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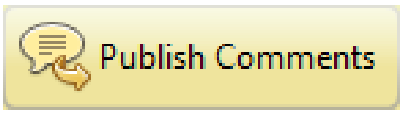

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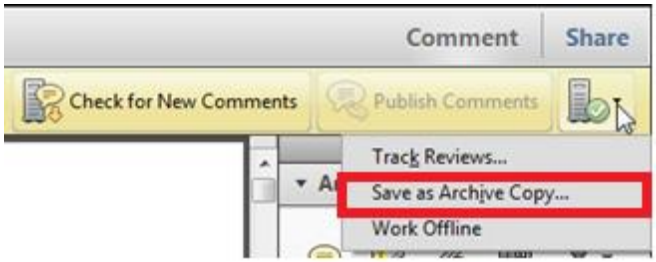

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REVIEW

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Solubilization of keratins and functional properties of their isolates and hydrolysates

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Abstract

The keratinous wastes of the textile industry and poultry slaughterhouses may be used as sources of soluble keratins or hydrolysates. This review presents methods for processing raw keratin-based materials into bioproducts with functional and bioactive properties suitable for biomedical, cosmetic, food, and agricultural applications. Soluble keratin can be obtained by thermal treatment in some organic solvents, reduction, or oxidation of the disulfide bonds. Recent studies have shown that keratins contain amino acid sequences with high biological activities such as antioxidant, angiotensin I converting enzyme inhibitory, dipeptidyl peptidase IV inhibitory, and antimicrobial. Peptides containing these sequences may find numerous applications as value-added products in the food industry. More research devoted to development of methods for conversion of animal by-products to novel products is needed. Further technological investigations to create large-scale production methods are also necessary.

Practical applications

The keratinous wastes represent a problematic by-product to the wool textile industry and poultry slaughterhouses due to the large volumes and their high pollutant load. They are usually incinerated or used for low value purposes such as fertilizers. This review focuses on the trends of application of keratin recovered from animal by-products. Biomaterials for regenerative medicine, cosmetic formulations, and biodegradable food packaging can be obtained as a result of keratin self-assembly. Several peptide sequences released by hydrolysis as bioactive peptides should be studied further for their in vivo antihypertensive, and antidiabetic effects, as well as functional ingredients in foods.

KEYWORDS

bioactive peptides, functional properties, keratin hydrolysates, keratin isolates

1 | INTRODUCTION

Keratins have biological activity, biocompatibility, biodegradability, and mechanical durability (Cardamone, 2010; Ferraro, Anton, & Santé-Lhoutellier, 2016; Reddy, Chen, & Yang, 2013) and are also capable of facilitating cell adhesion and proliferation (Rouse & Van Dyke, 2010). These properties have led to the development of keratin-based materials which can be suitable for numerous applications: biomedical (wound healing, drug delivery, tissue engineering, and medical devices) (Rouse & Van Dyke, 2010; Vasconcelos, Freddi, & Cavaco-Paulo, 2008), cosmetic materials (Nomura et al., 2005; Vermelho, Villa, De Almeida, de Souza Dias, & Dos Santos, 2008), food products (Goodwin, 1976), and agricultural uses (Veselá & Friedrich, 2009), as well as for food packaging (Song, Lee, Al Mijan, & Song, 2014).

The industrial applications of keratin-rich materials are limited due to difficulty in dissolving it due to the high level of cross-linking of the protein and tightly packed microfibrils (Reddy, Jiang, et al., 2013). In recent years, bioactive properties related to antioxidant (Ohba et al., 2003), angiotensin I converting enzyme (ACE) inhibitory (Karamać, Flaczyk, Wanasundara, & Amarowicz, 2005), dipeptidyl peptidase IV (DPP IV) inhibitory (Fontoura et al., 2014), antifungal (Gousterova et al., 2011), and antibacterial activity (Sundaram, Legadevi, Banu, Gayathri, & Palanisammy, 2015) have been found in keratin hydrolysates. Production of enzymatic hydrolysates as a source of bioactive peptides can contribute to develop

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nutritional or pharmaceutical applications (Di Bernardini et al., 2011; Gómez-Guillén, Giménez, López-Caballero, & Montero, 2011).

This paper reviews the processing and applications of keratin. The great potential of keratin as a fibrous protein with supramolecular organization in the form of α-helix which is an important factor affecting the characteristic mechanical properties and functionality, is discussed. The potential of keratin as a store house of bioactive peptides is also discussed.

