Collagen and Leather

REVIEW

Open Access

Developments for collagen hydrolysates as a multifunctional antioxidant in biomedical domains

Guiya Deng[1], Ke Huang[1], Xianchao Jiang[1], Kun Wang[1], Zihao Song[1], Yuchen Su[1], Chengming Li[1], Shuai Zhang[2], Shiqi Wang[3*] and Yaqin Huang[1*]

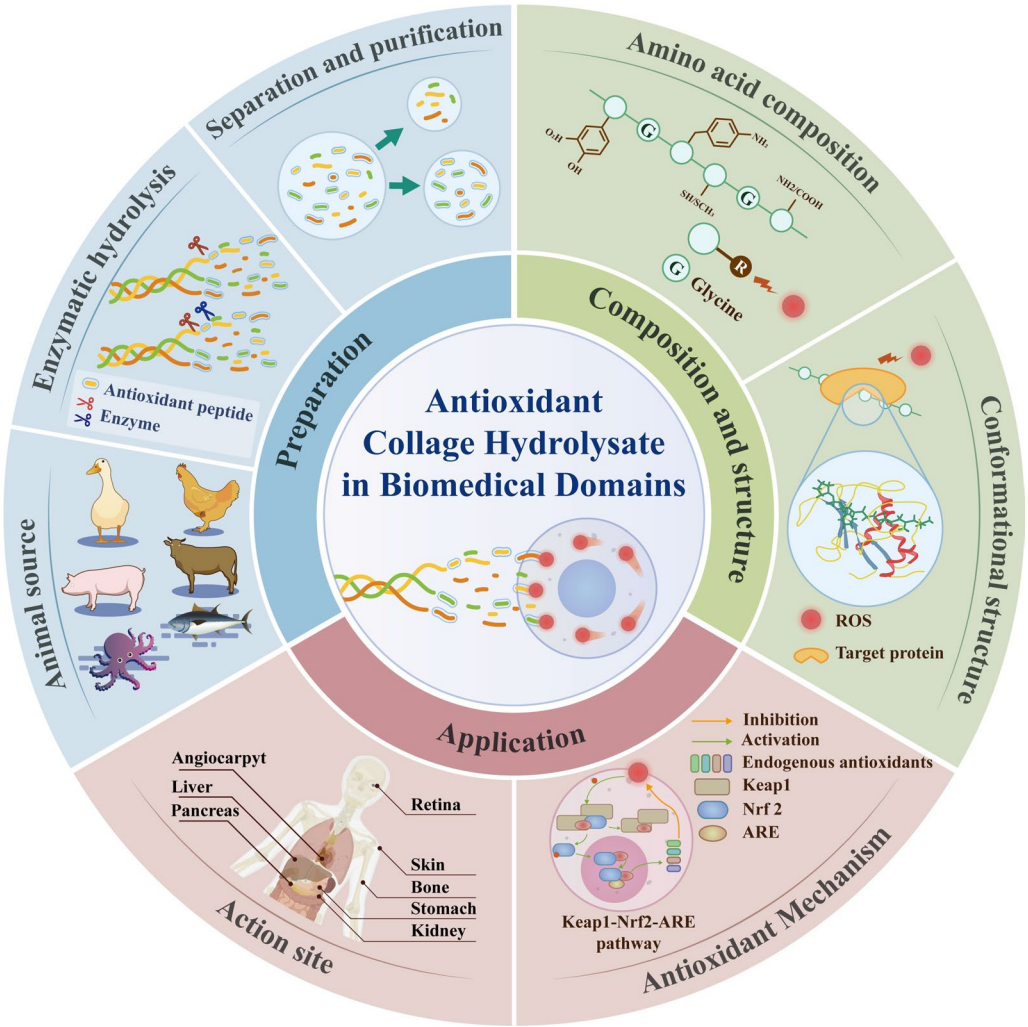
Abstract

Antioxidant collagen hydrolysates refers to the peptides mixture with antioxidant properties identified from hydrolyzed collagen. Due to its specific structural, biological and physicochemical properties, collagen hydrolysates have been explored as a multifunctional antioxidant in the biomedical field. In this review, we summarize recent advances in antioxidant collagen hydrolysates development. Initially, the preparation process of antioxidant collagen hydrolysates is introduced, including the production and separation methods. Then the effects and the mechanisms of amino acid composition and collagen peptide structure on the antioxidant activity of collagen hydrolysates are reviewed. Finally, the applications of antioxidant collagen hydrolysates in biomedical domains are summarized, with critical discussions about the advantages, current limitations and challenges to be resolved in the future.

Keywords Collagen hydrolysates, Antioxidant, Peptides, Biomedical applications

*Correspondence:
Shiqi Wang
shiqi.wang@helsinki.fi
Yaqin Huang
huangyq@mail.buct.edu.cn
Full list of author information is available at the end of the article

Graphical abstract



1 Introduction

Oxidative stress is caused by excessive production and accumulation of reactive oxygen species (ROS) in cells and tissues. ROS are usually produced in the cells of living organisms as a result of normal cellular metabolism and are fundamental to maintaining cellular homeostasis. Common ROS include superoxide radicals ($O_2^{\cdot -}$) and hydrogen peroxide (H_2O_2) [1]. While a basal concentration of ROS is indispensable for the manifestation of cellular functions, excessive levels of ROS cause damage to cellular macromolecules such as DNA, lipids, and proteins, eventually leading to necrosis and apoptotic cell death, which has harmful effects on tissues [2]. It explains why oxidative stress is detrimental to human health. When oxidative stress occurs, the balance of the redox system is damaged, resulting in the accumulation of a large number of intracellular oxidation products, which may be associated with accelerated aging, neurodegeneration, inflammation, tumor, diabetes, and other diseases [3–7].

Antioxidants, a class of compounds able to counteract oxidative stress and mitigate its effects on individuals' health, have gained enormous attention from the biomedical research community. A variety of endogenous antioxidants, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px), and the glutathione (GSH) system, are substances that counterbalance oxidants in the human body to resist oxidative damage [8, 9]. Once the endogenous antioxidant efficiency is insufficient to maintain the reduction–oxidation

homeostasis, the exogenous antioxidants play an important role in inhibiting oxidative stress. Exogenous antioxidants are divided into synthetic antioxidants and natural antioxidants. Natural antioxidants from animals and plants, compared with synthetic antioxidants, are generally considered to be more biocompatible and reliable in biomedical domains. Thus, practical and efficient natural antioxidants have gradually replaced the trend of synthetic antioxidants. In fact, after careful preclinical and clinical studies, some natural antioxidants, such as carotenoids, vitamins, natural flavonoids, and protein peptides, have now been proven to be potential therapeutic agents to prevent various human diseases, such as aging, and cardiovascular diseases [10]. Therefore, the discovery and development of new natural antioxidants will help against the harmful effects of oxidative stress under different pathological conditions.

Collagen is a well-known natural protein in animals with various essential biological, biochemical and biomedical functions [11]. Currently, researchers have identified 29 distinct types of collagens originating from biological tissues with significant differences in peptide sequences, structures, and functional properties [12]. Among these types, collagen types I, II and III are the most prevalent. The spatial structure of collagen consists of three α chains of peptides intertwining each other to form a right-handed superhelix [13]. The length of the helix and the composition of the non-helical region usually vary across different types of collagens [14]. Each polypeptide chain in procollagen has one N-terminal and one C-terminal propeptides, which shows the greatest sequence differences between species [15]. A central domain that forms the fibers in mature collagen has a characteristic amino acid sequence containing Gly-Xaa-Yaa repeats, where Xaa and Yaa could be any kind of amino acids [16]. Collagen peptides are inactive when folded in the native protein but exhibit various physiological functions once released [17]. Collagen hydrolysates could develop as a potential nutraceutical or valuable treatment agent obtained from collagen. Significantly, antioxidant collagen hydrolysates, as the important collagen derivatives, refers to the multiple peptides with antioxidation properties identified from collagen hydrolysates. Antioxidant collagen hydrolysates can be released from its precursors during food processing, gastric digestion and enzymatic hydrolysis through exogenous and endogenous proteases [18]. The past decade has witnessed a growing number of biomedical studies on collagen hydrolysates to alleviate diseases related to oxidative stress, such as hypertension, gastrointestinal disease and photoaging, by scavenging the ROS and boosting the inherent antioxidative system in the cells [19, 20]. Furthermore, antioxidant collagen hydrolysates can be extracted from the connective tissues of animals, such as skin, bone and fish scales, which are widely available and biologically safe. These advantages make it a promising candidate for biomedical antioxidation applications.

