution. *Journal of Chemical Technology & Biotechnology*, *94*(10), 3384–3392. https://doi.org/10.1002/jctb.6151

Fontoura, R., Daroit, D. J., Correa, A. P. F., Moresco, K. S., Santi, L., Beys-da-Silva, W. O., Yates, J. R., 3rd, Moreira, J. C. F., & Brandelli, A. (2019). Characterization of a novel antioxidant peptide from feather keratin hydrolysates. *New Biotechnology*, *49*, 71–76. https://doi.org/10.1016/j.nbt.2018.09.003

Friedman, M. (1999). Chemistry, biochemistry, nutrition, and microbiology of lysinoalanine, lanthionine, and histidinoalanine in food and other proteins. *Journal of Agricultural and Food Chemistry*, *47*(4), 1295–1319. https://doi.org/10.1021/jf981000+

Fukatsu, K. (1997). Change in amino acid composition of Cu(II)-wool complex by degradation with hydrogen peroxide. *Sen'i Gakkaishi*, *53*(4), 167–170. https://doi.org/10.2115/fiber.53.167

Gaidau, C., Epure, D. - G., Enascuta, C. E., Carsote, C., Sendrea, C., Proietti, N., Chen, W., & Gu, H. (2019). Wool keratin total solubilisation for recovery and reintegration—An ecological

approach. *Journal of Cleaner Production*, *236*. https://doi.org/10.1016/j.jclepro.2019.07.061

Gaidau, C., Stanca, M., Niculescu, M. D., Alexe, C. A., Becheritu, M., Horoias, R., Cioineag, C., Râpă, M., & Stanculescu, I. R. (2021). Wool keratin hydrolysates for bioactive additives preparation. *Materials (Basel)*, *14*(16). https://doi.org/10.3390/ma14164696

Garcia, M. T., Gathergood, N., & Scammells, P. J. (2005). Biodegradable ionic liquids: Part II. Effect of the anion and toxicology. *Green Chemistry*, *7*(1), 9–14. https://doi.org/10.1039/b411922c

Gegeckas, A., Gudiukaite, R., & Citavicius, D. (2014). Keratinolytic proteinase from *Bacillus thuringiensis* AD-12. *International Journal of Biological Macromolecules*, *69*, 46–51. https://doi.org/10.1016/j.ijbiomac.2014.05.024

Gegeckas, A., Gudiukaite, R., Debski, J., & Citavicius, D. (2015). Keratinous waste decomposition and peptide production by keratinase from *Geobacillus stearothermophilus* AD-11. *International Journal of Biological Macromolecules*, *75*, 158–165. https://doi.org/10.1016/j.ijbiomac.2015.01.031

Gervasi, M., Sisti, D., Amatori, S., Donati Zeppa, S., Annibalini, G., Piccoli, G., Vallorani, L., Benelli, P., Rocchi, M. B. L., Barbieri, E., Calavalle, A. R., Agostini, D., Fimognari, C., Stocchi, V., & Sestili, P. (2020). Effects of a commercially available branched-chain amino acid-alanine-carbohydrate-based sports supplement on perceived exertion and performance in high intensity endurance cycling tests. *Journal of the International Society of Sports Nutrition*, *17*(1), 6. https://doi.org/10.1186/s12970-020-0337-0

Ghosh, A., Clerens, S., Deb-Choudhury, S., & Dyer, J. M. (2014). Thermal effects of ionic liquid dissolution on the structures and properties of regenerated wool keratin. *Polymer Degradation and Stability*, *108*, 108–115. https://doi.org/10.1016/j.polymdegradstab.2014.06.007

Giteru, S. G., Cridge, B., Oey, I., Ali, A., & Altermann, E. (2020). In-vitro degradation and toxicological assessment of pulsed electric fields crosslinked zein-chitosan-poly(vinyl alcohol) biopolymeric films. *Food and Chemical Toxicology*, *135*, 111048. https://doi.org/10.1016/j.fct.2019.111048

Gousterova, A., Braikova, D., Goshev, I., Christov, P., Tishinov, K., Vasileva-Tonkova, E., Haertlé, T., & Nedkov, P. (2005). Degradation of keratin and collagen containing wastes by newly isolated thermoactinomycetes or by alkaline hydrolysis. *Letters in Applied Microbiology*, *40*(5), 335–340. https://doi.org/10.1111/j.1472-765X.2005.01692.x

Gousterova, A., Nustorova, M., Goshev, I., Christov, P., Braikova, D., Tishinov, K., Haertlé, T., & Nedkov, P. (2003). Alkaline hydrolysate of waste sheep wool aimed as fertilizer. *Biotechnology and Biotechnological Equipment*, *17*(2), 140–145. https://doi.org/10.1080/13102818.2003.10817072

Goyal, S., Dotter, M., Diestelhorst, E., Storck, J. L., Ehrmann, A., & Mahltig, B. (2022). Extraction of keratin from wool and its use as biopolymer in film formation and in electrospinning for composite material processing. *Journal of Engineered Fibers and Fabrics*, *17*. https://doi.org/10.1177/15589250221090499

Grandview Research. (2021). Protein ingredients market size, share & trends analysis report by product (plant proteins, animal/dairy proteins, microbe-based proteins, insect proteins), by application, by region, and segment forecasts, 2021–2028. https://www.grandviewresearch.com/industry-analysis/protein-ingredients-market

Grazziotin, A., Pimentel, F. A., Jong, E. V. D., & Brandelli, A. (2008). Poultry feather hydrolysate as a protein source for growing rats. *Brazilian Journal of Veterinary Research and Animal Science*, *45*, (Suppl.), 61–67. https://doi.org/10.11606/s1413-95962008000700008

Guidotti, G., Soccio, M., Posati, T., Sotgiu, G., Tiboni, M., Barbalinardo, M., Valle, F., Casettari, L., Zamboni, R., Lottia, N., & Aluigi, A. (2020). Regenerated wool keratin-polybutylene succinate nanofibrous mats for drug delivery and cells culture. *Polymer Degradation and Stability*, *179*, 109272. https://doi.org/10.1016/j.polymdegradstab.2020.109272

Gupta, R., & Ramnani, P. (2006). Microbial keratinases and their prospective applications: An overview. *Applied Microbiology and Biotechnology*, *70*(1), 21–33. https://doi.org/10.1007/s00253-005-0239-8

Hansen, J. Ø., Penn, M., Øverland, M., Shearer, K. D., Krogdahl, Å., Mydland, L. T., & Storebakken, T. (2010). High inclusion of partially deshelled and whole krill meals in diets for Atlantic salmon (Salmo salar). *Aquaculture*, *310*(1–2), 164–172. https://doi.org/10.1016/j.aquaculture.2010.10.003

Hassabo, A. G., Salama, M., Mohamed, A. L., & Popescu, C. (2014). Ultrafine wool and cotton powder and their characteristics. *Journal of Natural Fibers*, *12*(2), 141–153. https://doi.org/10.1080/15440478.2014.903819