2 | STRUCTURE AND OCCURANCE OF KERATINS

Keratins (gr. keras—horn) are major structural proteins of vertebrate epithelia. They occur in hair, bristles, wool, feathers, claws, and horns. They perform various functions such as waterproof, excretion of wastes and regulation of temperature, cushion to protect the deeper tissues against mechanical shock and infection (Ferraro et al., 2016). Keratins are very hard, visco-elastic, and resilient (Bonser, 1996). They undergo bundling and have higher Young's modulus than collagen (Eslahi, Dadashian, & Nejad, 2013). They are insoluble in water, weak acids, and alkalis, as well as in organic solvents (Ferraro et al., 2016). Keratins belong to the superfamily of intermediate filament (IF) proteins forming the cytoskeleton (Kornitłowicz-Kowalska & Bohacz, 2011). Their subunits consist of a central domain with α-helical structure and globular N- and C-terminal domains composed of 15–30 amino acid residues, and β-sheet regions (Fraser, MacRae, Parry, & Suzuki, 1986). The highly conserved central domains contain 310–315 residues arranged in repeating sequences (Bragulla & Homberger, 2009). Keratin subunits associate in a high-order structure forming a double-stranded superhelix, microfibrils, and macrofibrils embedded in an amorphic matrix (McKittrick et al., 2012). The right-handed α-helix of α-keratin is stabilized by hydrogen bonds and numerous disulfide bridges formed by cysteine residues that cause the insolubility of keratin. Therefore it is not easily degradable by common proteolytic enzymes such as trypsin, pepsin, and papain. A high cystine content amounting to 7–20% of the total amino acid residues is characteristic of keratins. They also contain about 0.5% methionine residues, as well as large proportion of glycine, serine, leucine, and glutamic acid. The amino acid sequence of keratin is very similar in different species (Bragulla & Homberger, 2009).

Keratins are heterogeneous proteins due to variation in amino acids composition (Table 1) and type of secondary structure. Twenty isoforms have been identified with molecular weights ranging from 40 to 70 kDa in human epithelial cells (Rodziewicz & Łaba, 2006). Wool, hair, and skin keratins with cystine content between 10 and 14% are soft and flexible, but keratins extracted from feathers, beaks, claws, and horns are hard, rigid, inflexible, and inextensible due to higher cystine content up to 22% (Cardamone, 2010). Keratin polypeptide chains can curl into two configurations: α-helix and β-sheet. Thus, keratins are also classified into four groups: α-keratin, β-keratin, feather keratin, and amorphic keratin (McKittrick et al., 2012). α-Keratins occur in mammals as the primary constituent of hair (fiber cortex), nails, hooves,

TABLE 1 Amino acid composition (% of total amino acid residues) of keratin from different sources

Amino acid	Buffalo horn and hoof (Noda, Imai, Kida, & Otagiri, 1996)	Cow hair (Coward-Kelly, Chang, Agbogbo, & Holtzapple, 2006)	Feathers (Moore, Martelli, Gandolfo, Pires, & Laurindo, 2006)	Wool (Cardamone, 2010)
Alanine	6.3	4.5	3.6	5.8
Arginine	6.8	11.0	5.4	7.8
Aspartic acid	6.7	6.6	4.7	4.1
Cysteine	3.7	nd	7.7	6.1
Glutamic acid	12.6	14.5	7.7	11.4
Glycine	12.3	5.5	6.2	2.9
Histidine	0.6	1.3	–	–
Isoleucine	3.0	4.2	4.3	3.9
Leucine	8.2	9.8	7.0	11.9
Lysine	2.7	5.5	0.6	2.9
Methionine	0.6	0.7	1.3	0.2
Phenylalanine	2.9	3.1	4.2	1.9
Proline	6.8	7.7	8.7	4.1
Serine	10.8	8.9	9.3	8.3
Threonine	5.6	7.5	3.5	5.6
Tyrosine	5.9	2.4	2.0	2.4
Valine	4.1	6.8	6.9	6.1

nd = not determined.