The increasing number of studies about antioxidant collagen hydrolysates has been published over the past decade. The reviews of collagen hydrolysates are mainly focused on the their rich biological activities for functional foods [21–23]. Herein, we aim to provide insights into the collagen hydrolysates as the multifunctional antioxidants to counteract oxidative stress in biomedical domains, with an emphasis on the physical–chemical properties and the applications of antioxidant collagen hydrolysates that are correlated with their properties in biomedical domains. Furthermore, the challenges and potential for increasing the pharmaceutical industrial use of antioxidant collagen hydrolysates are discussed.

2 Preparation of antioxidant collagen hydrolysates

Antioxidant collagen hydrolysates can be obtained from various animal species, varying according to their intrinsic characteristics, the extraction process, and specific hydrolysis conditions. The extraction of antioxidant collagen hydrolysates is divided into three main steps (Fig. 1). The first step is the pre-treatment of the raw material for purification: removal of lipids, miscellaneous proteins, and pigments. The second step, enzymatic hydrolysis of collagen, and it is crucial in the overall process of obtaining antioxidant collagen hydrolysates. The third step is to separate the fractions with higher antioxidant capacity from the obtained antioxidant collagen hydrolysates. In the following sections, we first discuss the collagen sources and summarize preparation methods of antioxidant collagen hydrolysates used in the biomedical domains.

2.1 Species as collagen sources

Collagen can be extracted from different connective tissues, such as skin, bone, and tendon. Different animal sources of collagen with multiple amino acid compositions determine the differences in intrinsic chemical or physical properties of collagen, which in turn affect the antioxidant activity of collagen hydrolysates [24, 25]. Examples of antioxidant collagen hydrolysates obtained from various animal sources in past five years and their potential use in biomedical domains are summarized in Table 1.

Skin and bone from porcine and bovine sources are widely used for obtaining collagen for industrial purposes. One of the major disadvantages of bovine collagen is that some people, especially children, are allergic to it, which limits its usage [35]. Just like the bovine source, the setback of zoonosis poses a risk of contamination, and

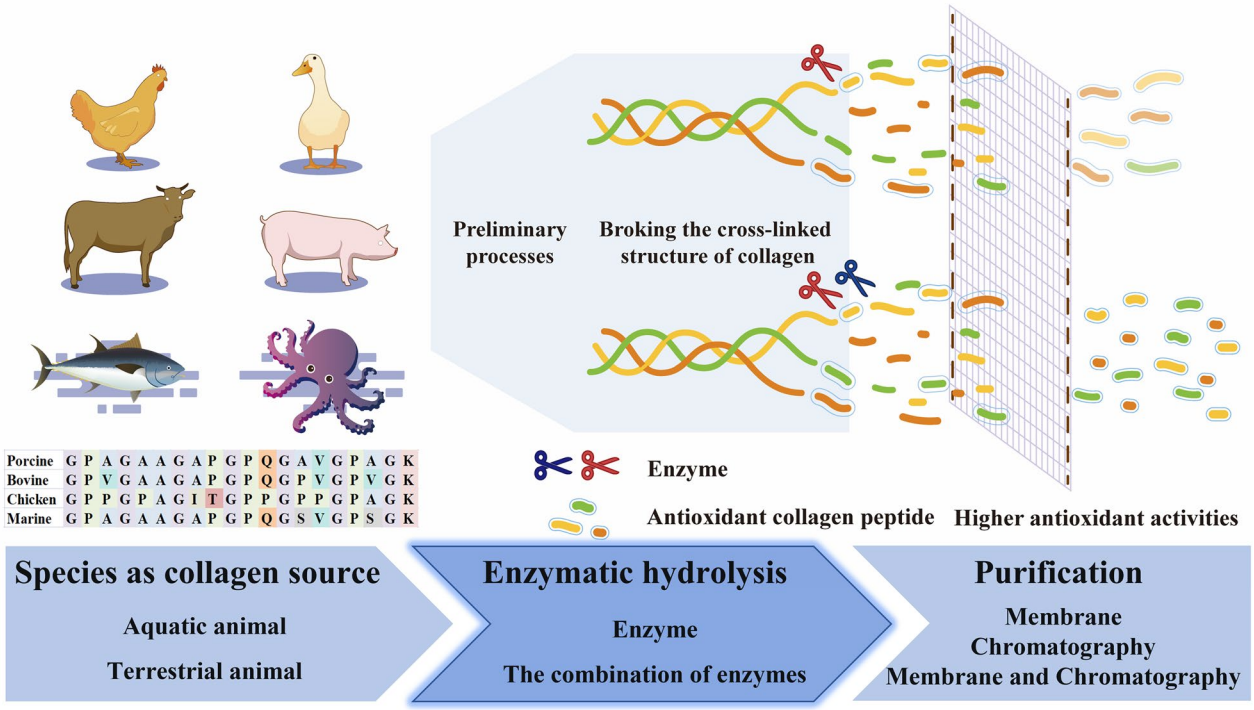


Fig. 1 Schematic representation of antioxidant collagen hydrolysates preparation process. The enzymatic hydrolysis of collagen is critical for the successful generation of antioxidant collagen hydrolysates

due to religious constraints, pigs are forbidden in certain counties and regions. Deer sinew, chicken bone, chicken skin, and chicken liver have also been discovered as raw materials for preparing antioxidant collagen hydrolysates used in medicine in recent years [30, 31, 36, 37].

Marine collagen hydrolysate has recently been reported as a novel natural antioxidant in biomedical applications as an alternative to conventional mammalian (such as bovine and porcine sources) collagen hydrolysates [38]. Aquatic collagen sources have many advantages over land animals, such as weak immunogenicity, biocompatibility, no religious and moral conflicts, and low chances of causing transmissible diseases. In the field of biomedicine, antioxidant collagen hydrolysates are mostly derived from aquatic fish, such as tilapia fish, crimson snapper, carp fish, siberian sturgeon, cypselurus melanurus, giant croaker, and cod [39–45]. During fish processing, the removal of collagen-containing materials (mainly skin, bones, and scales) could account for as much as 75% of the total catch weight generated after filleting [46]. Considering the biological activity, compared with fish bones, researchers have been more inclined to study the application of antioxidant collagen hydrolysates extracted from fish skin and scales in biomedical applications in recent years [47]. Another category of marine collagen falls under invertebrate animals. Antioxidant collagen hydrolysates isolated from invertebrate animals including squid [48], rhopilema hispidum, sea cucumber and cuttlefish, have been found with a variety of biomedical activities [34, 49, 50]. They can also serve as marine source of antioxidant collagen hydrolysates for medicinal use.