Hassabo, A. G., Salama, M., & Popescu, C. (2015). Characterizations of PVA composites based on recycled ultrafine cot-ton and wool powders. *Research and Reviews in BioSciences*, *10*(4), 147–158. https://www.tsijournals.com/articles/characterizations-of-pva-composites-based-on-recycled-ultrafine-cottonand-wool-powders.pdf

Hassan, M. A., Abol-Fotouh, D., Omer, A. M., Tamer, T. M., & Abbas, E. (2020). Comprehensive insights into microbial keratinases and their implication in various biotechnological and industrial sectors: A review. *International Journal of Biological Macromolecules*, *154*, 567–583. https://doi.org/10.1016/j.ijbiomac.2020.03.116

He, J., Xu, D., Li, J., Li, L., Li, W., Cui, W., & Liu, K. (2020). Highly efficient extraction of large molecular-weight keratin from wool in a water/ethanol co-solvent. *Textile Research Journal*, *90*(9-10), 1084–1093. https://doi.org/10.1177/0040517519885022

Hochberg, M. C., Martel-Pelletier, J., Monfort, J., Moller, I., Castillo, J. R., Arden, N., Berenbaum, F., Blanco, F. J., Conaghan, P. G., Doménech, G., Henrotin, Y., Pap, T., Richette, P., Sawitzke, A., du Souich, P., Pelletier, J. P., & Group, M. I. (2016). Combined chondroitin sulfate and glucosamine for painful knee osteoarthritis: A multicentre, randomised, double-blind, non-inferiority trial versus celecoxib. *Annals of Rheumatic Diseases*, *75*(1), 37–44. https://doi.org/10.1136/annrheumdis-2014-206792

Hooper, L., Abdelhamid, A., Ali, A., Bunn, D. K., Jennings, A., John, W. G., Kerry, S., Lindner, G., Pfortmueller, C. A., Sjöstrand, F., Walsh, N. P., Fairweather-Tait, S. J., Potter, J. F., Hunter, P. R., & Shepstone, L. (2015). Diagnostic accuracy of calculated serum osmolarity to predict dehydration in older people: adding value to pathology laboratory reports. *BMJ Open*, *5*(10), e008846. https://doi.org/10.1136/bmjopen-2015-008846

Hou, Y., Wu, Z., Dai, Z., Wang, G., & Wu, G. (2017). Protein hydrolysates in animal nutrition: Industrial production, bioactive peptides, and functional significance. *Journal of Animal Science and Biotechnology*, *8*(1), 24. https://doi.org/10.1186/s40104-017-0153-9

Houltham, S., Starck, C., & Stannard, S. (2014). Two week keratin-based protein supplementation is comparable in gastrointestinal handling to a Milk-based equivalent. *Journal of Human Nutrition and Food Science*, *2*(4), 1040. https://www.jscimedcentral.com/Nutrition/nutrition-2-1040.pdf

Huang, Y., Busk, P. K., Herbst, F. A., & Lange, L. (2015). Genome and secretome analyses provide insights into keratin decomposition by novel proteases from the non-pathogenic fungus Onygena corvina. *Applied Microbiology and Biotechnology*, *99*(22), 9635–9649. https://doi.org/10.1007/s00253-015-6805-9

Idris, A., Vijayaraghavan, R., Rana, U. A., Patti, A. F., & MacFarlane, D. R. (2014). Dissolution and regeneration of wool keratin in ionic liquids. *Green Chemistry*, *16*(5), 2857–2864. https://doi.org/10.1039/c4gc00213j

Ignowski, E., Winter, A. N., Duval, N., Fleming, H., Wallace, T., Manning, E., Koza, L., Huber, K., Serkova, N. J., & Linseman, D. A. (2018). The cysteine-rich whey protein supplement, Immunocal(R), preserves brain glutathione and improves cognitive, motor, and histopathological indices of traumatic brain injury in a mouse model of controlled cortical impact. *Free Radical Biology & Medicine*, *124*, 328–341. https://doi.org/10.1016/j.freeradbiomed.2018.06.026

IWTO. (2022). *International Wool Trade Organization: Market Information*. http://www.iwto.org/

Jayathilakan, K., Sultana, K., Radhakrishna, K., & Bawa, A. S. (2012). Utilization of byproducts and waste materials from meat, poultry and fish processing industries: A review. *Journal of Food Science and Technology*, *49*(3), 278–293. https://doi.org/10.1007/s13197-011-0290-7

Jiang, Z., Yuan, J., Wang, P., Fan, X., Xu, J., Wang, Q., & Zhang, L. (2018). Dissolution and regeneration of wool keratin in the deep eutectic solvent of choline chloride-urea. *International Journal of Biological Macromolecules*, *119*, 423–430. https://doi.org/10.1016/j.ijbiomac.2018.07.161

Jou, C., Chen, Y., Wang, H. - P., Lin, K., & Tai, H. (1999). Hydrolytic dissociation of hog-hair by microwave radiation. *Bioresource Technology*, *70*(1), 111–113. https://doi.org/10.1016/S0960-8524(99)00011-5

Kalman, D. S. (2014). Amino acid composition of an organic brown rice protein concentrate and isolate compared to soy and whey concentrates and isolates. *Foods*, *3*(3), 394–402. https://doi.org/10.3390/foods3030394

Kelly, R., Ellis, G., Macdonald, R., McPherson, R., Middlewood, P., Nuthall, M., Rao, G. F., Roddick-Lanzilotta, A., Sigurjonsson, G., & Singleton, D. (2007). US20070065506A1. U. S. Patent.

Kelly, R. J. M., & Marsh, C. (2012). US20120219667A1.

Khumalo, M., Sithole, B., & Tesfaye, T. (2020). Valorisation of waste chicken feathers: Optimisation of keratin extraction from waste chicken feathers by sodium bisulphite, sodium dodecyl sulphate and urea. *Journal of Environmental Management*, *262*, 110329. https://doi.org/10.1016/j.jenvman.2020.110329

Ki, C., Gang, E., Um, I., & Park, Y. (2007). Nanofibrous membrane of wool keratose/silk fibroin blend for heavy metal ion adsorption. *Journal of Membrane Science*, *302*(1–2), 20–26. https://doi.org/10.1016/j.memsci.2007.06.003

Kirsner, R. S., Cassidy, S., Marsh, C., Vivas, A., & Kelly, R. J. (2012). Use of a keratin-based wound dressing in the management of wounds in a patient with recessive dystrophic epidermolysis bullosa. *Advances in skin & wound care*, *25*(9), 400–403. https://doi.org/10.1097/01.ASW.0000419404.44947.de

Koleva, M., & Zheleva, D. (2022). Methods for obtaining of keratin hydrolysates from sheep wool. *Journal of Chemical Technology and Metallurgy*, *57*(1), 76–83. https://dl.uctm.edu/journal/node/j2022-1/9_21-08p76-83.pdf