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horns, quills, and the epidermal layer of the skin. They have α-helical tertiary structure and are rich in cystine residues ranging from 10 to 22%. They are divided into two subfamilies, the type I acidic microfibrillar component of ca. 40–50 kDa and the type II neutral/basic membranes of ca. 55–65 kDa (Marchisio, 2000). β-Keratins are found in reptiles and birds in scales, claws, beaks, feathers, and cuticle hair. They are difficult to extract and they do not form useful reconstituted structures such as gels, films, coatings, and fibers suitable for medical applications (wound healing, bone generation, hemostasis, and peripheral nerve repair (Ferraro et al., 2016; Hill, Brantley, & Van Dyke, 2010). They are rich in glycine, alanine, serine, and proline residues, but lack cysteine, thus the structure is stabilized only by hydrogen bonds. In feather keratin β-sheet and α-helix occur in 1/3 and 2/3, respectively (Marchisio, 2000). Feather keratins from various birds are similar with molecular weight of about 10 kDa and cystine content of about 8% which is lower than that in keratin from nail and hair (Akhatar & Edwards, 1997). They are composed of about 20 different types which vary only by few amino acids (Saravanan, 2012). The basic and acid residues are positioned in the N- and C-terminal regions, whereas the hydrophobic residues are located in the central portion. The chemical or enzymatic process of feather keratin degradation is not uniform due to its complex hierarchical structure (Ferraro et al., 2016). Amorphic keratins, so-called γ-keratins are a part of the matrix. These are globular proteins with high cystine content and molecular weight of about 15 kDa. γ-Keratins occur in the external layer of the hair cuticle (Hill et al., 2010).

The content and structure of various forms of keratin depend on the physiological function and type of organism in which the protein occurs (Wang, Yang, McKittrick, & Meyers, 2016). Structural diversity of keratins also occurs within the same skin appendages. An example of this is the hair in which the external layer of the cuticle contains more cystine than the internal layers which are less resistant to proteolytic enzymes (Kornitłowicz-Kowalska & Bohacz, 2011).

The physicochemical and biological features of keratins isolated from different sources are reflected in various functionalities of these proteins of which self-assembly is the most important (Dickerson et al., 2013). During thermodynamic equilibrium, the keratin molecules spontaneously arrange forming well-defined networks stabilized by noncovalent interactions. As a result of self-assembly, keratins can provide biomaterials for medicine, bioactive peptides, cosmetic formulations, and biodegradable films (Ferraro et al., 2016).

3 | SOLUBILIZATION OF KERATINS

3.1 | Introduction

The method of processing raw keratin-based materials depends on the intended use of the product of keratin solubilization. These include thermal treatment in some organic solvents, reduction or oxidation of the disulfide bonds, alkaline, acid or enzymatic hydrolysis, various hydrothermal methods, and a combination of thermo-chemical and enzymatic treatments (Chojnacka, Górecka, Michalak, & Górecki, 2011; Ferraro et al., 2016; Wolski, 1979).

3.2 | Production of keratin isolates

Obtaining keratin isolates containing native keratin is difficult in practice due to insolubility of the protein in solutions which do not cause its degradation (Yin, Li, He, Wang, & Wang, 2013). A method of solubilization of keratin was developed using organic solvents, for example, N,N-dimethylformamide (DMF) or dimethyl sulfoxide (DMSO). For extraction with DMSO, precipitation of dissolved protein with acetone or benzene is needed. When both solvents are removed, a sediment is dried for dietary purposes as protein preparation (Wolski, 1985). This method requires a long extraction time and high cost caused by the need for solvent recovery (Wolski, 1979). There are no changes in protein structure caused by this procedure and is often used by many researchers on laboratory scale to obtain a substrate for determination of keratinolytic activity (Wawrzkiewicz, Lobarzewski, & Wolski, 1987).

Reduction and oxidation of disulfide bonds belong to the common methods for keratin isolation. Reduction of keratin involves use of 2-mercaptoethanol (Balaji et al., 2012; Fujii & Li, 2008; Kakkar, Madhan, & Shanmugam, 2014; Reichl, 2009; Schrooyen, Dijkstra, Oberthür, Bantjes, & Feijen, 2001; Tanabe, Okitsu, & Yamauchi, 2004; Yamauchi, Yamauchi, Kusunoki, Kohda, & Konishi, 1996), dithiothreitol (DTT), dithioerythritol (Vasconcelos et al., 2008; Yang, Zhang, Yuan, & Cui, 2009), thioglycolic acid (Hill et al., 2010; Zabashta, Kasprova, Senchurov, & Grabovskii, 2012), glutathione (Schrooyen, Dijkstra, Oberthür, Bantjes, & Feijen, 2000), salts of hydrocyanic acid (Arai, Sakamoto, Naito, & Takahashi, 1989), bisulfites (Tonin et al., 2007), and *m*-bisulphites (Aluigi et al., 2007; Vasconcelos et al., 2008) to solubilize the protein. Many keratins can remain trapped within the protective structure, and usually a hydrogen-bond breaking agent, such as urea, thiourea, transition metal hydroxides, surfactants, and combinations thereof, are included in the extractant to unfold or denature the protein (Torchinsky, 1981). Aqueous solutions of tris(hydroxymethyl)aminomethane in concentrations between 0.1 and 1.0 M, and urea solutions 0.1–10 M are used (Schrooyen et al., 2000). The keratin solution is dialyzed to remove the reagents. During dialysis, extensive protein aggregation may occur but is often prevented by addition of sodium dodecylsulfate (SDS) (Schrooyen et al., 2001). Upon reduction, the disulfide bonds are broken to give cysteine thiol (reduced keratin) and cysteine-S-sulphonate (Bunte salt) residues:

$$\text{keratin–Cys–S–S–Cys–keratin} + SO_3^{2-} \rightarrow \text{keratin–Cys–S}^- + \text{keratin–Cys–SSO}_3^-$$

where keratin–Cys–S– is the reduced keratin and keratin–Cys–SSO_3^- is the Bunte salt (Maclaren & Milligan, 1995). If keratins are extracted by reduction, the resulting products are referred to as kerateines which are less polar, less soluble in water, but more stable in acidic and alkaline solutions. They can re-cross-link, and remain in vivo for weeks to months longer than the oxidized derivatives (Hill et al., 2010).

When oxidation is applied to extract keratin, strong oxidants are used, such as hydrogen peroxide (Breinl & Baudisch, 1907), potassium permanganate (Lissizin, 1928), ammonium copper hydroxide (Nagai & Nishikawa, 1970), and organic peracids (de Guzman et al., 2011). The disulfide bonds are converted to sulfonic acid groups and cysteic acid derivatives are formed, which are referred to as "keratoses":

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$$RS{-}SR + [O] \rightarrow R{-}SO_2H + R{-}SO_3H$$

These keratoses are hygroscopic, water soluble, nondisulfide cross-linkable, and degrade relatively quickly in vivo in days to weeks (Hill et al., 2010).

3.3 | Chemical hydrolysis

During chemical hydrolysis, some amino acids are lost (Zhang, Li, et al., 2013). Keratins can be easily solubilized by hydrolysis in strong acids or alkalis, but they cannot be recovered except as amino acids or peptides, peptones, and proteoses, the properties of which differ significantly from those of the native keratin. The thermo-chemical treatment of keratinous materials with alkali leads to degradation of asparagine, arginine, serine, threonine, and glutamine (Chojnacka et al., 2011). The solubilization of keratin wool at temperatures above 70°C in a pH range of 9–11 for 4–12 hr in the presence of excess alkali can cause conversion of disulfide groups to cystyl residues (CysS–):

$$2\ Cys{-}S{-}S{-}Cys + 4\ OH^- \leftrightarrow 3\ Cys{-}S^- + Cys{-}SO_2^- + 2\ H_2O\ \text{(Cardamone, 2010)}$$

and subsequently the conversion of the cystyl residues into thioether groups, giving lanthionyl residues (Cys–S–Cys) (Cardamone, 2010). Preliminary alkaline treatment of wool in the sheep skin unhairing process also leads to the formation of two unnatural amino acids lysinoalanine and ornithinoalanine (Money, 1996). These products are a result of keratin hydrolysis under alkaline conditions during unhairing by the lime-sulfide method. Moreover, the treatment of keratins with reducing agents in strong alkaline solutions creates conditions that destroy the cystine and hydroxy amino acid residues (Koleva, Danalev, Ivanova, Vezenkov, & Vassilev, 2009).

Keratins can also be solubilized in alkaline solutions of metallic sulfides. These reagents are generally used in cosmetic depilatories and removal of hair from hides in the tanning industry (Jones & Mecham, 1948). Furthermore, alkaline hydrolysis with prolonged exposure at elevated temperature produces low molecular weight peptide fragments with poor mechanical properties. This product has limited biomedical application (Smith, Blanchard, & Lankford, 1994).