2.2 Pre-processing raw material to improve the quality of collagen hydrolysates

The main purpose of the pre-treatment of raw materials before extracting antioxidant collagen hydrolysates is to improve the quality of collagen hydrolysates obtained by removing impurities (such as lipids, pigments, and non-collagenous proteins) and breaking the cross-linked structure of collagen. The chemical hydrolysis of different raw materials is preceded by alkaline or acidic treatment, or both, each of which reports specific advantages. The alkaline pre-treatment removes unwanted products, such as non-collagenous proteins and pigments, and reduces the endogenous effects of proteases [51, 52]. After pre-treatment with alkali solutions, the cross-linked structure of collagen in the raw material was broken, resulting in a significant decrease in the molecular weight of collagen. This facilitates subsequent collagen dissolution in water to prepare antioxidant collagen hydrolysates. In this regard, two

Table 1 The molecular weight of antioxidant collagen hydrolysates isolated from different animal sources for mitigating oxidative stress

Animal Source	Molecular Weight (Da)	Antioxidant activity	Application	
Bullfrog (skin)	686–6511	DPPH radical scavenging[a]	Anti-hypertension	[26]
Takifugu Bimaculatus (skin)	< 1000	Increasing superoxide dismutase activity		[27]
		Increasing catalase activity		
Bigeye tuna (skin)	< 3000	DPPH radical scavenging	Anti-photoaging	[28]
		Hydroxyl radical scavenging[a]		
		Superoxide anion radical scavenging		
Bigeye tuna (bone)	< 3000	DPPH radical scavenging		[28]
		Hydroxyl radical scavenging		
		Superoxide anion radical scavenging[a]		
Skipjack tuna (skins)	< 3500	DPPH radical scavenging		[29]
Chicken (bone)	< 3000	Increasing superoxide dismutase activity		[30]
		Increasing catalase activity		
		Increasing GSH-Px activity		
		Decreasing MDA[b] content		
Deer (sinew)	5000–13000	ABTS radical scavenging[a]		[31]
Redlip Croaker (scales)	< 1000	DPPH radical scavenging	Prevent Liver Injury	[32]
		Hydroxyl radical scavenging		
Monkfish (swim bladder)	417.46	DPPH radical scavenging		[33]
	427.45	ABTS radical scavenging		
		Hydroxyl radical scavenging		
		Superoxide anion radical scavenging		
Cuttlefish (skin)	< 3000	DPPH radical scavenging		[34]
		ABTS radical scavenging		

[a] DPPH, ABTS, Hydroxyl and Superoxide anion radical scavenging: The common free radical scavenging capacity in vitro experiments to evaluate the antioxidant activity of collagen hydrolysates

[b] Malondialdehyde (MDA): One of the end products of lipid peroxidation

alkali solvents are reported extensively, i.e., NaOH and $Ca(OH)_2$. NaOH is preferred over $Ca(OH)_2$ because it causes comparatively more swelling to assist the subsequent chemical extraction process by promoting the mass transfer rate in a tissue matrix. Most of the researchers would choose NaOH as the raw materials pre-treatment reagent for preparing antioxidant collagen hydrolysates. For example, Cao et al. [30] pre-treated the chicken bone in 0.05 M NaOH to swell the bone, and remove non-collagen proteins, fat, and minerals. Nalinanon et al. [53] used NaOH initial pre-treatment and then butyl alcohol for removing fatty acids from raw materials of collagen during the pre-treatment stages. Regarding acid pre-treatment, Hong et al. [54] reported that formic acid can improve the hydrolysis of highly cross-linked collagen from waste hens and produce small collagen peptides from other aged vertebrate collagens. Organic acids (lactic, citric acid or a 1:1 mixture of both) could also be used as a pre-treatment reagent to remove residues (scales, bones, meat or fat) [55].

2.3 The design of the hydrolysis reaction to obtain antioxidant collagen hydrolysates

Hydrolysis of collagen is a crucial step in the whole process of obtaining antioxidant collagen hydrolysates. To date, designing suitable enzymatic hydrolysis reactions is the most effective way to hydrolyze the collagen to obtain the antioxidant as the catalytic proteolysis reactions is characterized by high specificity, selectivity, and efficiency. Alcalase, papain, and neutrase protease are often used as the efficient catalyst for enzymatic hydrolysis of collagen to obtain antioxidant collagen hydrolysates in biomedical research [30, 42]. Considering the variety of raw materials and the actual lab environment, researchers need to optimize the experimental conditions for enzymatic hydrolysis. For example, the optimal extraction conditions for a high antioxidant collagen hydrolysate from cuttlefish skin using alcalase were based on the single-factor analysis. A common process condition optimization method, Box-Behnken design, was used to optimize the operating variables such as temperature, pH, and enzyme quantity [34]. Moreover, a combination

of enzymes leverages the advantages of multiple enzymes, resulting in higher antioxidant activities of collagen hydrolysates. For example, Vijayan et al. obtained a collagen hydrolysate with high antioxidant activity by hydrolyzing acid-soluble collagen extracted from great hammerhead shark with the mixture of pepsin, papain, and protease at the optimum hydrolytic condition [51].

Bacterial collagenase have received much attentions due to its unique subunits, ability to bind collagen and open entangled polypeptide chains compared to ordinary proteases, and have variety of enzyme cleavage sites than other collagenase-type matrix metalloproteinases (MMPs) [56]. Kim et al. [57] studied and acquired the antioxidant collagen hydrolysate using non-pathogenic Bacillus collagenase-type protease to ameliorate age-associated sarcopenia. Bacillus cereus is a recognized safe microorganism with the ability to secrete collagenase with high activity, but the main problem is that the wild-type collagenase lacks stability and coexists in multiple isoenzyme forms, which may adversely affect the production process. Therefore, Fang's team [56, 58] constructed a heterologous expression system based on genetic engineering for Bacillus cereus to obtain stable collagenase for preparing the collagen hydrolysate with high antioxidant activity.

In addition to exploring new enzymes, microwave-assisted hydrolysis of collagen is another processing method for improving the production of antioxidant collagen hydrolysates. The conventional hydrolysis process is lengthy and usually insufficient. Microwave radiation penetrates into the protein molecule's interior, exposes the hidden enzymatic sites, and enables a more complete hydrolysis. Thus, microwave-assisted enzymatic hydrolysis of protein can significantly shorten the total hydrolysis time and increase the yield of the peptides by strengthening the movement and collision of molecules [59]. Moreover, microwave radiation can significantly increase the content and antioxidant activity of collagen hydrolysates with low molecular weights [60]. However, at high microwave power, excessive molecular collision will cause denaturation of protease and reduce the antioxidant activities of collagen hydrolysates [61, 62].

2.4 Separation of collagen hydrolysates with high antioxidant

After hydrolysis, the antioxidant collagen hydrolysates is a peptides mixture, including partially unhydrolyzed protein, polypeptides with different chain lengths, hydrophobicity and net charge, and free amino acids. Developing appropriate purification methods would help to evaluate the structure and activities of antioxidant collagen peptides more accurately. At present, the separation technology of antioxidant collagen hydrolysates in biomedical domains mainly include chromatography, membrane separation, and combinations of these technologies.

2.4.1 Ultrafiltration

The first step in the purification process of antioxidant collagen hydrolysates is usually ultrafiltration membrane separation, due to its advantage of simple operation, low energy consumption and environmental friendliness [63]. Furthermore, ultrafiltration is an effective purification method to obtain low molecular weight peptides from crude hydrolysates. Reportedly, these low molecular weight peptides (2–20 amino acids) are more biologically active than their larger polypeptide/proteins counterparts. For example, Norizah et al. [64] separated three antioxidant peptide components from chicken skin gelatin hydrolysate by using different ultrafiltration membranes (Mw = 10, 5, 2 kDa, respectively). The low molecular weight (Mw < 2 kDa) peptides showed better antioxidant properties than the original gelatin hydrolysate stock solution. Hong et al. [65] obtained the antioxidant collagen hydrolysate (Mw < 3 kDa) with collagenase inhibition effects comparable to vitamin C through ultrafiltration, which may play an important role in anti-aging activities. They proved that the degree of hydrolysis (DH) may significantly affect the molecular weight and the exposure of the terminal amino groups of the resulting product [65].

