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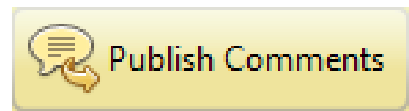
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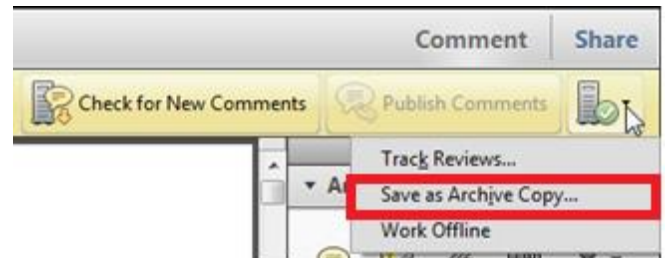
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# Solubilization of keratins and functional properties of their isolates and hydrolysates

Izabela Sinkiewicz  | Hanna Staroszczyk | Agata Śliwińska

Department of Food Chemistry, Technology and Biotechnology, Gdansk University of Technology, G. Narutowicza 11/12, 80-233 Gdańsk, Poland

## Correspondence

Department of Food Chemistry, Technology and Biotechnology, Gdansk University of Technology, G. Narutowicza 11/12, 80-233 Gdańsk, Poland.  
Email: izabela.sinkiewicz@pg.edu.pl

## 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 (Vesela & 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, & Palanisamy, 2015) have been found in keratin hydrolysates. Production of enzymatic hydrolysates as a source of bioactive peptides can contribute to develop

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

56 This paper reviews the processing and applications of keratin. The  
57 great potential of keratin as a fibrous protein with supramolecular orga-  
58 nization in the form of  $\alpha$ -helix which is an important factor affecting  
59 the characteristic mechanical properties and functionality, is discussed.  
60 The potential of keratin as a store house of bioactive peptides is also  
61 discussed.

## 62 2 | STRUCTURE AND OCCURANCE OF 63 KERATINS

64 Keratins (gr. keras—horn) are major structural proteins of vertebrate  
65 epithelia. They occur in hair, bristles, wool, feathers, claws, and horns.  
66 They perform various functions such as waterproof, excretion of  
67 wastes and regulation of temperature, cushion to protect the deeper  
68 tissues against mechanical shock and infection (Ferraro et al., 2016).  
69 Keratins are very hard, visco-elastic, and resilient (Bonser, 1996). They  
70 undergo bundling and have higher Young's modulus than collagen  
71 (Eslahi, Dadashian, & Nejad, 2013). They are insoluble in water, weak  
72 acids, and alkalis, as well as in organic solvents (Ferraro et al., 2016).  
73 Keratins belong to the superfamily of intermediate filament (IF)  
74 proteins forming the cytoskeleton (Kornitowicz-Kowalska & Bohacz,  
75 2011). Their subunits consist of a central domain with  $\alpha$ -helical  
76 structure and globular N- and C-terminal domains composed of 15–30  
77 amino acid residues, and  $\beta$ -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 amorphous matrix (McKittrick et al., 2012). The right-handed  $\alpha$ -helix  
of  $\alpha$ -keratin is stabilized by hydrogen bonds and numerous disulfide  
bridges formed by cysteine residues that cause the insolubility of kera-  
tin. 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 cys-  
tine content up to 22% (Cardamone, 2010). Keratin polypeptide chains  
can curl into two configurations:  $\alpha$ -helix and  $\beta$ -sheet. Thus, keratins are  
also classified into four groups:  $\alpha$ -keratin,  $\beta$ -keratin, feather keratin,  
and amorphous keratin (McKittrick et al., 2012).  $\alpha$ -Keratins occur in  
mammals as the primary constituent of hair (fiber cortex), nails, hooves, 103

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.