Kornillowicz-Kowalska, T., & Bohacz, J. (2011). Biodegradation of keratin waste: Theory and practical aspects. *Waste Management*, *31*(8), 1689–1701. https://doi.org/10.1016/j.wasman.2011.03.024

Kozich, V., & Stabler, S. (2020). Lessons learned from inherited metabolic disorders of sulfur-containing amino acids metabolism. *Journal of Nutrition*, *150*(Suppl. 1), 2506S–2517S. https://doi.org/10.1093/jn/nxaa134

Kras, J. V., Dong, L., & Winkelstein, B. A. (2013). The prostaglandin E2 receptor, EP2, is upregulated in the DRG after painful cervical facet joint injury in the rat. *Spine*, *38*(3), 217. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3500406/pdf/nihms-399172.pdf

Kshetri, P., Roy, S. S., Chanu, S. B., Singh, T. S., Tamreihao, K., Sharma, S. K., Ansari, M. A., & Prakash, N. (2020). Valorization of chicken feather waste into bioactive keratin hydrolysate by a newly purified keratinase from Bacillus sp. RCM-SSR-102. *Journal of Environmental Management*, *273*, 111195. https://doi.org/10.1016/j.jenvman.2020.111195

Kui, W., Li, R., Ma, J., Jian, Y., & Che, J. (2016). Extracting keratin from wool by using L-cysteine. *Green Chemistry*, *18*(2), 476–481. https://doi.org/10.1039/C5GC01254F

Kumar, D. M., Priya, P., Balasundari, S. N., Devi, G., Rebecca, A. I. N., & Kalaichelvan, P. (2012). Production and optimization of feather protein hydrolysate from Bacillus sp. MPTK6 and its antioxidant potential. *Middle-East Journal of Scientific Research*, *11*(7), 900–907. http://www.idosi.org/mejsr/mejsr11%287%2912/9.pdf

Kumar, V., Atherton, P., Smith, K., & Rennie, M. J. (2009). Human muscle protein synthesis and breakdown during and after exercise. *Journal of Applied Physiology (1985)*, *106*(6), 2026–2039. https://doi.org/10.1152/japplphysiol.91481.2008

Lange, L., Huang, Y., & Busk, P. K. (2016). Microbial decomposition of keratin in nature—A new hypothesis of industrial relevance. *Applied Microbiology and Biotechnology*, *100*(5), 2083–2096. https://doi.org/10.1007/s00253-015-7262-1

Lazarus, B. S., Chadha, C., Velasco-Hogan, A., Barbosa, J. D. V., Jasiuk, I., & Meyers, M. A. (2021). Engineering with keratin: A functional material and a source of bioinspiration. *iScience*, *24*(8), 102798. https://doi.org/10.1016/j.isci.2021.102798

Lee, Y. S., Phang, L. - Y., Ahmad, S. A., & Ooi, P. T. (2016). Microwave-alkali treatment of chicken feathers for protein hydrolysate production. *Waste and Biomass Valorization*, *7*(5), 1147–1157. https://doi.org/10.1007/s12649-016-9483-7

Li, B., Sun, Y., Yao, J., Wu, H., Shen, Y., Zhi, C., & Li, J. (2022). An environment-friendly chemical modification method for thiol groups on polypeptide macromolecules to improve the performance of regenerated keratin materials. *Materials & Design*, *217*, 110611. https://doi.org/10.1016/j.matdes.2022.110611

Li, J., Li, Y., Zhang, Y., Liu, X., Zhao, Z., Zhang, J., Han, Y., & Zhou, D. (2013). Toxicity study of isolated polypeptide from wool hydrolysate. *Food and Chemical Toxicology*, *57*, 338–345. https://doi.org/10.1016/j.fct.2013.03.047

Li, N., Rivera-Bermudez, M. A., Zhang, M., Tejada, J., Glasson, S. S., Collins-Racie, L. A., Lavallie, E. R., Wang, Y., Chang, K. C., Nagpal, S., Morris, E. A., Flannery, C. R., & Yang, Z. (2010). LXR

modulation blocks prostaglandin E2 production and matrix degradation in cartilage and alleviates pain in a rat osteoarthritis model. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(8), 3734–3739. https://doi.org/10.1073/pnas.0911377107

Li, R., & Wang, D. (2013). Preparation of regenerated wool keratin films from wool keratin-ionic liquid solutions. *Journal of Applied Polymer Science*, *127*(4), 2648–2653. https://doi.org/10.1002/app.37527

Li, Y., Lin, J., Zhi, X., Li, P., Jiang, X., & Yuan, J. (2018). Triple stimuli-responsive keratin nanoparticles as carriers for drug and potential nitric oxide release. *Materials Science and Engineering: C*, *91*, 606–614. https://doi.org/10.1016/j.msec.2018.05.073

Liu, R., Li, L., Liu, S., Li, S., Zhu, X., Yi, M., & Liao, X. (2018). Structure and properties of wool keratin/poly (vinyl alcohol) blended fiber. *Advances in Polymer Technology*, *37*(8), 2756–2762. https://doi.org/10.1002/adv.21948

Liu, Y., Wang, X., Song, Y., & Li, R. (2021). The synthesis of binary DES and its application in the pretreatment of wool keratin extraction by the L-cysteine redox method. *Journal of Natural Fibers*, *19*(15), 10872–10882. https://doi.org/10.1080/15440478.2021.2002776

Lopes, F. C., Silva, L. A., Tichota, D. M., Daroit, D. J., Velho, R. V., Pereira, J. Q., Corrêa, A. P., & Brandelli, A. (2011). Production of proteolytic enzymes by a keratin-degrading *Aspergillus niger*. *Enzyme Research*, *2011*, 487093. https://doi.org/10.4061/2011/487093

Lopez-Santamarina, A., Mondragon, A. D. C., Lamas, A., Miranda, J. M., Franco, C. M., & Cepeda, A. (2020). Animal-origin prebiotics based on chitin: An alternative for the future? A critical review. *Foods*, *9*(6), 782. https://doi.org/10.3390/foods9060782

Maciel, J. L., Werlang, P. O., Daroit, D. J., & Brandelli, A. (2016). Characterization of protein-rich hydrolysates produced through microbial conversion of waste feathers. *Waste and Biomass Valorization*, *8*(4), 1177–1186. https://doi.org/10.1007/s12649-016-9694-y

Martínez-Alvarez, O., Chamorro, S., & Brenes, A. (2015). Protein hydrolysates from animal processing by-products as a source of bioactive molecules with interest in animal feeding: A review. *Food Research International*, *73*, 204–212. https://doi.org/10.1016/j.foodres.2015.04.005

Matyasovsky, J., Sedliacik, J., Valachová, K., Novak, I., Jurkovic, P., Duchovic, P., Micusik, M., Kleinova, A., & Soltes, L. (2017). Antioxidant effects of keratin hydrolysates. *Journal of the American Leather Chemists Association*, *112*(10), 327–337. https://journals.uc.edu/index.php/JALCA/article/view/3715