Nevertheless, many studies have shown that molecular weight was not directly correlated with antioxidant activity. Table 1 summarizes the molecular weights of some antioxidant collagen hydrolysates used in biomedical applications. For example, some antioxidant collagen peptides with approximate molecular weight, GRW(417.46 Da) and GPGPT(427.45 Da), showed different antioxidant capacities [33]. It has been also noted that some high molecular weight antioxidant collagen hydrolysates also demonstrated the potential to prevent or treat diseases associated with oxidative stress [31]. Although the molecular weight of antioxidant collagen hydrolysate from bigeye tuna bone (DH = 21.96 ± 0.16%) is greater than that from bigeye tuna skin (DH = 34.28 ± 0.44%), the bone collagen hydrolysate showed better anti-photoaging effects [28]. This can be explained by the differences in the amino acid sequence of the peptides rather than of DH [66, 67]. Although ultrafiltration is useful in separating antioxidant collagen hydrolysates with low molecular weight, it may cause a reduction in yield. Nowadays, more and more studies combine ultrafiltration with chromatography to separate collagen hydrolysates and obtain components with higher antioxidant activity [42].

2.4.2 Chromatography separation technology

Chromatography has played a key role in the purification of antioxidant collagen hydrolysates for decades. The principle of chromatography technology is based on the differences in the physical and chemical properties of the mixed peptide components, so that they are distributed differently in the fixed phase and the mobile phase, so as to achieve the purpose of separation. The common chromatographic separation technologies for separating and purifying the biomedical antioxidant collagen hydrolysates mainly include gel filtration chromatography (GFC), ion-exchange chromatography (IEC), and reverse-phase high-performance liquid chromatography (RP-HPLC), etc. [32, 42, 68] The separation principles, advantages and disadvantages of these common chromatographic separation techniques are shown in Table 2.

Gel filtration chromatography (GFC) is also known as molecular sieve or particle size exclusion chromatography. As mentioned in Table 2, the separation principle of GFC is based on the molecular size of peptides or proteins. The separation efficiency of this technology is mainly affected by many factors, such as packing type and the column volume. The method has the advantages of simple operation, no pollution, low cost, and has been widely used in the purification of antioxidant collagen hydrolysates. Generally, GFC is used to obtain low molecular weight peptides. Low molecular weight peptides have been found to exhibit potent antioxidant activity compared to larger peptides and proteins [23]. This is primarily due to their ability to easily enter into the oxidant/antioxidant system, allowing them to interact with reactive oxygen species (ROS) and terminate free radical chain reactions. It has been proved that the antioxidant activity of collagen hydrolysate obtained by gel filtration increase by two folds compared with the unseparated collagen hydrolysates, and the antioxidant activity of small molecular weight collagen hydrolysate obtained by gel filtration is better than that of the unseparated collagen hydrolysates in most cases [72].

The application of ion-exchange chromatography (IEC) in the separation, structure determination, and detection of proteins and peptides has been increased. IEC can separate and purify biologically active collagen peptides according to their net charges. By adjusting pH, the net charge of collagen peptides is tunable, thereby making them easily separated by IEC. For example, Banerjee et al. [73] separated and purified a collagen hydrolysate with high antioxidant properties from bovine Achilles tendon by IEC, which has the potential to act as an antioxidant in case of a free radical overload for human health. Furthermore, the combination of IEC and GFC has been used for the separation of collagen hydrolysates to improve antioxidant activity against fatigue caused by oxidative stress [74]. This is due to certain low molecular weight peptides containing charged residues such as Glu, Lys, and Arg have been shown to possess antioxidant and metal-chelating activity [75]. This metal-chelating ability is particularly important in preventing metal ion-induced oxidative stress. The composition of amino acids in peptides also play a significant role in their antioxidant activity. Peptides containing acidic and basic amino acids have been reported to exhibit excellent antioxidant properties [76]. These amino acids can contribute to the chelation of metal ions, scavenge free radicals, and inhibit oxidative damage. However, this technique has disadvantages, including cost-effectiveness, method complexity, and sensitivity toward pH and metal ions [77].

Reverse-phase high-performance liquid chromatography (RP-HPLC) is increasingly used in the field of biomedicine to purify antioxidant collagen hydrolysates. This technique separates peptide mixtures based on their differing hydrophobicities, with less hydrophobic components eluted first, followed by more hydrophobic fractions. In addition, peptides containing hydrophobic amino acids may enhance their solubility at the water–lipid interface, allowing for better interaction with free radicals located within cellular membranes [78]. This improved interaction helps in neutralizing oxidative

Table 2 Characteristics of the separation method to obtain antioxidant collagen hydrolysates

Separation method	Advantage	Disadvantage	Characteristics of antioxidant peptides
GFC	Gentle operating conditions Wide temperature range Maintains physicochemical properties	Uncharged and weak adsorption Affected by packing type and column volume	Low molecular weight peptide [69]
IEC	High adsorption capacity Gentle elution conditions High recovery rate High specificity	High cost Complicated process Sensitive to pH and metal ions	Peptides with acidic and basic amino acid residues [70]
RP-HPLC	High separation efficiency Reproducibility High analytical accuracy	Affected by pH and temperature Suitable for low molecular weight peptides	Peptides with hydrophobic amino acids [71]

species and protecting cellular components from damage. For example, Ding et al. [79] identified a major antioxidant collagen hydrolysate fraction with high antioxidant activity using RP-HPLC's elution profile. RP-HPLC has many advantages, including good stability, high separation efficiency, and wide applicability. Furthermore, RP-HPLC can separate the antioxidant collagen hydrolysates initially obtained by GFC or IEC to acquire peptide fractions with higher antioxidant activity [79, 80]. In addition, it is worth noting that the separation effect of RP-HPLC is affected by many factors, such as pH value, flow rate, and temperature. Therefore, it is necessary to repeat this step several times to ensure accurate sequence identification of different components of antioxidant collagen hydrolysates [81].

Low molecular weight peptides with specific amino acid sequences, containing charged and/or hydrophobic residues, have demonstrated potent antioxidant activity and are being explored for various applications in the field of oxidative stress and cellular protection [82]. Overall, the antioxidant activity of peptides is closely linked to their amino acid composition and structures. Combining the principles of these common separation techniques with the peptide characteristics could facilitate the preparation of antioxidant collagen peptides with higher activity.

3 Influence of composition and structure of collagen hydrolysates on antioxidation properties

The antioxidant activity of collagen hydrolysates containing peptides of different chain lengths or molecular weights is affected by the structure and amino acid composition of these peptides [83]. In the following sections, we discuss the structure–function relationship in antioxidant collagen hydrolysates at different levels: amino acid residues, primary peptide sequences, secondary and higher-level structures.

3.1 Effects of amino acid composition on the antioxidant activities of collagen hydrolysates

Collagen is a fibrous protein composed of amino acids. The most abundant amino acids found in collagen are glycine, proline, and hydroxyproline, which together account for approximately 50% of the total amino acid content in collagen [84]. Other amino acids in collagen include alanine, lysine and arginine, et al. It is important to note that the exact amino acid composition of collagen can vary depending on the specific collagen type determined by the animal source and animal tissue. Even through the basic composition, with glycine, proline and hydroxyproline being the major constituents, remains relatively consistent, different types of collagens may have different proportions of amino acids [85]. The amino acid composition of collagen significantly influences the bioactivity of peptides in antioxidant collagen hydrolysates [86]. Generally, amino acids residues mitigate oxidation by capturing free radicals as hydrogen donors, chelating oxidative metal ions, and inhibiting lipid peroxidation reactions, etc. The phenolic hydroxyl group of Tyr and the imidazole group of His can directly act as hydrogen donors to trap free radicals, thus achieving the effect of scavenging free radicals. Acidic (Asp and Glu) and basic (Lys) amino acid residues can form complexes with metal ions through their charged side chain groups, thereby chelating the metal ions. Hydrophobic amino acid residues (Leu, Val, Ala, Pro) increase antioxidant activities of peptides because their aliphatic hydrocarbon side chains may help to promote the interactions between the peptide and free radicals produced during the lipid peroxidation due to the improved solubility of peptides in the lipid phase. Furthermore, the sulfhydryl-containing amino acids (Cys) in collagen peptide can scavenge free radicals by providing hydrogen atoms that are very active in the thiol group. In addition, Met residues within collagen peptide chains were demonstrated to provide an active site for the scavenging of free radicals due to the S atom on the Met being oxidized to Met sulfoxide by supplying electrons [79].