104 horns, quills, and the epidermal layer of the skin. They have  $\alpha$ -helical  
105 tertiary structure and are rich in cystine residues ranging from 10 to  
106 22%. They are divided into two subfamilies, the type I acidic microfibril-  
107 lar component of ca. 40–50 kDa and the type II neutral/basic mem-  
108 branes of ca. 55–65 kDa (Marchisio, 2000).  $\beta$ -Keratins are found in  
109 reptiles and birds in scales, claws, beaks, feathers, and cuticle hair. They  
110 are difficult to extract and they do not form useful reconstituted struc-  
111 tures such as gels, films, coatings, and fibers suitable for medical appli-  
112 cations (wound healing, bone generation, hemostasis, and peripheral  
113 nerve repair (Ferraro et al., 2016; Hill, Brantley, & Van Dyke, 2010).  
114 They are rich in glycine, alanine, serine, and proline residues, but lack  
115 cysteine, thus the structure is stabilized only by hydrogen bonds. In  
116 feather keratin  $\beta$ -sheet and  $\alpha$ -helix occur in 1/3 and 2/3, respectively  
117 (Marchisio, 2000). Feather keratins from various birds are similar with  
118 molecular weight of about 10 kDa and cystine content of about 8%  
119 which is lower than that in keratin from nail and hair (Akhatar &  
120 Edwards, 1997). They are composed of about 20 different types which  
121 vary only by few amino acids (Saravanan, 2012). The basic and acid  
122 residues are positioned in the N- and C-terminal regions, whereas the  
123 hydrophobic residues are located in the central portion. The chemical  
124 or enzymatic process of feather keratin degradation is not uniform due  
125 to its complex hierarchical structure (Ferraro et al., 2016). Amorphous  
126 keratins, so-called  $\gamma$ -keratins are a part of the matrix. These are globu-  
127 lar proteins with high cystine content and molecular weight of about  
128 15 kDa.  $\gamma$ -Keratins occur in the external layer of the hair cuticle (Hill  
129 et al., 2010).

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

137 The physicochemical and biological features of keratins isolated  
138 from different sources are reflected in various functionalities of these  
139 proteins of which self-assembly is the most important (Dickerson et al.,  
140 2013). During thermodynamic equilibrium, the keratin molecules  
141 spontaneously arrange forming well-defined networks stabilized by  
142 noncovalent interactions. As a result of self-assembly, keratins can  
143 provide biomaterials for medicine, bioactive peptides, cosmetic formu-  
144 lations, and biodegradable films (Ferraro et al., 2016).

### 145 3 | SOLUBILIZATION OF KERATINS

#### 146 3.1 | Introduction

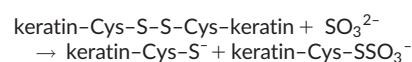
147 The method of processing raw keratin-based materials depends on the  
148 intended use of the product of keratin solubilization. These include  
149 thermal treatment in some organic solvents, reduction or oxidation of  
150 the disulfide bonds, alkaline, acid or enzymatic hydrolysis, various  
151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

#### 3.2 | Production of keratin isolates

154

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

168 Reduction and oxidation of disulfide bonds belong to the common  
169 methods for keratin isolation. Reduction of keratin involves use of 2-  
170 mercaptoethanol (Balaji et al., 2012; Fujii & Li, 2008; Kakkar, Madhan,  
171 & Shanmugam, 2014; Reichl, 2009; Schrooyen, Dijkstra, Oberthür,  
172 Bantjes, & Feijen, 2001; Tanabe, Okitsu, & Yamauchi, 2004; Yamauchi,  
173 Yamauchi, Kusunoki, Kohda, & Konishi, 1996), dithiothreitol (DTT),  
174 dithioerythritol (Vasconcelos et al., 2008; Yang, Zhang, Yuan, & Cui,  
175 2009), thioglycolic acid (Hill et al., 2010; Zabashta, Kasprova,  
176 Senchurov, & Grabovskii, 2012), glutathione (Schrooyen, Dijkstra,  
177 Oberthür, Bantjes, & Feijen, 2000), salts of hydrocyanic acid (Arai,  
178 Sakamoto, Naito, & Takahashi, 1989), bisulfites (Tonin et al., 2007), and  
179 *m*-bisulphites (Aluigi et al., 2007; Vasconcelos et al., 2008) to solubilize  
180 the protein. Many keratins can remain trapped within the protective  
181 structure, and usually a hydrogen-bond breaking agent, such as urea,  
182 thiourea, transition metal hydroxides, surfactants, and combinations  
183 thereof, are included in the extractant to unfold or denature the protein  
184 (Torchinsky, 1981). Aqueous solutions of tris(hydroxymethyl)amino-  
185 methane in concentrations between 0.1 and 1.0 M, and urea solutions  
186 0.1–10 M are used (Schrooyen et al., 2000). The keratin solution is  
187 dialyzed to remove the reagents. During dialysis, extensive protein  
188 aggregation may occur but is often prevented by addition of sodium  
189 dodecylsulfate (SDS) (Schrooyen et al., 2001). Upon reduction, the  
190 disulfide bonds are broken to give cysteine thiol (reduced keratin) and  
191 cysteine-S-sulphonate (Bunte salt) residues:



192 where keratin-Cys-S- is the reduced keratin and keratin-Cys-SSO<sub>3</sub><sup>-</sup> is  
193 the Bunte salt (Maclaren & Milligan, 1995). If keratins are extracted by  
194 reduction, the resulting products are referred to as kerateines which  
195 are less polar, less soluble in water, but more stable in acidic and alka-  
196 line solutions. They can re-cross-link, and remain in vivo for weeks to  
197 months longer than the oxidized derivatives (Hill et al., 2010).

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

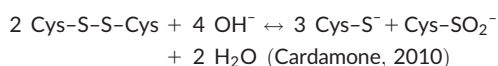




203 These keratoses are hygroscopic, water soluble, nondisulfide  
204 cross-linkable, and degrade relatively quickly in vivo in days to weeks  
205 (Hill et al., 2010).

### 206 3.3 | Chemical hydrolysis

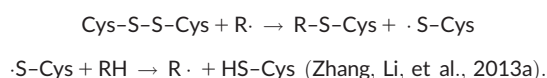
207 During chemical hydrolysis, some amino acids are lost (Zhang, Li, et al.,  
208 2013). Keratins can be easily solubilized by hydrolysis in strong acids or  
209 alkalis, but they cannot be recovered except as amino acids or peptides,  
210 peptones, and proteoses, the properties of which differ significantly  
211 from those of the native keratin. The thermo-chemical treatment of  
212 keratinous materials with alkali leads to degradation of asparagine,  
213 arginine, serine, threonine, and glutamine (Chojnacka et al., 2011). The  
214 solubilization of keratin wool at temperatures above 70°C in a pH  
215 range of 9–11 for 4–12 hr in the presence of excess alkali can cause  
216 conversion of disulfide groups to cystyl residues (CysS<sup>-</sup>):



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

227 Keratins can also be solubilized in alkaline solutions of metallic sul-  
228 fides. These reagents are generally used in cosmetic depilatories and  
229 removal of hair from hides in the tanning industry (Jones & Mecham,  
230 1948). Furthermore, alkaline hydrolysis with prolonged exposure at ele-  
231 vated temperature produces low molecular weight peptide fragments  
232 with poor mechanical properties. This product has limited biomedical  
233 application (Smith, Blanchard, & Lankford, 1994).

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



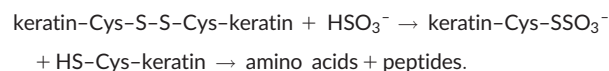
244 The degree of acid hydrolysis of keratin ranges from about 33 to  
245 46% (Karamać et al., 2005; Zhang, Li, et al., 2013). Acid-derived keratin  
246 hydrolysates have higher glass transition and lower decomposition

temperatures than pristine wool fibers (Katoh, Shibayama, Tanabe, & 247  
Yamauchi, 2004; Vasconcelos et al., 2008). They are nontoxic and bio- 248  
compatible and therefore can have potential application as biomaterials 249  
for wound healing and drug delivery. During acid hydrolysis of wool 250  
keratin, most of the hydrogen bonds are broken down which results in 251  
the amorphous structure of wool keratin polypeptides (Tung & Daoud, 252  
2009). Hence the content of both  $\alpha$ -helix and  $\beta$ -sheet structures in 253  
wool keratin are decreased as the total crystallinity of wool is the sum 254  
of  $\alpha$ - and  $\beta$ -crystallinity (Cao & Billows, 1999). The products of acid 255  
hydrolysis are more amorphous keratin polypeptides than alkaline- 256  
derived keratin hydrolysates (Zhang, Li, et al., 2013). 257