Acidic hydrolysis is highly efficient, but it is not recommended because of the loss of some amino acids, for example, serine, threonine, tyrosine, and cystine, as well as conversion of asparagine, glutamine, and tryptophan to other products. Furthermore, the bonds between valine and isoleucine are gradually disrupted (Chojnacka et al., 2011). Keratin can be solubilized in formic acid (Aluigi et al., 2007), hydrochloric acid (Zhang, Li, et al., 2013), and sulfuric acid (Kurbanoglu & Kurbanoglu, 2007) using appropriately high temperature. During acid hydrolysis of wool keratin, disulfide, and partial peptide bonds are destroyed:

$$Cys{-}S{-}S{-}Cys + R\cdot \rightarrow R{-}S{-}Cys + \cdot S{-}Cys$$

$$\cdot S{-}Cys + RH \rightarrow R\cdot + HS{-}Cys\ \text{(Zhang, Li, et al., 2013a)}.$$

The degree of acid hydrolysis of keratin ranges from about 33 to 46% (Karamać et al., 2005; Zhang, Li, et al., 2013). Acid-derived keratin hydrolysates have higher glass transition and lower decomposition temperatures than pristine wool fibers (Katoh, Shibayama, Tanabe, & Yamauchi, 2004; Vasconcelos et al., 2008). They are nontoxic and biocompatible and therefore can have potential application as biomaterials for wound healing and drug delivery. During acid hydrolysis of wool keratin, most of the hydrogen bonds are broken down which results in the amorphous structure of wool keratin polypeptides (Tung & Daoud, 2009). Hence the content of both α-helix and β-sheet structures in wool keratin are decreased as the total crystallinity of wool is the sum of α- and β-crystallinity (Cao & Billows, 1999). The products of acid hydrolysis are more amorphous keratin polypeptides than alkaline-derived keratin hydrolysates (Zhang, Li, et al., 2013).

The hydrothermal methods for obtaining soluble keratin are expensive and destroy certain amino acids, for example, lysine, methionine, and tryptophan (Grazziotin, Pimentel, De Jong, & Brandelli, 2006). They result in products with poor digestibility and variable nutritional quality (Chojnacka et al., 2011). These processes are performed at 100–150°C and 1.5×10^5 Pa (Grazziotin et al., 2006) and alkali or acid are often added. These hydrolysates have been used in feeding of poultry, rainbow trout, shrimp, and salmon after supplementation with essential amino acids (Bertsch & Coello, 2005).

3.4 | Enzymatic hydrolysis

The enzymatic and/or microbiological methods for solubilization of keratin waste are cheap and run under mild conditions (Chojnacka et al., 2011). These methods are an alternative to environmentally harmful chemical methods used most often in keratin isolation. Keratinases are extracellular serine proteases or metalloproteases produced by bacteria, actinomycetes, and fungi (Brandelli, 2008). The characteristics of keratinases produced by some microorganisms are shown in Table 2. These enzymes convert insoluble keratin to feedstuffs, fertilizers, and films, and also materials suitable for cosmetic and pharmaceutical applications (Brandelli, Daroit, & Riffel, 2010). The mechanism of microbial keratinolysis is not completely known. The process of keratin degradation proposed by Kunert (1976) is for dermatophytes and consists of sulfitolysis and proteolysis:

$$keratin{-}Cys{-}S{-}S{-}Cys{-}keratin + HSO_3^- \rightarrow keratin{-}Cys{-}SSO_3^- + HS{-}Cys{-}keratin \rightarrow amino\ acids + peptides.$$

In the first stage, disulfide bonds are disrupted by sulfite produced by the fungus which leads to protein denaturation (Kunert, 1976) and proteolysis by endopeptidases. On the other hand, Yamamura, Morita, Hasan, Yokoyama, and Tamiya (2002) proposed a two-stage process of keratin degradation involving disulfide reductase and serine protease produced by *Stenotrophomonas* sp. D-1 from deer fur. Keratin reduced by disulfide reductase is hydrolyzed by protease to amino acids and peptides. Some bacteria, actinomycetes, keratinophilic fungi, and larvae of the common clothes moth (*Tineola bisselliella* Hummel) use native keratin as the sole source of carbon, nitrogen, sulfur, and energy (Kornitowicz-Kowalska & Bohacz, 2011). *Bacillus licheniformis*, *Bacillus pumilus*, *Bacillus cereus*, and *Bacillus subtilis*, and *Stenotrophomonas* sp., *Fervidobacterium pannavorans*, and *Fervidobacterium islandicum* were isolated from plumage and bird feathers, and fermented feather waste

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TABLE 2 Characteristic of keratinases from some microorganisms