McCarthy, A., O'Callaghan, Y., & O'Brien, N. (2013). Protein hydrolysates from agricultural crops—Bioactivity and potential for functional food development. *Agriculture*, *3*(1), 112–130. https://doi.org/10.3390/agriculture3010112

McLeay, Y., Stannard, S., Houltham, S., & Starck, C. (2017). Dietary thiols in exercise: oxidative stress defence, exercise performance, and adaptation. *Journal of the International Society of Sports Nutrition*, *14*, 12. https://doi.org/10.1186/s12970-017-0168-9

McPherson, R. A., & Hardy, G. (2011). Clinical and nutritional benefits of cysteine-enriched protein supplements. *Current Opinion in Clinical Nutrition & Metabolic Care*, *14*(6), 562–568. https://doi.org/10.1097/MCO.0b013e32834c1780

Minamoto, Y., Minamoto, T., Isaiah, A., Sattasathuchana, P., Buono, A., Rangachari, V. R., McNeely, I. H., Lidbury, J., Steiner, J. M., & Suchodolski, J. S. (2019). Fecal short-chain fatty acid concentrations and dysbiosis in dogs with chronic enteropathy. *Journal of Veterinary Internal Medicine*, *33*(4), 1608–1618. https://doi.org/10.1111/jvim.15520

Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food—An international consensus. *Food & Function*, *5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j

Miniaci, M. C., Irace, C., Capuozzo, A., Piccolo, M., Di Pascale, A., Russo, A., Lippiello, P., Lepre, F., Russo, G., & Santamaria, R. (2016). Cysteine prevents the reduction in keratin synthesis induced by iron deficiency in human keratinocytes. *Journal of Cellular Biochemistry*, *117*(2), 402–412. https://doi.org/10.1002/jcb.25286

Mizani, M., Aminlari, M., & Khodabandeh, M. (2016). An effective method for producing a nutritive protein extract powder from Shrimp-head waste. *Food Science and Technology International*, *11*(1), 49–54. https://doi.org/10.1177/1082013205051271

Mothé, M. G., Viana, L. M., & Mothé, C. G. (2017). Thermal property study of keratin from industrial residue by extraction, processing and application. *Journal of Thermal Analysis and Calorimetry*, *131*(1), 417–426. https://doi.org/10.1007/s10973-017-6845-8

Mokrejs, P., Krejci, O., & Svoboda, P. (2011). Producing keratin hydrolysates from sheep wool. *Oriental Journal of Chemistry*, *27*(4), 1303–1309. http://www.orientjchem.org/?p=11745

Moore, D. R. (2019). Maximizing post-exercise anabolism: The case for relative protein intakes. *Frontiers in Nutrition*, *6*, 147. https://doi.org/10.3389/fnut.2019.00147

Moore, K. E., Mangos, D. N., Slattery, A. D., Raston, C. L., & Boulos, R. A. (2016). Wool deconstruction using a benign eutectic melt. *RSC Advances*, *6*(24), 20095–20101. https://doi.org/10.1039/c5ra26516a

Nagai, Y., & Nishikawa, T. (2014). Solubilization of chicken feather keratin by ammonium copper hydroxide (Schweitzer's Reagent). *Agricultural and Biological Chemistry*, *34*(4), 575–584. https://doi.org/10.1080/00021369.1970.10859648

Nahed, F., Manel, G., Ines, D., Alya, S. K., Abdelfattah, E. F., & Moncef, N. (2012). Improvement of antioxidant potential in rats consuming feathers protein hydrolysate obtained by fermentation of the keratinolytic bacterium, *Bacillus pumilus* A1. *African Journal of Biotechnology*, *11*(4), 938–949. https://doi.org/10.5897/ajb11.1741

Naik, R., Wen, G., Ms, D., Hureau, S., Uedono, A., Wang, X., Liu, X., Cookson, P. G., & Smith, S. V. (2010). Metal ion binding properties of novel wool powders. *Journal of Applied Polymer Science*, *115*(3), 1642–1650. https://doi.org/10.1002/app.31206

Nchienzia, H. A., Morawicki, R. O., & Gadang, V. P. (2010). Enzymatic hydrolysis of poultry meal with endo- and exopeptidases. *Poultry Science*, *89*(10), 2273–2280. https://doi.org/10.3382/ps.2008-00558

Okoro, O. V., Jafari, H., Hobbi, P., Nie, L., Alimoradi, H., & Shavandi, A. (2022). Enhanced keratin extraction from wool waste using a deep eutectic solvent. *Chemical Papers*. https://doi.org/10.1007/s11696-021-02029-4

Oluba, O. M., Okongwu, C., Lawa, T., & Akpor, B. O. (2019). Growth performance and toxicological assessments of chicken feather protein hydrolysate as fish meal substitute in rat diet. *Asian Journal of Scientific Research*, *12*(3), 450–461. https://doi.org/10.3923/ajsr.2019.450.461

Oosthuyse, T., & Millen, A. M. (2016). Comparison of energy supplements during prolonged exercise for maintenance of cardiac function: Carbohydrate only versus carbohydrate plus whey or casein hydrolysate. *Applied Physiology, Nutrition, and Metabolism*, *41*(6), 674–683. https://doi.org/10.1139/apnm-2015-0491

Ossai, I. C., Shahul Hamid, F., & Hassan, A. (2022). Valorisation of keratinous wastes: A sustainable approach towards a circular economy. *Waste Management*, *151*, 81–104. https://doi.org/10.1016/j.wasman.2022.07.021

Øverland, M., Sørensen, M., Storebakken, T., Penn, M., Krogdahl, Å., & Skrede, A. (2009). Pea protein concentrate substituting fish meal or soybean meal in diets for Atlantic salmon (Salmo salar)—Effect on growth performance, nutrient digestibility, carcass composition, gut health, and physical feed quality. *Aquaculture*, *288*(3-4), 305–311. https://doi.org/10.1016/j.aquaculture.2008.12.012

Ozel, N., & Elibol, M. (2021). A review on the potential uses of deep eutectic solvents in chitin and chitosan related processes. *Carbohydrate Polymers*, *262*, 117942. https://doi.org/10.1016/j.carbpol.2021.117942

Pasupuleti, V. K., Holmes, C., & Demain, A. L. (2008). Applications of protein hydrolysates in biotechnology. In V. Pasupuleti, A. Demain (Eds.), *Protein hydrolysates in biotechnology* (pp. 1–9). Springer, Dordrecht. https://doi.org/10.1007/978-1-4020-6674-0_1

Patkowska-Sokoła, B., Dobrzański, Z., Osman, K., Bodkowski, R., & Zygadlik, K. (2009). The content of chosen chemical elements in wool of sheep of different origins and breeds. *Archives Animal Breeding*, *52*(4), 410–418. https://doi.org/10.5194/aab-52-410-2009