The hydrothermal methods for obtaining soluble keratin are expen- 258  
sive and destroy certain amino acids, for example, lysine, methionine, 259  
and tryptophan (Grazziotin, Pimentel, De Jong, & Brandelli, 2006). They 260  
result in products with poor digestibility and variable nutritional quality 261  
(Chojnacka et al., 2011). These processes are performed at 100–150°C 262  
and  $1.5 \times 10^5$  Pa (Grazziotin et al., 2006) and alkali or acid are often 263  
added. These hydrolysates have been used in feeding of poultry, rain- 264  
bow trout, shrimp, and salmon after supplementation with essential 265  
amino acids (Bertsch & Coello, 2005). 266

### 267 3.4 | Enzymatic hydrolysis

The enzymatic and/or microbiological methods for solubilization of ker- 268  
atin waste are cheap and run under mild conditions (Chojnacka et al., 269  
2011). These methods are an alternative to environmentally harmful 270  
chemical methods used most often in keratin isolation. Keratinases are 271  
extracellular serine proteases or metalloproteases produced by bacte- 272  
ria, actinomycetes, and fungi (Brandelli, 2008). The characteristics of 273  
keratinases produced by some microorganisms are shown in Table 2. 274  
These enzymes convert insoluble keratin to feedstuffs, fertilizers, and 275  
films, and also materials suitable for cosmetic and pharmaceutical appli- 276  
cations (Brandelli, Daroit, & Riffel, 2010). The mechanism of microbial 277  
keratinolysis is not completely known. The process of keratin degrada- 278  
tion proposed by Kunert (1976) is for dermatophytes and consists of 279  
sulfitolysis and proteolysis: 280



In the first stage, disulfide bonds are disrupted by sulfite produced 281  
by the fungus which leads to protein denaturation (Kunert, 1976) and 282  
proteolysis by endopeptidases. On the other hand, Yamamura, Morita, 283  
Hasan, Yokoyama, and Tamiya (2002) proposed a two-stage process of 284  
keratin degradation involving disulfide reductase and serine protease 285  
produced by *Stenotrophomonas* sp. D-1 from deer fur. Keratin reduced 286  
by disulfide reductase is hydrolyzed by protease to amino acids and 287  
peptides. Some bacteria, actinomycetes, keratinophilic fungi, and larvae 288  
of the common clothes moth (*Tineola bisselliella* Hummel) use native 289  
keratin as the sole source of carbon, nitrogen, sulfur, and energy 290  
(Kornitowicz-Kowalska & Bohacz, 2011). *Bacillus licheniformis*, *Bacillus* 291  
*pumilus*, *Bacillus cereus*, and *Bacillus subtilis*, and *Stenotrophomonas* sp., 292  
*Fervidobacterium pannavorans*, and *Fervidobacterium islandicum* were 293  
isolated from plumage and bird feathers, and fermented feather waste 294

TABLE 2 Characteristic of keratinases from some microorganisms

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

AQ7

295 (Burt & Ichida, 1999; Ichida et al., 2001; Williams & Shih, 1989).  
 296 Keratinolytic species of actinomycetes, particularly from the genus  
 297 *Streptomyces*, and some species from *Thermoactinomyces* occur in  
 298 feathers, hairs, nails, and horns. The keratinophilic fungi live in the soil,  
 299 birds, mammals, avian nests, bird plumage, mammalian hair, communal  
 300 waste water, waste sediments, communal waste, and polluted water.  
 301 They are represented by dermatophytes (some species of *Trichophyton*  
 302 and *Microsporum*), and two genera: *Chryso sporium* and *Myceliophthora*  
 303 (Korniłowicz-Kowalska & Bohacz, 2011).