Structural modifications may increase the collagen peptides' stability and enhance the bioactivity. For example, collagen hydrolysate chelates tend to possess more stable characteristics and higher antioxidant properties due to their unique structure formed by binding minerals with collagen peptides. However, most reported peptide-mineral chelates generally have a relatively low mineral-binding capacity. The amino acid residues with hydroxyl groups, such as threonine, serine, tyrosine, and other amino acid residues, can be phosphorylated, and phosphorylation can enhance the binding ability of collagen peptides to minerals [87]. Luo et al. [88] prepared collagen peptides from fish bones, phosphorylated collagen peptides and chelated them with calcium ions. The phosphorylation significantly improved the chelation degree of collagen peptides with calcium ions and enhanced the free radical scavenging capability. Although calcium-chelated collagen peptides were associated with higher absorption and bioavailability, and the exact mechanism of enhanced antioxidant activity needs to be further studied.

Nowadays, the amino acid composition of different animal sources as well as different types of collagens are available in some protein databases (For example, The Universal Protein Resource). Thus, analyzing the amino acid composition of collagen is helpful to select superior raw materials for preparing antioxidant collagen

hydrolysates. The controllable preparation of collagen hydrolysates could be achieved by utilizing enzyme-specific cleavage sites and the characteristics of separation techniques (Table 2), resulting in an increased probability for the exposure of effective amino acid residues, thereby improving the antioxidant properties of collagen hydrolysates.

3.2 Relationship between peptide structures and antioxidant activities in collagen hydrolysates

3.2.1 Peptide sequence

Generally, amino acid sequence analysis of peptides should not only identify the amino acid composition, including the type and number of amino acids, but also determine the arrangement of amino acids. Apart from the amino acid composition, the amino acid sequence is an important factor that contributes to the antioxidant activity of peptides. In other words, peptides with similar amino acid composition but different sequences do not necessarily exhibit similar activity. For example, the relative antioxidant activities of three tripeptides (GHH, HGH and HHG) are 1.089, 0.8318, and 0.3170, respectively [89]. Notably, the physicochemical characteristics of the residuals at the C- and N-termini affect the antioxidant activity of peptides. Previous studies have shown that the presence of C-terminal aromatic amino acids and N-terminal hydrophobic amino acids can significantly enhance the antioxidant activity of collagen peptides [90]. It is also found that Glu and Lys at the N-terminal of collagen peptide have potential antioxidant effects [74]. In addition, C-terminal Gly-Leu has been reported to play an important role in inhibiting intercellular MMP-1 activity and ROS production [91]. Moreover, collagen peptides with Tyr, Phe, Lys and Arg residues present at the C-terminus are preferred, considering their antioxidant contribution [92, 93].

Some key amino acid sequences of antioxidant collagen hydrolysates from different animal sources have been revealed (Table 3). The sequences can be used to explore new animal sources of antioxidant collagen hydrolysates. Shared peptide describes common amino acid sequences shared by several animals. In contrast, marker peptide is the amino acid sequence that are unique to a certain animal relative to other animals. For example, as shown in Table 3, Shi et al. [94] found that PAGPRGPA in the antioxidant collagen hydrolysate from three-spot seahorse can effectively attenuates ethanol-induced oxidative stress in Human hepatic LO2 cells. According to the

Table 3 The main shared or marker peptides from antioxidant collagen hydrolysate and their biomedical applications

Peptide sequence	Animal source	Shared peptide/ Marker peptide	Other animal species found in BLAST result	In vitro/In vivo	Application	
FTGML	Grass carp scale	Marker peptide	–	B16F10 melanoma cell	Skin diseases	[95]
PAGPRGPA	Three-spot seahorse	Shared peptide	Bos taurus Oncorhynchus mykiss Equus sp. Xenopus tropicalis	LO2 cell	Liver diseases	[94]
YGDEY	Tilapia fish	Shared peptide	Mus musculus Bos taurus	HepG2 cell		[39]
GPEGPMGLE	Redlip croaker	Shared peptide	Bos taurus Caenorhabditis elegans			[32]
EGPFGPEG		Shared peptide	Bos taurus Xenopus laevis			
FAGPPGGDGQPGAK	Salmo salar	Shared peptide	Caenorhabditis elegans Bos taurus Oncorhynchus mykiss Equus sp. Xenopus tropicalis Gallus gallus	(ApoE-/-) mouse model	Atherosclerosis	[52]
IAGPAGPRGPSGPA		Shared peptide	Caenorhabditis elegans Bos taurus Oncorhynchus mykiss Equus sp. Xenopus tropicalis Gallus gallus			

searching result of animal species containing this peptide in the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/), PAGPRGPA is the shared sequence in several animal species, such as Bos taurus, Oncorhynchus mykiss, Equus sp.and Xenopus tropicalis. Collagen hydrolysates containing this shared peptide extracted from these animal sources may also have the potential to alleviate ethanol-induced oxidative stress in Human hepatic LO2 cells. Conversely, FTGML is a bioactive oligopeptide with tyrosinase inhibitory activity derived from gelatin hydrolysate of grass carp scales, which can reduce melanin production in mouse B16F10 melanoma cells to prevent or relief melanoma. Through BLAST search, it was found that no common animal source shared this peptide with grass carp, indicating that this peptide is the marker peptide of grass carp. Therefore, sequence analysis using peptide database can help to reveal the relationship between animal origin and antioxidant collagen hydrolysates activity, and predict the antioxidant activity of collagen hydrolysate based on existing data (antioxidant peptides reported). In addition, these previously reported antioxidant collagen peptide sequences can be used for the artificial design and preparation of peptides with antioxidant activities. By manipulating single amino acid residues in the sequence, we can enhance the antioxidant properties of existing peptides and even evolve new sequences that are not existing in nature.

3.2.2 Conformational structures

Despite the extensive studies on the primary sequence of antioxidant collagen peptides, the investigation of how the secondary structures affect their antioxidant properties has not been well documented to our best knowledge. Nevertheless, spatial conformation plays an important role in the antioxidant activity effect [96]. Conformational changes induced by temperature, pH, and salt concentration may lead to the exposure of critical amino acid residuals, such as Cys, Tyr, and His, which exert antioxidant effects [97]. For example, Ding et al. [79] reported that the Met residue, on top of a collagen peptide clump and exposed to the environment, provided a precise active site for free radical scavenging, while another Met residue, which is inside the peptide clump, would hardly contact with the dissociated radical.

The secondary structure (α-helix, β-sheet, β-turn, and random coil) also affects the antioxidant properties of these peptides. Zhang et al. [87] speculated that the increase in antioxidant activity of collagen peptides after magnesium chelation may be related to the increase of β turn and the decrease of random coil, which enables the aromatic amino acids that can serve as proton/hydrogen donors to be directly exposed to the hydrophilic environment to remove free radicals. Besides, the appropriate changes in secondary structure could help to promote exposure to active groups in amino acids, so that the peptides could exhibit better free radical scavenging capacity [98].