Source of keratinase	Molecular mass (kDa)	Optimum pH	Optimum temperature (°C)	References
Aspergillus fumigatus TKF1	24	6.0	50	Paul et al. (2014)
Aspergillus parasiticus	36	7.0	50	Anitha and Palanivelu (2013)
Bacillus licheniformis PWD-1	33	7.5	50	Lin, Lee, Casale, and Shih (1992)
Bacillus pumilus A1	–	9.0	55–60	Fakhfakh-Zouari, Haddar, Hmidet, Frikha, and Nasri (2010)
Bacillus subtilis S14	–	8.0	50	Silva, Macedo, and Termignoni (2014)
Brevibacillus sp.	83.2	12.5–13.0	45	Rai and Mukherjee (2011)
Chryseobacterium indologenes A22	–	7.5	45	Bach, Daroit, Corrêa, and Brandelli (2011)
Chryseobacterium sp. kr6	64	8.5	50	Riffel et al. (2007)
Fervidobacterium islandiucum AW-1	>200	9.0	100	Nam et al. (2002)
Microsporum canis	33	8.0	35–45	Descamps et al. (2003)
Microsporum gypseum	33	8.0	35	Raju, Neogi, Saumya, and Goud (2007)
Stenotrophomonas sp. D-1	40	7.0	30	Yamamura et al. (2002)
Streptomyces fradiae	24	8.0	50	Galas and Kałuźewska (1991)
Streptomyces gulbargensis	46	9.0	45	Syed, Lee, Li, Kim, and Agasar (2009)
Streptomyces thermoviolaceus SD8	40	8.0	55	Chitte, Nalawade, and Dey (1999)
Trichophyton mentagrophytes	38	5.5	55	Muhsin and Aubaid (2001)

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(Burtt & Ichida, 1999; Ichida et al., 2001; Williams & Shih, 1989). Keratinolytic species of actinomycetes, particularly from the genus *Streptomyces*, and some species from *Thermoactinomyces* occur in feathers, hairs, nails, and horns. The keratinophylic fungi live in the soil, birds, mammals, avian nests, bird plumage, mammalian hair, communal waste water, waste sediments, communal waste, and polluted water. They are represented by dermatophytes (some species of *Trichophyton* and *Microsporum*), and two genera: *Chrysosporium* and *Myceliophthora* (Korniłłowicz-Kowalska & Bohacz, 2011).

Another method used to dissolve keratins is a combination of enzymatic and chemical treatment (Mokrejs, Svoboda, Hrncirik, Janacova, & Vasek, 2011). Reports on application of thermo-chemical treatment of keratins have appeared recently, however these methods occur in different experimental layout, aimed in aiding subsequent enzymatic digestion (Łaba et al., 2015).

4 | BIOACTIVE PROPERTIES OF KERATIN PRODUCTS

4.1 | Introduction

Hydrolyzed proteins from many sources such as milk casein, soybean, rice bran, quinoa seed protein, canola, egg yolk protein, and muscle proteins have been reported to be sources of biologically active peptides (Gómez-Guillén et al., 2011) (Table 3). These peptides, sequences of 2–30 amino acids, are inactive in the parent protein and can be released during gastrointestinal digestion, enzymatic processing or microbial fermentation (Di Bernardini et al., 2011; Ferraro et al., 2016; Gómez-Guillén et al., 2011). After liberation, they display biological activities, for example, antioxidant, ACE inhibitory, and antimicrobial. Keratins have also been shown to be a source of bioactive peptides by Ferraro et al. (2016) and Lasekan, Bakar, and Hashim (2013).

4.2 | Antioxidant activity

Reports on the antioxidant properties of hydrolysates or peptides from various proteins are abundant, but only a few from keratin. The antioxidant peptides often contain hydrophobic amino acid residues, proline, histidine, tyrosine, and tryptophan (Brandelli, Daroit, & Corrêa, 2015). Ohba et al. (2003) reported high antioxidant activity in the enzymatic hydrolysate of a mixture of horn and hoof, and chicken feather. They suggested that the large amounts of cysteine in keratin were responsible for this activity. Fakhfakh et al. (2011) also found high antioxidant activity in the hydrolysate obtained after fermentation of chicken feather with the bacterium *Bacillus pumilus* A1. The keratin wastes showed stronger antioxidant activity than the collagen wastes using the DPPH radical scavenging assay. The authors suggested that the use of feather protein hydrolysate in fish feed formulations could be suitable for improving the biological properties of the feed. Kumar et al. (2012) produced feather protein hydrolysate with a high DPPH free radical-scavenging activity which was similar to that shown by Fakhfakh et al. (2011) using the strain *Bacillus pumilus* A1. Fontoura et al. (2014) obtained hydrolysates from raw chicken feathers with the bacterium *Chryseobacterium* sp. kr6 which displayed in vitro antioxidant properties. These hydrolysates might be used as a source of bioactive constituent for feed, food, and drug production. An antioxidative