Pikosky, M. A., Gaine, P. C., Martin, W. F., Grabarz, K. C., Ferrando, A. A., Wolfe, R. R., & Rodriguez, N. R. (2006). Aerobic exercise training increases skeletal muscle protein turnover in healthy adults at rest. *The Journal of Nutrition*, *136*(2), 379–383. https://doi.org/10.1093/jn/136.2.379

Plowman, J. E., Flanagan, L. M., Paton, L. N., Fitzgerald, A. C., Joyce, N. I., & Bryson, W. G. (2003). The effect of oxidation or alkylation on the separation of wool keratin proteins by two-dimensional gel electrophoresis. *Proteomics*, *3*(6), 942–950. https://doi.org/10.1002/pmic.200300419

Pongkai, P., Saisavoey, T., Sangtanoo, P., Sangvanich, P., & Karnchanatat, A. (2017). Effects of protein hydrolysate from chicken feather meal on tyrosinase activity and melanin formation in B16F10 murine melanoma cells. *Food Science and Biotechnology*, *26*(5), 1199–1208. https://doi.org/10.1007/s10068-017-0186-z

Poole, A. J., Church, J. S., & Huson, M. G. (2009). Environmentally sustainable fibers from regenerated protein. *Biomacromolecules*, *10*(1), 1–8. https://doi.org/10.1021/bm8010648

Pourjavaheri, F., Ostovar Pour, S., Jones, O. A. H., Smooker, P. M., Brkljača, R., Sherkat, F., Blanch, E. W., Gupta, A., & Shanks, R. A. (2019). Extraction of keratin from waste chicken feathers using sodium sulfide and l-cysteine. *Process Biochemistry*, *82*, 205–214. https://doi.org/10.1016/j.procbio.2019.04.010

Qiu, J., Wilkens, C., Barrett, K., & Meyer, A. S. (2020). Microbial enzymes catalyzing keratin degradation: Classification, structure, function. *Biotechnology Advances*, *44*, 107607. https://doi.org/10.1016/j.biotechadv.2020.107607

Queiroga, A. C., Pintado, M. E., & Malcata, F. X. (2012). Potential use of wool-associated Bacillus species for biodegradation of keratinous materials. *International Biodeterioration & Biodegradation*, *70*, 60–65. https://doi.org/10.1016/j.ibiod.2011.12.013

Rafii, M., Elango, R., Ball, R. O., Pencharz, P. B., & Courtney-Martin, G. (2018). Metabolic availability of the limiting amino acids lysine and tryptophan in cooked White African cornmeal assessed in healthy young men using the indicator amino acid oxidation technique. *The Journal of Nutrition*, *148*(6), 917–924. https://doi.org/10.1093/jn/nxy039

Rajabinejad, H., Zoccola, M., Patrucco, A., Montarsolo, A., Rovero, G., & Tonin, C. (2017). Physicochemical properties of keratin extracted from wool by various methods. *Textile Research Journal*, *88*(21), 2415–2424. https://doi.org/10.1177/0040517517723028

Rajkhowa, R., Zhou, Q., Tsuzuki, T., Morton, D. A. V., & Wang, X. (2012). Ultrafine wool powders and their bulk properties. *Powder Technology*, *224*, 183–188. https://doi.org/10.1016/j.powtec.2012.02.052

Ramirez, D. O. S., Carletto, R. A., Tonetti, C., Giachet, F. T., Varesano, A., & Vineis, C. (2017). Wool keratin film plasticized by citric acid for food packaging. *Food Packaging and Shelf Life*, *12*, 100–106. https://doi.org/10.1016/j.fpsl.2017.04.004

Ranjit, E., Hamlet, S., George, R., Sharma, A., & Love, R. (2021). Biofunctional approaches of wool-based keratin for tissue engineering. *Journal of Science: Advanced Materials and Devices*. https://doi.org/10.1016/j.jsamd.2021.10.001

Rippon, J. A. (2013). The structure of wool. In D. M. Lewis, J. A. Rippon (Eds.), *The coloration of wool and other keratin fibres* (pp. 1–42). https://doi.org/10.1002/9781118625118.ch1

Rosewald, M., Hou, F. Y. S., m Mututuvari, T., Harkins, A., & d Tran, C. (2014). Cellulose-chitosan-keratin composite materials: synthesis, immunological and antibacterial properties. *ECS Transactions*, *64*(4), 499–505.

Santos, R. M., Firmino, A. A., De Sa, C. M., & Felix, C. R. (1996). Keratinolytic activity of Aspergillus fumigatus Fresenius. *Current Microbiology*, *33*(6), 364–370. https://doi.org/10.1007/s002849900129

Sarwar, G. (1997). The protein digestibility–corrected amino acid score method overestimates quality of proteins containing antinutritional factors and of poorly digestible proteins supplemented with limiting amino acids in rats. *The Journal of Nutrition*, *127*(5), 758–764. https://doi.org/10.1093/jn/127.5.758

Savige, W. E. (2016). The dispersion of wool protein by thiols in acid solution. *Textile Research Journal*, *30*(1), 1–10. https://doi.org/10.1177/004051756003000101

Seghir, B. B., Amor, I. B., Beki, A., Souici, I., Hemmamia, H., Soumeia, Z., Eddine, L. S., & Rebiai, A. (2020). Preparation and optimization of keratin from sheep wool using response surface methodology. *Asian Journal of Research in Chemistry*, *13*(3), 157. https://doi.org/10.5958/0974-4150.2020.00031.0

Seidel, U., Huebbe, P., & Rimbach, G. (2019). Taurine: A regulator of cellular redox homeostasis and skeletal muscle function. *Molecular Nutrition & Food Research*, *63*(16), e1800569. https://doi.org/10.1002/mnfr.201800569

Sharma, S., Gupta, A., Chik, S., Kee, C. G., Mistry, B. M., Kim, D. H., & Sharma, G. (2017). Characterization of keratin microparticles from feather biomass with potent antioxidant and anticancer activities. *International Journal of Biological Macromolecules*, *104*(Pt A), 189–196. https://doi.org/10.1016/j.ijbiomac.2017.06.015

Shavandi, A., Bekhit, A. E.-D. A., Carne, A., & Bekhit, A. (2016). Evaluation of keratin extraction from wool by chemical methods

for bio-polymer application. *Journal of Bioactive and Compatible Polymers*, *32*(2), 163–177. https://doi.org/10.1177/0883911516662069

Shavandi, A., Carne, A., Bekhit, A. A., & Bekhit, A. E.-D. A. (2017). An improved method for solubilisation of wool keratin using peracetic acid. *Journal of Environmental Chemical Engineering*, *5*(2), 1977–1984. https://doi.org/10.1016/j.jece.2017.03.043

Shavandi, A., Jafari, H., Zago, E., Hobbi, P., Nie, L., & De Laet, N. (2021). A sustainable solvent based on lactic acid and l-cysteine for the regeneration of keratin from waste wool. *Green Chemistry*, *23*(3), 1171–1174. https://doi.org/10.1039/D0GC04314A