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

## 310 4 | BIOACTIVE PROPERTIES OF KERATIN 311 PRODUCTS

### 312 4.1 | Introduction

313 Hydrolyzed proteins from many sources such as milk casein, soybean,  
 314 rice bran, quinoa seed protein, canola, egg yolk protein, and muscle  
 315 proteins have been reported to be sources of biologically active pep-  
 316 tides (Gómez-Guillén et al., 2011) (Table 3). These peptides, sequences  
 317 of 2–30 amino acids, are inactive in the parent protein and can be  
 318 released during gastrointestinal digestion, enzymatic processing or  
 319 microbial fermentation (Di Bernardini et al., 2011; Ferraro et al., 2016;

Gómez-Guillén et al., 2011). After liberation, they display biological  
 320 activities, for example, antioxidant, ACE inhibitory, and antimicrobial.  
 321 Keratins have also been shown to be a source of bioactive peptides by  
 322 Ferraro et al. (2016) and Lasekan, Bakar, and Hashim (2013).  
 323

### 324 4.2 | Antioxidant activity

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

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 Ala-Tyr-Phe-Tyr-Pro-Glu-Leu Tyr-Gln-Lys-Phe-Pro-Gln-Tyr	Contreras et al. (2009)
Bovine $\alpha$ -lactalbumin	Ile-Asn-Tyr-Trp	Sadat et al. (2011)	Leu-Ala-His-Lys-Ala-Leu Trp-Leu-Ala-His-Lys Val-Gly-Ile-Asn-Tyr-Trp-Leu-Ala-His-Lys	Pihlanto-Leppälä et al. (1998) Pihlanto-Leppälä, Koskinen, Piilola, Tupasela, and Korhonen (2000)
Bovine $\beta$ -lactoglobulin	Phe-Asn-Pro-Thr-Gln Leu-Gln-Lys-Trp Leu-Asp-Thr-Asp-Tyr-Lys-Lys Val-Ala-Gly-Thr-Trp-Tyr Trp-Tyr-Ser-Leu	Contreras, Hernández-Ledesma, Amigo, Martín-Álvarez, and Recio (2011) Power et al. (2014) Zhang, Wu, Ling, and Lu (2013)	Ile-Ile-Ala-Glu-Lys Ile-Pro-Ala-Val-Phe-Lys Ala-Leu-Pro-Met-His-Ile-Arg	Power, Fernández, Norris, Riera, and FitzGerald (2014) Mullally, Meisel, and FitzGerald (1997)
Bovine skin gelatin	Gly-Pro-Hyp-Gly-Pro-Hyp-Gly-Pro-Hyp-Gly	Kim, Byun, Park, and Shahidi (2001) and Kim, Kim, Byun, Park, and Ito (2001)	Gly-Pro-Val Gly-Pro-Leu	Kim, Byun, et al. (2001) and Kim, Kim, et al. (2001)
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 Leu-Glu-Leu-His-Lys-Leu-Arg-Ser-Ser-His-Trp-Phe-Ser-Arg-Arg	Park, Jung, Nam, Shahidi, and Kim (2001)	-	-
Egg white protein	Ala-His Val-His-His Val-His-His-Ala-Asn-Glu-Asn	Tsuge, Eikawa, Nomura, Yamamoto, and Sugisawa (1991)	Arg-Ala-Asp-His-Pro-Phe-Leu Tyr-Ala-Glu-Glu-Arg-Tyr-Pro-Ile-Leu	Miguel, Recio, Gómez-Ruiz, Ramos, and Lopez-Fandino (2004)
Fish skin gelatin ( <i>Jonius belengerii</i> )	His-Gly-Pro-Leu-Gly-Pro-Leu	Mendis, Rajapakse, and Kim (2005)	-	-
Pacific codfish gelatin	-	-	Thr-Cys-Ser-Pro Thr-Gly-Gly-Gly-Asn-Val	Ngo et al. (2011)
Porcine actomyosin	Asp-Leu-Tyr-Ala Ser-Leu-Tyr-Ala Val-Trp	Arihara (2006)	-	-
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)



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

### 352 4.3 | ACE inhibitory activity

353 Antihypertensive peptides can lower blood pressure through inhibition  
354 ACE. Many years of research have been devoted to the synthesis of  
355 ACE inhibitors used widely for therapeutic purposes to prevent hyper-  
356 tension (Gómez-Guillén et al., 2011). However, they have side effects  
357 such as coughing, poor taste, skin rashes, and angioneurotic edema  
358 (Atkinson & Robertson, 1979). Therefore, research has focused on  
359 identifying natural sources of ACE inhibitors with no side effects. Many  
360 antihypertensive/ACE inhibitory peptides have been isolated from  
361 casein, collagen, lactalbumin, myosin, ovalbumin, and serum albumin  
362 (Brandelli et al., 2015; Contreras, Carrón, Montero, Ramos, & Recio,  
363 2009; Pihlanto-Leppälä, Rokka, & Korhonen, 1998; Saiga et al., 2008).