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TABLE 3 Bioactive peptides from different proteins

Source	Antioxidant peptides	Reference	ACE inhibitory peptides	Reference
Bovine casein	Tyr-Phe-Tyr-Pro-Glu-Leu	Suetsuna, Ukeda, and Ochi (2000)	Arg-Tyr-Leu-Gly-Tyr	Contreras et al. (2009)
			Ala-Tyr-Phe-Tyr-Pro-Glu-Leu	
			Tyr-Gln-Lys-Phe-Pro-Gln-Tyr	
Bovine α-lactalbumin	Ile-Asn-Tyr-Trp	Sadat et al. (2011)	Leu-Ala-His-Lys-Ala-Leu	Pihlanto-Leppälä et al. (1998)
			Trp-Leu-Ala-His-Lys	Pihlanto-Leppälä, Koskinen, Piilola, Tupasela, and Korhonen (2000)
			Val-Gly-Ile-Asn-Tyr-Trp-Leu-Ala-His-Lys	
Bovine β-lactoglobulin	Phe-Asn-Pro-Thr-Gln	Contreras, Hernández-Ledesma, Amigo, Martín-Álvarez, and Recio (2011)	Ile-Ile-Ala-Glu-Lys	Power, Fernández, Norris, Riera, and FitzGerald (2014)
	Leu-Gln-Lys-Trp		Ile-Pro-Ala-Val-Phe-Lys	
	Leu-Asp-Thr-Asp-Tyr-Lys-Lys		Ala-Leu-Pro-Met-His-Ile-Arg	Mullally, Meisel, and Fitz-Gerald (1997)
	Val-Ala-Gly-Thr-Trp-Tyr	Power et al. (2014)		
	Trp-Tyr-Ser-Leu	Zhang, Wu, Ling, and Lu (2013)		
Bovine skin gelatin	Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly	Kim, Byun, Park, and Shahidi (2001) and Kim, Kim, Kim, Byun, Park, and Ito (2001)	Gly-Pro-Val	Kim, Byun, et al. (2001) and Kim, Kim, et al. (2001)
			Gly-Pro-Leu	
Chicken feather keratin	Ser-Asn-Leu-Cys-Arg-Pro-Cys-Gly	Wan et al. (2016)	–	–
Chicken leg collagen	–	–	Gly-Ala-Hyp-Gly-Leu-Hyp-Gly-Pro	Saiga et al. (2008)
Egg yolk protein	Leu-Met-Ser-Tyr-Met-Trp-Ser-Thr-Ser-Met	Park, Jung, Nam, Shahidi, and Kim (2001)	–	–
	Leu-Glu-Leu-His-Lys-Leu-Arg-Ser-Ser-His-Trp-Phe-Ser-Arg-Arg			
Egg white protein	Ala-His	Tsuge, Eikawa, Nomura, Yamamoto, and Sugisawa (1991)	Arg-Ala-Asp-His-Pro-Phe-Leu	Miguel, Recio, Gómez-Ruiz, Ramos, and Lopez-Fandino (2004)
	Val-His-His		Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu	
	Val-His-His-Ala-Asn-Glu-Asn			
Fish skin gelatin (*Jonius belengerii*)	His-Gly-Pro-Leu-Gly-Pro-Leu	Mendis, Rajapakse, and Kim (2005)	–	–
Pacific codfish gelatin	–	–	Thr-Cys-Ser-Pro	Ngo et al. (2011)
			Thr-Gly-Gly-Gly-Asn-Val	
Porcine actomyosin	Asp-Leu-Tyr-Ala	Arihara (2006)	–	–
	Ser-Leu-Tyr-Ala			
	Val-Trp			
Porcine skin collagen	Gln-Gly-Ala-Arg	Li, Chen, Wang, Ji, and Wu (2007)	–	–
Porcine skin gelatin	–	–	Gly-Phe-Hyp-Gly-Pro	Ichimura, Yamanaka, Otsuka, Yamashita, and Maruyama (2009)

J_ID: Customer A_ID: JFBC12494 Cadmus Art: JFBC12494 Ed. Ref. No.: JFBC-09-17-0591.R2 Date: 28-December-17 Stage: Page: 7

peptide had been isolated from chicken feather hydrolysate obtained by bacterial fermentation and identified as Ser-Asn-Leu-Cys-Arg-Pro-Cys-Gly (Wan, Dong, Yang, & Feng, 2016). Polypeptides from bovine hair exhibited significant antioxidant activity and remarkable food protection. These polypeptides could be a new natural antioxidant used in oil and oil-rich food (Zeng, Zhang, Zhang, & Shi, 2013).