Recently, structure–activity relationship studies using molecular docking simulation have been reported for the development of therapeutic antioxidant collagen peptides. It is a powerful technology to reveal the interaction between antioxidant collagen peptides and catalytic sites of target proteins associated with oxidative stress. Ma et al. [99] have been reported that LSGYGP from fish skin inhibited activities of MMPs associated with a classical antioxidant pathway by docking the active sites of MMP-1 and MMP-9. The site of interaction between antioxidant collagen peptide and target protein associated with oxidative stress is visible in the 3D structural perspective via the simulation, and thus providing crucial insights into the antioxidation mechanisms. The length of the peptide chain is closely related to the structure and amino acid composition of the peptide. Therefore, the study of the relationship between the length of peptide chain and antioxidant activity needs to consider the effects of both the structure and amino acid composition of the peptide. Molecular docking simulation might provide a new idea to solve this problem at the theoretical level.

In summary, based on the current studies, it is still difficult to make conclusive claims about how secondary and higher order structures of collagen peptides affect their antioxidant activity. Thus, more experimental research and simulation studies are needed in the future to advance the understanding in this field.

4 Antioxidant collagen hydrolysates for biomedical research

The research on antioxidant collagen hydrolysates in the biomedical field mainly aims to cure or attenuate various disorders and diseases caused by excessive oxidative stress. These disorders and diseases include oxidation-induced skin aging, osteoarthritis, osteoporosis, oxidation-related liver injury, atherosclerosis, metabolic syndrome (hypertension and type II diabetes), gastric ulceration, retina diseases, and acute kidney injury. As shown in Table 4, the common role of antioxidant collagen hydrolysates is to scavenge ROS, decrease lipid peroxidation, and increase cellular antioxidant enzyme activity to defend against oxidative stress by regulating major antioxidant pathways, such as NF-κB, Keap1-Nrf2-ARE, MAPK, and PI3K/AKT/mTOR pathways. In this section, we introduce the role of oxidative stress in several medical ailments, and how antioxidant collagen

Table 4 The molecular mechanisms of antioxidant collagen hydrolysates against oxidative stress in different diseases

Medical impairments	Species	In vitro/ In vivo models	Molecular mechanisms (Signaling pathways)	
Liver diseases	Three-spot seahorse	LO2 cell model of ethanol-induced liver injury in vitro	MAPK; Keap/Nrf2	[94]
	Tilapia fish	HepG2 cell model of ethanol-induced liver injury in vitro	MAPK; Akt; NF-κB	[39]
	Skate	HFD-induced obese mice in vivo	NF-κB; Nrf2;	[100]
Skin diseases	Skipjack tuna	UVB-irradiated HaCaT cell model in vitro	Nrf2	[101]
	Donkey	Hs68 cell in vitro	MAPK	[102]
	Grass carp scale	B16F10 melanoma cell in vitro	cAMP-PI3K/Akt; MAPK	[95]
Inflammatory bowel disease	Catfish	Caco-2 cell in vitro; DSS-induced acute colitis model in vivo	NF-κB	[103]

hydrolysates alleviates oxidative stress and contributes to the treatment.

4.1 Skin diseases

The skin is one of the largest organs of the human body covering the surface of the whole body. Skin aging and skin diseases associated with oxidative stress are common medical impairments in dermatology that are extensively investigated with antioxidant collagen hydrolysates.

Skin aging includes chronological and photo-aging, which is affected by internal and external factors. Chen et al. [40] have found that antioxidant collagen hydrolysates played an anti-chronological aging role by reducing the accumulation of peroxide products and improving the activity of antioxidant enzymes. Regarding photo-aging, the main cause is ROS generated by ultraviolet (UV) radiation that mediates the expression of matrix metalloproteinases (MMPs) and type-I pro-collagen through multiple signal pathways. Particularly, mitogen-activated protein kinase (MAPK) signaling pathway is involved, resulting in a wide range of fundamental cellular process variations, including cell growth, differentiation, survival, apoptosis, migration, inflammation, and response to environmental stresses. Antioxidant collagen hydrolysates inhibit the rapid phosphorylation of ERK, JNK, and p38 in the MAPK signaling pathway (Fig. 2A), thus decreases MMPs expression and protects the extracellular matrix (ECM) in the skin [99, 104]. Furthermore, as shown in Fig. 2B**,** the antioxidant collagen hydrolysate can reverse type I collagen reduction by activating the transforming growth factor β (TGF-β)/Smad signaling pathway [105, 106]. Although TGF-β/Smad pathways are not directly associated with oxidative stress, the increased collagen biosynthesis helps to recover ECM, and relieve skin damage. Additionally, the collagen hydrolysate could suppress UV-induced damages by down-regulating the pro-inflammatory NF-κB pathway [106]. The down-regulation led to less inflammatory cytokines production and reduced ROS level, which in turn attenuates the skin damage.

The biological effect of antioxidant collagen hydrolysates extracted from different sources has been evaluated by several in vitro studies. Collagen hydrolysate as a natural antioxidant was beneficial beyond UV-induced damage and could be protective against other oxidative skin damage, since it significantly reduced ROS generation [31, 62]. The anti-aging effects of collagen hydrolysates from different animal sources are different. Wang et al. [107] observed that hen skin collagen hydrolysate is superior to porcine, bovine, and tilapia skin collagen hydrolysates in the protection of UVA-induced damage in human dermal fibroblasts (HDF). Hen collagen hydrolysate improved viability and pro-collagen I production, alleviated the expression of apoptotic genes and reduced ROS production (Fig. 2C). The decline of ROS plays a role in photo-aging activities and is possibly via the suppression of MMPs production [108]. In normal physiological conditions, the formation of oxidants is balanced by efficient removal of antioxidative enzymes, including SOD, CAT, and GSH-Px. Tilapia fish skin collagen hydrolysates significantly reduced intercellular ROS generation and increased SOD activity in UVB-induced mouse embryonic fibroblasts (MEFs) [99]. SOD is the initial enzyme in the enzymatic defense system in vivo and converts superoxide radicals into H_2O_2, which can directly or indirectly damage skin cells and induce cell apoptosis or necrosis. A few studies have evaluated the protective effect of collagen hydrolysates with the high radical scavenging activity and lipid peroxidation inhibiting capability against H_2O_2 injury in human umbilical vein endothelial cells (HUVECs) by reducing the contents of ROS promoting and cell proliferation [42, 44]. Additionally, previous