364 Keratin has also been shown to be a source of ACE inhibitory pep-  
365 tides, although it has not been studied with regard to this activity as  
366 much as other proteins. ACE inhibitory activity has been shown in  
367 keratin hydrolysates from poultry feathers (Karamać et al., 2005). The  
368 activity of acid hydrolysates from keratin waste was lower (49.6% inhi-  
369 bition) than that of collagen hydrolysates (72.3% inhibition). Increase in  
370 ACE inhibitory activity with increase of the concentration of proline  
371 and hydroxyproline had been observed (Gómez-Guillén et al., 2011).  
372 Ohba et al. (2003) reported that the enzymatic hydrolysate of a mixture  
373 of horn and hoof also exhibited low ACE inhibitory activity. ACE inhi-  
374 bitory activity increased with decreasing molecular weight of hydroly-  
375 sates. The hydrolysates obtained from raw chicken feathers with the  
376 bacterium *Chryseobacterium* sp. kr6 also had ACE inhibitory activity  
377 (Fontoura et al., 2014). The keratin hydrolysates were able to inhibit  
378 65% ACE activity and was comparable to ACE inhibitory activity of  
379 soybean hydrolysates and milk protein hydrolysates. Enzyme specificity  
380 influences the biological activity of protein hydrolysates (Gómez-  
381 Guillén et al., 2011). High hydrophobic and aromatic amino acid  
382 residues content of 50–60% of the total amino acid residues is charac-  
383 teristic of keratins (Fontoura et al., 2014). Hydrophobic amino acids at  
384 the C-terminal tripeptide sequence contribute to the ACE inhibitory  
385 activity of peptides (Haque & Chand, 2008).

### 386 4.4 | Other activities

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

Gousterova et al. (2011) found that feather hydrolysate obtained 395  
using a mixed culture of *Thermoactinomyces* strains showed good activ- 396  
ity against plant pathogenic fungi *Fusarium solani*, *Fusarium oxysporum*, 397  
*Mucor* sp., and *Aspergillus niger*. It was suggested that the feather 398  
hydrolysate could be used as an alternative soil amendment for restor- 399  
ing contaminated soils, accelerating ryegrass growth, and improving the 400  
quality of agricultural soils. 401

Sundaram et al. (2015) observed antibacterial activity of keratin 402  
hydrolysate and keratin nanoparticles. The radius of inhibition zone for 403  
keratin hydrolysate against *Staphylococcus aureus* and *Escherichia coli* 404  
was 7.5 mm and 9 mm, respectively, at 100 µg/mL. They reported that 405  
the inhibition zone formulated for keratin nanoparticles was higher 406  
than that for keratin hydrolysates. 407

## 5 | CONCLUSIONS AND PERSPECTIVES 408

Keratin extracted from waste is a source of bioactive compounds for 409  
biological, food, and biomaterial applications. There is more information 410  
on the nonbiological functions of keratins than bioactive properties. 411  
Thus there is a need for further research devoted to selecting enzyme 412  
systems that convert keratin waste into bioactive peptides which could 413  
be used for formation of useful novel bioproducts. Literature shows an 414  
increasing number of reports on the use of various enzymes and 415  
conditions to obtain bioactive peptides from keratin. The peptides with 416  
antioxidant and antimicrobial activities could possibly be used as addi- 417  
tives in functional food products. Similarly, the fragments of keratin 418  
with ACE inhibitory and DPP IV inhibitory activity could be suitable for 419  
food and pharmaceutical applications. Therefore, advanced research on 420  
safety of these future bioproducts, maintenance of their bioactivity in 421  
humans mechanism of action, and industrial production are necessary. 422

### ORCID 423

Izabela Sinkiewicz  <http://orcid.org/0000-0001-5221-552X> 424

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