4.3 | ACE inhibitory activity

Antihypertensive peptides can lower blood pressure through inhibition ACE. Many years of research have been devoted to the synthesis of ACE inhibitors used widely for therapeutic purposes to prevent hypertension (Gómez-Guillén et al., 2011). However, they have side effects such as coughing, poor taste, skin rashes, and angioneurotic edema (Atkinson & Robertson, 1979). Therefore, research has focused on identifying natural sources of ACE inhibitors with no side effects. Many antihypertensive/ACE inhibitory peptides have been isolated from casein, collagen, lactalbumin, myosin, ovalbumin, and serum albumin (Brandelli et al., 2015; Contreras, Carrón, Montero, Ramos, & Recio, 2009; Pihlanto-Leppälä, Rokka, & Korhonen, 1998; Saiga et al., 2008).

Keratin has also been shown to be a source of ACE inhibitory peptides, although it has not been studied with regard to this activity as much as other proteins. ACE inhibitory activity has been shown in keratin hydrolysates from poultry feathers (Karamać et al., 2005). The activity of acid hydrolysates from keratin waste was lower (49.6% inhibition) than that of collagen hydrolysates (72.3% inhibition). Increase in ACE inhibitory activity with increase of the concentration of proline and hydroxylproline had been observed (Gómez-Guillén et al., 2011). Ohba et al. (2003) reported that the enzymatic hydrolysate of a mixture of horn and hoof also exhibited low ACE inhibitory activity. ACE inhibitory activity increased with decreasing molecular weight of hydrolysates. The hydrolysates obtained from raw chicken feathers with the bacterium *Chryseobacterium* sp. kr6 also had ACE inhibitory activity (Fontoura et al., 2014). The keratin hydrolysates were able to inhibit 65% ACE activity and was comparable to ACE inhibitory activity of soybean hydrolysates and milk protein hydrolysates. Enzyme specificity influences the biological activity of protein hydrolysates (Gómez-Guillén et al., 2011). High hydrophobic and aromatic amino acid residues content of 50–60% of the total amino acid residues is characteristic of keratins (Fontoura et al., 2014). Hydrophobic amino acids at the C-terminal tripeptide sequence contribute to the ACE inhibitory activity of peptides (Haque & Chand, 2008).

4.4 | Other activities

Keratins have also been shown to be a source of bioactive peptides with other biological activities. Fontoura et al. (2014) showed that the hydrolysates obtained from raw chicken feathers had the ability to inhibit DPP IV activity by 44%. This activity was found only in whey hydrolysates which positively affect blood glucose control and insulinotropic responses in humans. Bioactive peptides from whey proteins stimulate the secretion gut hormones, and also act as DPP IV inhibitors in vivo (Jakubowicz & Froy, 2013).

Gousterova et al. (2011) found that feather hydrolysate obtained using a mixed culture of *Thermoactinomyces* strains showed good activity against plant pathogenic fungi *Fusarium solani*, *Fusarium oxysporum*, *Mucor* sp., and *Aspergillus niger*. It was suggested that the feather hydrolysate could be used as an alternative soil amendment for restoring contaminated soils, accelerating ryegrass growth, and improving the quality of agricultural soils.

Sundaram et al. (2015) observed antibacterial activity of keratin hydrolysate and keratin nanoparticles. The radius of inhibition zone for keratin hydrolysate against *Staphylococcus aureus* and *Escherichia coli* was 7.5 mm and 9 mm, respectively, at 100 μg/mL. They reported that the inhibition zone formulated for keratin nanoparticles was higher than that for keratin hydrolysates.

5 | CONCLUSIONS AND PERSPECTIVES

Keratin extracted from waste is a source of bioactive compounds for biological, food, and biomaterial applications. There is more information on the nonbiological functions of keratins than bioactive properties. Thus there is a need for further research devoted to selecting enzyme systems that convert keratin waste into bioactive peptides which could be used for formation of useful novel bioproducts. Literature shows an increasing number of reports on the use of various enzymes and conditions to obtain bioactive peptides from keratin. The peptides with antioxidant and antimicrobial activities could possibly be used as additives in functional food products. Similarly, the fragments of keratin with ACE inhibitory and DPP IV inhibitory activity could be suitable for food and pharmaceutical applications. Therefore, advanced research on safety of these future bioproducts, maintenance of their bioactivity in humans mechanism of action, and industrial production are necessary.

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