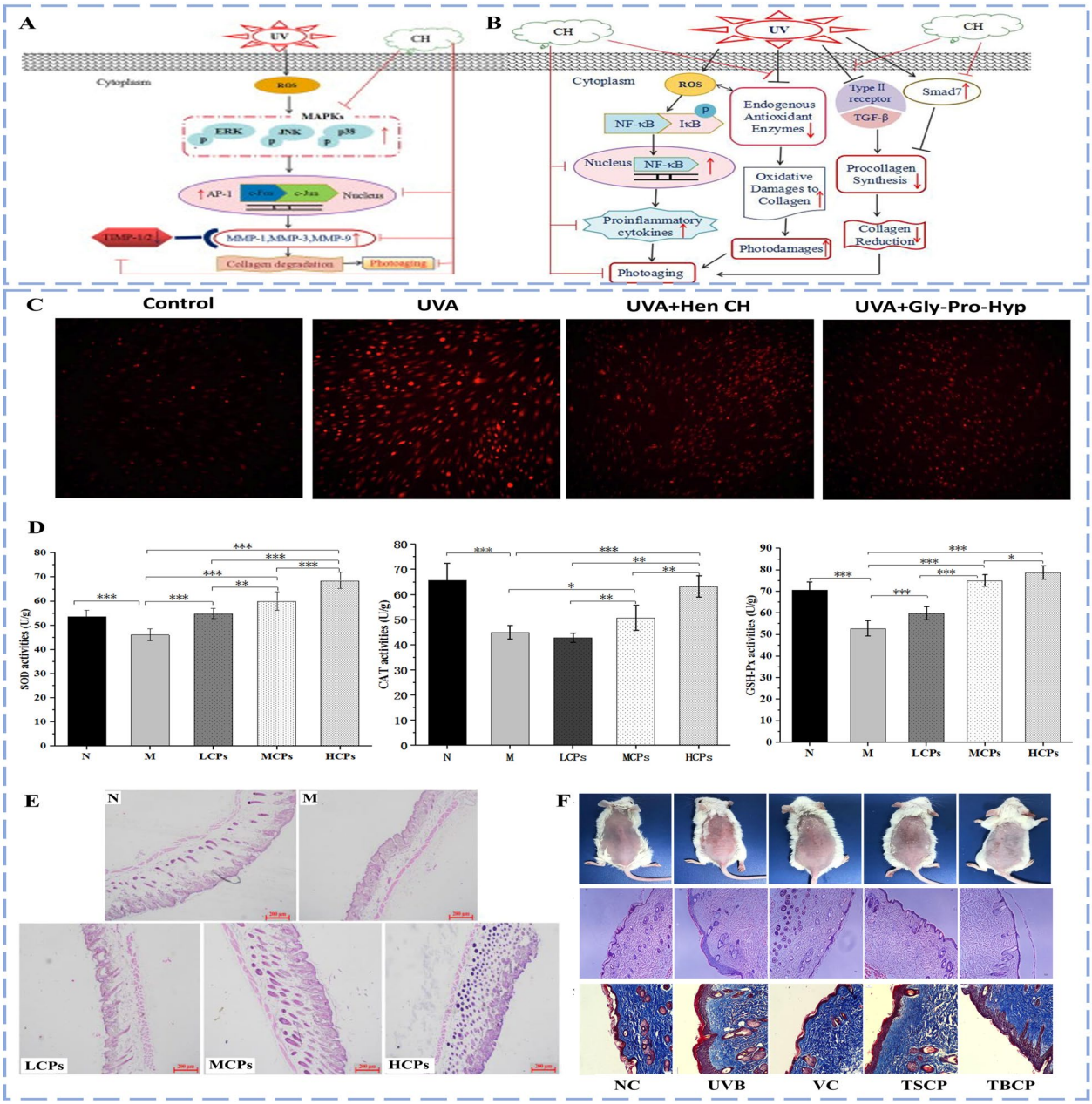


Fig. 2 Antioxidant collagen hydrolysates that suppress UV irradiation-induced damage to skin. The anti-photoaging mechanisms schematic of antioxidant collagen hydrolysates via, **A** suppressing MAPK signaling pathway and, **B** improving transforming growth factor-β/Smad signaling pathway. CH means collagen hydrolysates. Reproduced with permission [104, 106]. Copyright 2016, Elsevier. **C** In vitro evaluation of UVA-induced intracellular ROS by dihydroethidium staining. Representative images of DHE staining (magnification 200X). Hen collagen hydrolysates and corresponding peptides (Gly-Pro-Hyp) reduced ROS levels. Reproduced with permission [107]. Copyright 2019, Elsevier. **D** In vivo evaluation of endogenous antioxidants, including SOD, CAT and GSH-Px, treated by antioxidant collagen hydrolysates at different concentrations (N: the normal control group, M: the model group, LCPs: low concentration group, MCPs: medium concentration group and HCPs: high concentration group) [30]. Copyright 2022, The Author(s), under the terms of the Creative Commons CC-BY license. **E** The histomorphology of UV-induced aged murine skin tissue by H&E staining [30]. Copyright 2022, The Author(s), under the terms of the Creative Commons CC-BY license. **F** In vivo evaluation of antioxidant collagen hydrolysates on mice skin repair, including mice dorsal skin digital photographs, H&E staining images, and Masson's trichrome staining images in different groups. (NC: the normal control group, UVB: the model group, VC: Vitamin C group, TSCP: the skin collagen hydrolysate group and TBCP: the bone collagen hydrolysate group) Reproduced with permission [28]. Copyright 2022, Elsevier

results indicated that H_2O_2 is produced during melanogenesis, leading to advanced oxidative stress in melanocytes. Hu et al. [95] demonstrated a strong correlation between antioxidant activity and anti-melanin in vitro and revealed that the grass carp scale collagen peptide (FTGML) reduced the involvement of H_2O_2 in melanin synthesis.

It is worth noting that the in vitro skin-protective potential observed in antioxidant collagen hydrolysates also applied in vivo. Antioxidant collagen hydrolysates ingestion can alleviate the UV-irradiation-induced oxidative stress in vivo, and significantly increase SOD, CAT and GSH-Px activity in skin tissues (Fig. 2D) [30]. The condition of aging skin in mice improved after oral administration of the antioxidant collagen hydrolysate, maintaining a smooth, orderly, and complete structure (Fig. 2E). The results demonstrated that the antioxidant collagen hydrolysate had significant protective effects on chronologically aged skin. Moreover, antioxidant collagen hydrolysates obtained from different animal tissues have different degrees of protection against skin photoaging in mice. Bone collagen hydrolysate exhibited better effectiveness than skin collagen hydrolysate in improving UVB-induced mice skin damage by inhibiting the activation of p-ERK and p-JNK proteins in photo-aging skin and down-regulating MMP-1 protein expression, thereby suppressing collagen degradation (Fig. 2F). Unlike bone collagen hydrolysate, skin collagen hydrolysate improved UVB-induced mice skin damage by inhibiting p38 activation and promoting TGF-β1 expression via TGF-β signaling pathway [28].

4.2 Cardiovascular diseases

4.2.1 Hypertension

Hypertension is one of the major risk factor associated with cardiovascular diseases such as myocardial infarction, heart failure, and vascular dementia. Oxidative stress caused by increased free radical generation or weakened antioxidant mechanism is closely related to the occurrence and progression of hypertension and damage to targeted organs [109]. Antioxidant therapy can lower blood pressure, protect endothelial cells, reduce vascular wall remodeling, improve vascular function, and slow down the development of hypertension and its complications. Angiotensin-I-converting enzyme (ACE) is a crucial enzyme or receptor that catalyzes the generation of potent vasopressor angiotensin II (ANG II), which is one of the strongest vasoconstrictors known in the renin angiotensin system. ANG II induces apoptosis by enhancing oxidative stress and inflammation, which is related to the excessive production of ROS, and the decrease of endothelial nitric oxide synthase (eNOS) expression [110]. This means that reducing ROS production and restoring NO synthesis may be an effective strategy against ANG II-induced endothelial injury.

The collagen peptide FNLRMQ extracted from Takifugu bimaculatus skin by Shuilin et al. could cause NO production by activating the Akt/Enos pathway, thereby affecting the cell dysfunction induced by ANG II. In addition, FNLRMQ can also promote the nuclear Nrf2 level, thereby activating the Nrf2-/HO-1 pathway and protecting human umbilical vein endothelial cells from damage. Nrf2 is the most important regulator of cellular antioxidant activity and mediates antioxidant responses to inflammation and oxidative stress [27]. Another study showed that the antioxidant collagen hydrolysate from blue mussel byssus exhibited the highest ACE inhibition potencies, thereby having the potential for the management of cardiovascular diseases [111]. Alemán et al. [48] demonstrated the presence of Leu residues in the antioxidant collagen peptide sequence (GPLGLLGFLGPLGLS), which appears to play an important role in their ACE-inhibitory activity. However, in vivo studies need to be carried out to validate these effects.