Shavandi, A., Silva, T. H., Bekhit, A. A., & Bekhit, A. E. A. (2017). Keratin: dissolution, extraction and biomedical application. *Biomaterials Science*, *5*(9), 1699–1735. https://doi.org/10.1039/c7bm00411g

Shirsath, A. P., & Henchion, M. M. (2021). Bovine and ovine meat co-products valorisation opportunities: A systematic literature review. *Trends in Food Science & Technology*, *118*, 57–70. https://doi.org/10.1016/j.tifs.2021.08.015

Shorland, F. B., & Gray, J. M. (1970). The preparation of nutritious protein from wool. *British Journal of Nutrition*, *24*(3), 717–725. https://doi.org/10.1079/bjn19700073

Sierpinski, P., Garrett, J., Ma, J., Apel, P., Klorig, D., Smith, T., Koman, L. A., Atala, A., & Van Dyke, M. (2008). The use of keratin biomaterials derived from human hair for the promotion of rapid regeneration of peripheral nerves. *Biomaterials*, *29*(1), 118–128. https://doi.org/10.1016/j.biomaterials.2007.08.023

Sinkiewicz, I., Śliwińska, A., Staroszczyk, H., & Kołodziejska, I. (2016). Alternative methods of preparation of soluble keratin from chicken feathers. *Waste and Biomass Valorization*, *8*(4), 1043–1048. https://doi.org/10.1007/s12649-016-9678-y

Sinkiewicz, I., Staroszczyk, H., & Śliwińska, A. (2018). Solubilization of keratins and functional properties of their isolates and hydrolysates. *Journal of Food Biochemistry*, *42*(2). https://doi.org/10.1111/jfbc.12494

Smith, E. L., Abbott, A. P., & Ryder, K. S. (2014). Deep eutectic solvents (DESs) and their applications. *Chemical Reviews*, *114*(21), 11060–11082. https://doi.org/10.1021/cr300162p

Smith, N. W., Fletcher, A. J., Hill, J. P., & McNabb, W. C. (2022). Modeling the contribution of meat to global nutrient availability. *Frontiers in Nutrition*, *9*, 766796. https://doi.org/10.3389/fnut.2022.766796

Steenken, I., & Zahn, H. (2016). Determining the dehydroalanine and amide contents of wool as a means of estimating damage caused by alkaline treatments. *Textile Research Journal*, *54*(7), 429–433. https://doi.org/10.1177/004051758405400703

Stellavato, A., Restaino, O. F., Vassallo, V., Finamore, R., Ruosi, C., Cassese, E., De Rosa, M., & Schiraldi, C. (2019). Comparative analyses of pharmaceuticals or food supplements containing chondroitin sulfate: Are their bioactivities equivalent? *Advances in Therapy*, *36*(11), 3221–3237. https://doi.org/10.1007/s12325-019-01064-8

Su, C., Gong, J. - S., Qin, J., He, J. - M., Zhou, Z. - C., Jiang, M., Xu, Z. H., & Shi, J. - S. (2020). Glutathione enables full utilization of wool wastes for keratin production and wastewater decolorization. *Journal of Cleaner Production*, *270*, 122092. https://doi.org/10.1016/j.jclepro.2020.122092

Suh, H. J., & Lee, H. K. (2001). Characterization of a keratinolytic serine protease from Bacillus subtilis KS-1. *Journal of Protein Chemistry*, *20*(2), 165–169. https://doi.org/10.1023/a:1011075707553

Sun, P., Liu, Z. - T., & Liu, Z. - W. (2009). Chemically modified chicken feather as sorbent for removing toxic Chromium(VI) ions. *Industrial & Engineering Chemistry Research*, *48*(14), 6882–6889. https://doi.org/10.1021/ie900106h

Suzuki, K., Cheng, X. C., Kano, H., Shimizu, T., & Sato, Y. (1998). Influence of low protein diets on water intake and urine and nitrogen excretion in growing pigs. *Animal Science and Technology*, *69*, 267–270. https://www.jstage.jst.go.jp/article/chikusan1924/69/3/69_3_267/_pdf/-char/en

Swift, J. A., & Smith, J. R. (2001). Microscopical investigations on the epicuticle of mammalian keratin fibres. *Journal of Microscopy*, *204*(Pt 3), 203–211. https://doi.org/10.1046/j.1365-2818.2001.00957.x

Taheri, A., Anvar, S., Ahari, H., & Fogliano, V. (2013). Comparison the functional properties of protein hydrolysates from poultry by-products and rainbow trout (*Onchorhynchus mykiss*) viscera. *Iranian Journal of Fisheries Sciences*, *12*(1), 154–169. https://jifro.ir/article-1-879-en.pdf

Tantamacharik, T., Carne, A., Shavandi, A., & Bekhit, A. E.-D. A. (2021). Keratin as an alternative protein in food and nutrition. In A. E.-D. A. Bekhit, W. W. Riley, & M. A. Hussain (Eds.), *Alternative proteins* (1st ed., pp. 173–214). https://doi.org/10.1201/9780429299834-7

Taskin, M., & Kurbanoglu, E. B. (2011). Evaluation of waste chicken feathers as peptone source for bacterial growth. *Journal of Applied Microbiology*, *111*(4), 826–834. https://doi.org/10.1111/j.1365-2672.2011.05103.x

Taskin, M., Unver, Y., Firat, A., Ortucu, S., & Yildiz, M. (2016). Sheep wool protein hydrolysate: A new peptone source for microorganisms. *Journal of Chemical Technology & Biotechnology*, *91*(6), 1675–1680. https://doi.org/10.1002/jctb.4971

Thomson, R. L., & Buckley, J. D. (2011). Protein hydrolysates and tissue repair. *Nutrition Research Reviews*, *24*(2), 191–197. https://doi.org/10.1017/S0954422411000084

Toldra, F., Mora, L., & Reig, M. (2016). New insights into meat by-product utilization. *Meat Science*, *120*, 54–59. https://doi.org/10.1016/j.meatsci.2016.04.021

Toldra, F., Reig, M., & Mora, L. (2021). Management of meat by- and co-products for an improved meat processing sustainability. *Meat Science*, *181*, 108608. https://doi.org/10.1016/j.meatsci.2021.108608

Tomlinson, S. R., Kehr, C. W., Lopez, M. S., Schlup, J. R., & Anthony, J. L. (2014). Solubility of the corn protein zein in imidazolium-based ionic liquids. *Industrial & Engineering Chemistry Research*, *53*(6), 2293–2298. https://doi.org/10.1021/ie403659x

Tonin, C., Aluigi, A., Vineis, C., Varesano, A., Montarsolo, A., & Ferrero, F. (2007). Thermal and structural characterization of poly (ethylene-oxide)/keratin blend films. *Journal of Thermal Analysis and Calorimetry*, *89*(2), 601–608.

Tonin, C., Zoccola, M., Aluigi, A., Varesano, A., Montarsolo, A., Vineis, C., & Zimbardi, F. (2006). Study on the conversion of wool keratin by steam explosion. *Biomacromolecules*, *7*(12), 3499–3504. https://doi.org/10.1021/bm060597w

Tozer, R. G., Tai, P., Falconer, W., Ducruet, T., Karabadjian, A., Bounous, G., Molson, J. H., & Droge, W. (2008). Cysteine-rich protein reverses weight loss in lung cancer patients receiving chemotherapy or radiotherapy. *Antioxidants & Redox Signaling*, *10*(2), 395–402. https://doi.org/10.1089/ars.2007.1919

Tran, C. D., & Mututuvari, T. M. (2015). Cellulose, Chitosan, and keratin composite materials. Controlled drug release. *Langmuir*, *31*(4), 1516–1526. https://doi.org/10.1021/la5034367

Tuysuz, E., Ozkan, H., Arslan, N. P., Adiguzel, A., Baltaci, M. O., & Taskin, M. (2021). Bioconversion of waste sheep wool to microbial peptone by *Bacillus licheniformis* EY2. *Biofuels, Bioproducts and Biorefining*, *15*(5), 1372–1384. https://doi.org/10.1002/bbb.2232

Ulrich, J. F., & Jakel, N. T. (2003). United States Patent No. US6648930B2.

Valenzuela, P. L., Mata, F., Morales, J. S., Castillo-Garcia, A., & Lucia, A. (2019). Does beef protein supplementation improve body composition and exercise performance? A systematic review and meta-analysis of randomized controlled trials. *Nutrients*, *11*(6), 1429. https://doi.org/10.3390/nu11061429

Van Dyke, M. (2016). Keratin. In N. M. Neves & R. L. Reis (Eds.), *Biomaterials from nature for advanced devices and therapies* (pp. 93–105). https://doi.org/10.1002/9781119126218.ch6

Verma, A., Singh, H., Anwar, S., Chattopadhyay, A., Tiwari, K. K., Kaur, S., & Dhilon, G. S. (2017). Microbial keratinases: industrial enzymes with waste management potential. *Critical Reviews in Biotechnology*, *37*(4), 476–491. https://doi.org/10.1080/07388551.2016.1185388

Vidal, A. R., Cansian, R. L., Mello, R. O., Demiate, I. M., Kempka, A. P., Dornelles, R. C. P., Rodriguez, J. M. L., & Campagnol, P. C. B. (2022). Production of collagens and protein hydrolysates with antimicrobial and antioxidant activity from sheep slaughter by-products. *Antioxidants (Basel)*, *11*(6), 1173. https://doi.org/10.3390/antiox11061173

Villa, A. L., Aragao, M. R., Santos Dos, E. P., Mazotto, A. M., Zingali, R. B., de Souza, E. P., & Vermelho, A. B. (2013). Feather keratin hydrolysates obtained from microbial keratinases: effect on hair fiber. *BMC Biotechnology*, *13*, 15. https://doi.org/10.1186/1472-6750-13-15

Vineis, C., Varesano, A., Varchi, G., & Aluigi, A. (2019). Extraction and characterization of keratin from different biomasses. In S. Swati & K. Ashok (Eds.), *Keratin as a Protein Biopolymer* (pp. 35–76). Springer.

von Holstein, I. C., Penkman, K. E., Peacock, E. E., & Collins, M. J. (2014). Wet degradation of keratin proteins: linking amino acid, elemental and isotopic composition. *Rapid Communications in Mass Spectrometry*, *28*(19), 2121–2133. https://doi.org/10.1002/rcm.6999

Wan, X., Liu, S., Xin, X., Li, P., Dou, J., Han, X., Kang, I. -. K., Yuan, J., Chi, B., & Shen, J. (2020). S-nitrosated keratin composite mats with NO release capacity for wound healing. *Chemical Engineering Journal*, *400*, 125964. https://doi.org/10.1016/j.cej.2020.125964

Wang, B., Yang, W., McKittrick, J., & Meyers, M. A. (2016). Keratin: Structure, mechanical properties, occurrence in biological organisms, and efforts at bioinspiration. *Progress in Materials Science*, *76*, 229–318. https://doi.org/10.1016/j.pmatsci.2015.06.001

Wang, D., & Tang, R. - C. (2018). Dissolution of wool in the choline chloride/oxalic acid deep eutectic solvent. *Materials Letters*, *231*, 217–220. https://doi.org/10.1016/j.matlet.2018.08.056

Wang, J., Hao, S., Luo, T., Cheng, Z., Li, W., Gao, F., Guo, T., Gong, Y., & Wang, B. (2017). Feather keratin hydrogel for wound repair: Preparation, healing effect and biocompatibility evaluation. *Colloids and Surfaces B: Biointerfaces*, *149*, 341–350. https://doi.org/10.1016/j.colsurfb.2016.10.038

Wang, K., Li, R., Ma, J. H., Jian, Y. K., & Che, J. N. (2016). Extracting keratin from wool by using l-cysteine. *Green Chemistry*, *18*(2), 476–481. https://doi.org/10.1039/c5gc01254f

Wang, Q., Zhang, L., Wang, Q., Liu, Y., & Zhu, P. (2020). Study on dissolution and recovery of waste wool by sodium sulfide system. *Ferroelectrics*, *562*(1), 114–124. https://doi.org/10.1080/00150193.2020.1760599

Wang, Y. - X., & Cao, X. - J. (2012). Extracting keratin from chicken feathers by using a hydrophobic ionic liquid. *Process Biochemistry*, *47*(5), 896–899. https://doi.org/10.1016/j.procbio.2012.02.013

Wen, G., Naik, R., Cookson, P. G., Smith, S. V., Liu, X., & Wang, X. G. (2010). Wool powders used as sorbents to remove Co2+ ions from aqueous solution. *Powder Technology*, *197*(3), 235–240. https://doi.org/10.1016/j.powtec.2009.09.021

Wolber, F. M., McGrath, M., Jackson, F., Wylie, K., & Broomfield, A. (2016). Cysteic acid in dietary keratin is metabolized to glutathione and liver taurine in a rat model of human digestion. *Nutrients*, *8*(2), 104. https://doi.org/10.3390/nu8020104

Woods, A. (2012). *Global view of the apparel market. WOOL422/522 Wool marketing and clip preparation*. The Australian Wool Education Trust licensee for educational activities. Retrieved from https://www.woolwise.com/wp-content/uploads/2017/07/WOOL-422-522-12-T-16.pdf

Wools of New Zealand. (2022). *North Island Sale*. https://www.cpwool.co.nz/wp-content/uploads/2022/08/Market-Report-N08.pdf

Xie, H., Li, S., & Zhang, S. (2005). Ionic liquids as novel solvents for the dissolution and blending of wool keratin fibers. *Green Chemistry*, *7*(8), 606–608. https://doi.org/10.1039/b502547h

Xu, H., Ma, Z., & Yang, Y. (2014). Dissolution and regeneration of wool via controlled disintegration and disentanglement of highly crosslinked keratin. *Journal of Materials Science*, *49*(21), 7513–7521. https://doi.org/10.1007/s10853-014-8457-z

Xu, K., Wang, Y., Huang, Y., Li, N., & Wen, Q. (2015). A green deep eutectic solvent-based aqueous two-phase system for protein extracting. *Analytica Chimica Acta*, *864*, 9–20. https://doi.org/10.1016/j.aca.2015.01.026

Xu, W., Guo, W., & Li, W. (2003). Thermal analysis of ultrafine wool powder. *Journal of Applied Polymer Science*, *87*(14), 2372–2376. https://doi.org/10.1002/app.11991

Xu, W., Ke, G., Wu, J., & Wang, X. (2006). Modification of wool fiber using steam explosion. *European Polymer Journal*, *42*(9), 2168–2173. https://doi.org/10.1016/j.eurpolymj.2006.03.026

Yamauchi, K., Yamauchi, A., Kusunoki, T., Kohda, A., & Konishi, Y. (1996). Preparation of stable aqueous solution of keratins, and physiochemical and biodegradational properties of films. *Journal of Biomedical Materials Research*, *31*(4), 439–444. https://doi.org/10.1002/(SICI)1097-4636(199608)31:4⟨439::AID-JBM1⟩3.0.CO;2-M

Ye, J. P., Gong, J. S., Su, C., Liu, Y. G., Jiang, M., Pan, H., Li, R. Y., Geng, Y., Xu, Z. H., & Shi, J. S. (2020). Fabrication and characterization of high molecular keratin based nanofibrous membranes for wound healing. *Colloids and Surfaces B: Biointerfaces*, *194*, 111158. https://doi.org/10.1016/j.colsurfb.2020.111158

Yu, H., Li, W., Liu, X., Li, C., Ni, H., Wang, X., Huselstein, C., & Chen, Y. (2017). Improvement of functionality after chitosan-modified zein biocomposites. *Journal of Biomaterials Science, Polymer Edition*, *28*(3), 227–239. https://doi.org/10.1080/09205063.2016.1262159

Zaghloul, T. I., Embaby, A. M., & Elmahdy, A. R. (2011). Key determinants affecting sheep wool biodegradation directed by a keratinase-producing Bacillus subtilis recombinant strain. *Biodegradation*, *22*(1), 111–128. https://doi.org/10.1007/s10532-010-9381-9

Zeng, C.-h., & Lu, Q. (2014). Study on the recovery of waste wool by combining reduction and metallic salt methods. *Advanced Materials Research*, 881–883, 551–555. https://doi.org/10.4028/www.scientific.net/AMR.881-883.551

Zhang, C., Xia, L., Zhang, J., Liu, X., & Xu, W. (2020). Utilization of waste wool fibers for fabrication of wool powders and keratin: A review. *Journal of Leather Science and Engineering*, *2*(1). https://doi.org/10.1186/s42825-020-00030-3

Zhang, J., Li, Y., Li, J., Zhao, Z., Liu, X., Li, Z., Han, Y., Hu, J., & Chen, A. (2013). Isolation and characterization of biofunctional keratin particles extracted from wool wastes. *Powder Technology*, *246*, 356–362. https://doi.org/10.1016/j.powtec.2013.05.037

Zhang, N., Wang, Q., Yuan, J., Cui, L., Wang, P., Yu, Y., & Fan, X. (2018). Highly efficient and eco-friendly wool degradation by L-cysteine-assisted esperase. *Journal of Cleaner Production*, *192*, 433–442. https://doi.org/10.1016/j.jclepro.2018.05.008

Zhang, N., Wu, Z., Jiang, Z., Zhou, M., Yu, Y., Wang, P., & Wang, Q. (2022). pH Mediated L-cysteine aqueous solution for wool reduction and urea-free keratin extraction. *Journal of Polymers and the Environment*, *30*(7), 2714–2726. https://doi.org/10.1007/s10924-022-02383-8

Zhang, Z., Xu, L., Liu, W., Yang, Y., Du, Z., & Zhou, Z. (2014). Effects of partially replacing dietary soybean meal or cottonseed meal with completely hydrolyzed feather meal (defatted rice bran as the carrier) on production, cytokines, adhesive gut bacteria, and disease resistance in hybrid tilapia (Oreochromis niloticus female symbol x Oreochromis aureus male symbol). *Fish and Shellfish Immunology*, *41*(2), 517–525. https://doi.org/10.1016/j.fsi.2014.09.039

Zhang, Z., Zhang, X., Nie, Y., Wang, H., Zheng, S., & Zhang, S. (2017). Effects of water content on the dissolution behavior of wool keratin using 1-ethyl-3-methylimidazolium dimethylphosphate. *Science China Chemistry*, *60*(7), 934–941. https://doi.org/10.1007/s11426-016-9019-8

Zheng, S., Nie, Y., Zhang, S., Zhang, X., & Wang, L. (2015). Highly efficient dissolution of wool keratin by dimethylphosphate ionic liquids. *ACS Sustainable Chemistry & Engineering*, *3*(11), 2925–2932. https://doi.org/10.1021/acssuschemeng.5b00895

Zhou, W., & Freed, C. R. (2004). Tyrosine-to-cysteine modification of human alpha-synuclein enhances protein aggregation and cellular toxicity. *Journal of Biological Chemistry*, *279*(11), 10128–10135. https://doi.org/10.1074/jbc.M307563200

Zoccola, M., Aluigi, A., Patrucco, A., Vineis, C., Forlini, F., Locatelli, P., Sacchi, M. C., & Tonin, C. (2012). Microwave-assisted chemical-free hydrolysis of wool keratin. *Textile Research Journal*, *82*(19), 2006–2018. https://doi.org/10.1177/0040517512452948

Zoccola, M., Aluigi, A., & Tonin, C. (2009). Characterisation of keratin biomass from butchery and wool industry wastes. *Journal of Molecular Structure*, *938*(1–3), 35–40. https://doi.org/10.1016/j.molstruc.2009.08.036

Zoccola, M., Montarsolo, A., Mossotti, R., Patrucco, A., & Tonin, C. (2015). Green hydrolysis as an emerging technology to turn wool waste into organic nitrogen fertilizer. *Waste and Biomass Valorization*, *6*(5), 891–897. https://doi.org/10.1007/s12649-015-9393-0

Zou, T. B., He, T. P., Li, H. B., Tang, H. W., & Xia, E. Q. (2016). The structure-activity relationship of the antioxidant peptides from natural proteins. *Molecules*, *21*(1), 72. https://doi.org/10.3390/molecules21010072

How to cite this article: Giteru, S. G., Ramsey, D. H., Hou, Y., Cong, L., Mohan, A., & Bekhit, A. E. - D. A. (2022). Wool keratin as a novel alternative protein: A comprehensive review of extraction, purification, nutrition, safety, and food applications. *Comprehensive Reviews in Food Science and Food Safety*, 1–45. https://doi.org/10.1111/1541-4337.13087