4.2.2 Atherosclerosis (AS)

AS is an underlying disease for most cardiovascular diseases, whose development is modulated by oxidative stress [112]. Oxidative endothelial dysfunction is a trigger of atherosclerosis [113]. Collagen hydrolysates (containing antioxidant peptides and anti-inflammatory peptides) showed a great potential for attenuating AS. For example, collagen hydrolysate from salmo salar skin could inhibit arterial intima thickening and plaques formation in vivo by regulating serum inflammatory cytokine levels (IL-6 and TNF-α), and oxidative stress (MDA and CAT) without any side effects (Fig. 3A) [52]. As shown in Fig. 3B, after oral administration of collagen hydrolysate, the lipid plaques in the aortic intima and the atherosclerotic plaques in thoracic arterial walls in mice were significantly inhibited, exhibiting a comparable effect with positive control aspirin. Subsequently, Hidehito et al. [114] first tried to determine the molecular mechanism of anti-atherosclerotic effect of collagen tripeptide. They claimed that collagen tripeptide may exert a protective effect on Human Aortic Endothelial Cells (HAECs) (Fig. 3C), at least in part, by recovering some genes that were inhibited by ROS treatment, such as the interleukin-3 receptor subunit alpha (IL3RA) (Fig. 3D), thereby slowing the development of atherosclerosis and other vascular dysfunctions. However, at this stage, the understanding of the effective action sites of collagen hydrolysates and the signaling pathways that stimulate gene expression is still incomplete. Therefore, further research on the structure–activity relationship of collagen hydrolysates is needed to better elucidate the molecular mechanism and potential

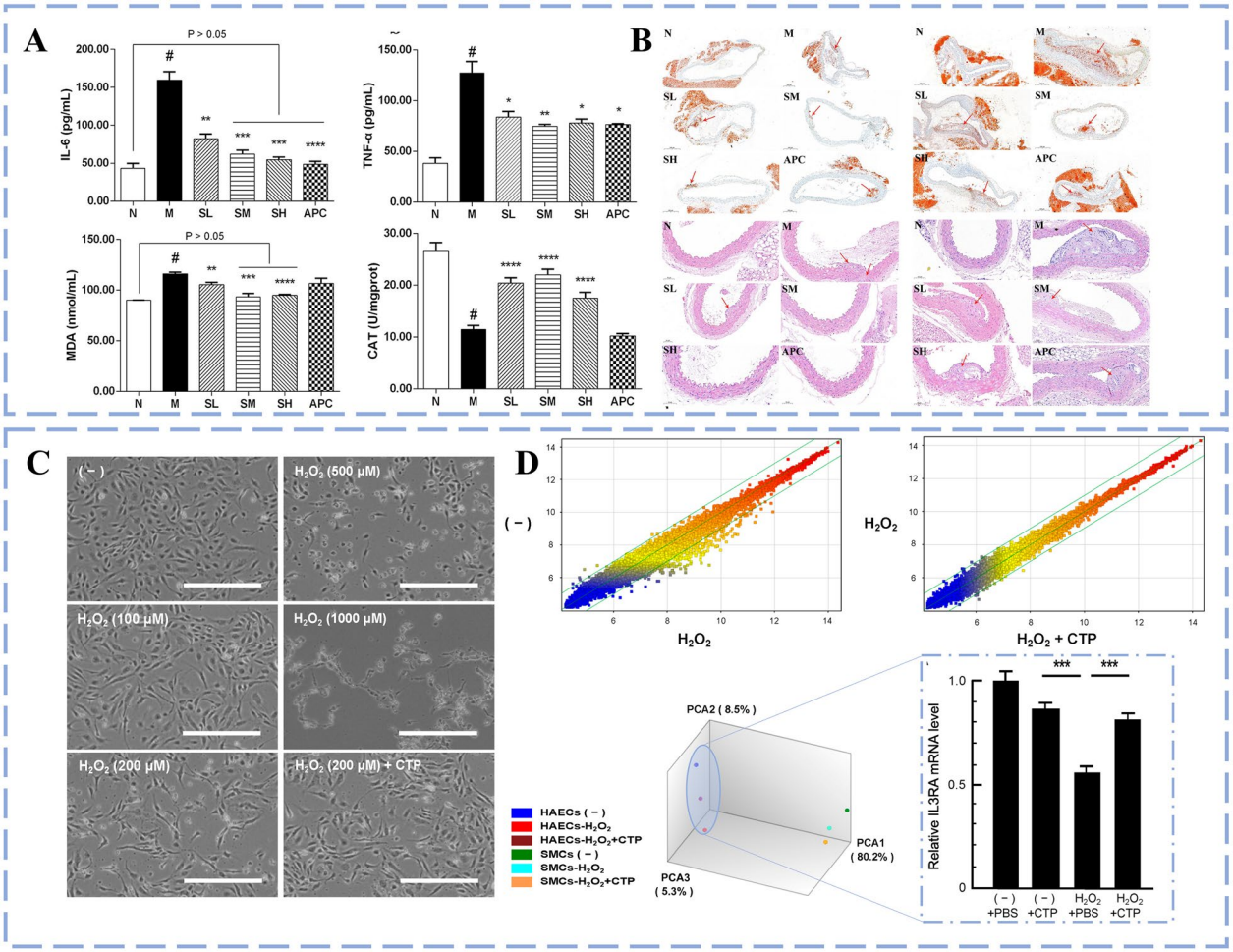


Fig. 3 Examples of antioxidant collagen hydrolysates that attenuates AS. **A** In vivo evaluation of anti-atherosclerotic effect of antioxidant collagen hydrolysates in different concentration groups, including IL-6, TNF-α, SOD, and MDA levels (N: the normal control group, M: the diseased model group, SL: low concentration group, SM: medium concentration group, SH: high concentration group and APC: Aspirin group). Reproduced with permission [52]. Copyright 2022, Elsevier. **B** In vivo evaluation of effects of antioxidant collagen hydrolysates on histopathological changes of aorta in mice in the early (14 weeks) and mid-stage (17 weeks) progression of atherosclerosis, including Oil Red-O staining images and H&E staining images in different concentration groups. Reproduced with permission [52]. Copyright 2022, Elsevier. **C** In vitro evaluation of anti-atherosclerotic effect of antioxidant collagen hydrolysates with the cell morphology digital photographs in different groups (The untreated group, H_2O_2-treared group, and H_2O_2 + CTP-treated group). [114] Copyright 2021, The Author(s), under the terms of the Creative Commons CC-BY license. **D** Gene expression profiling and global transcriptome analyses using principal component analysis (PCA) of the untreated, H_2O_2-treared, and H_2O_2 + CTP-treated HAECs to explore the partial mechanism of action in vitro, including the representative phase contrast of gene expression analysis images, global transcriptome analyses and IL3RA suppression level in different groups. (CTP: the antioxidant collagen hydrolysate group, and PBS: control group) [114]. Copyright 2021, The Author(s), under the terms of the Creative Commons CC-BY license

signaling pathways involved in the anti-atherosclerotic effect. Moreover, clinical trials are needed to further confirm the benefits of collagen hydrolysates for humans.

4.3 Liver diseases

Persistent oxidative stress in the liver is the leading cause of liver diseases such as fatty liver, liver injury, hepatitis, liver fibrosis, and liver cancer. At the molecular level, by analyzing the expression of differential genes, Du et al. found collagen hydrolysate could protect liver from ethyl carbamate-induced oxidative damage due to the modulation of ferroptosis and glutathione metabolism pathways (Fig. 4A) [34]. Furthermore, the antioxidant and protective effects of collagen hydrolysate against liver diseases are related to different signaling pathways. For instance, collagen hydrolysates have been reported to prevent acute liver injury via attenuation of oxidative stress by mediating the three classical signaling pathways, Keap1/Nrf2-ARE, PI3K/AKT, and MAPKs (Fig. 4B) [59].

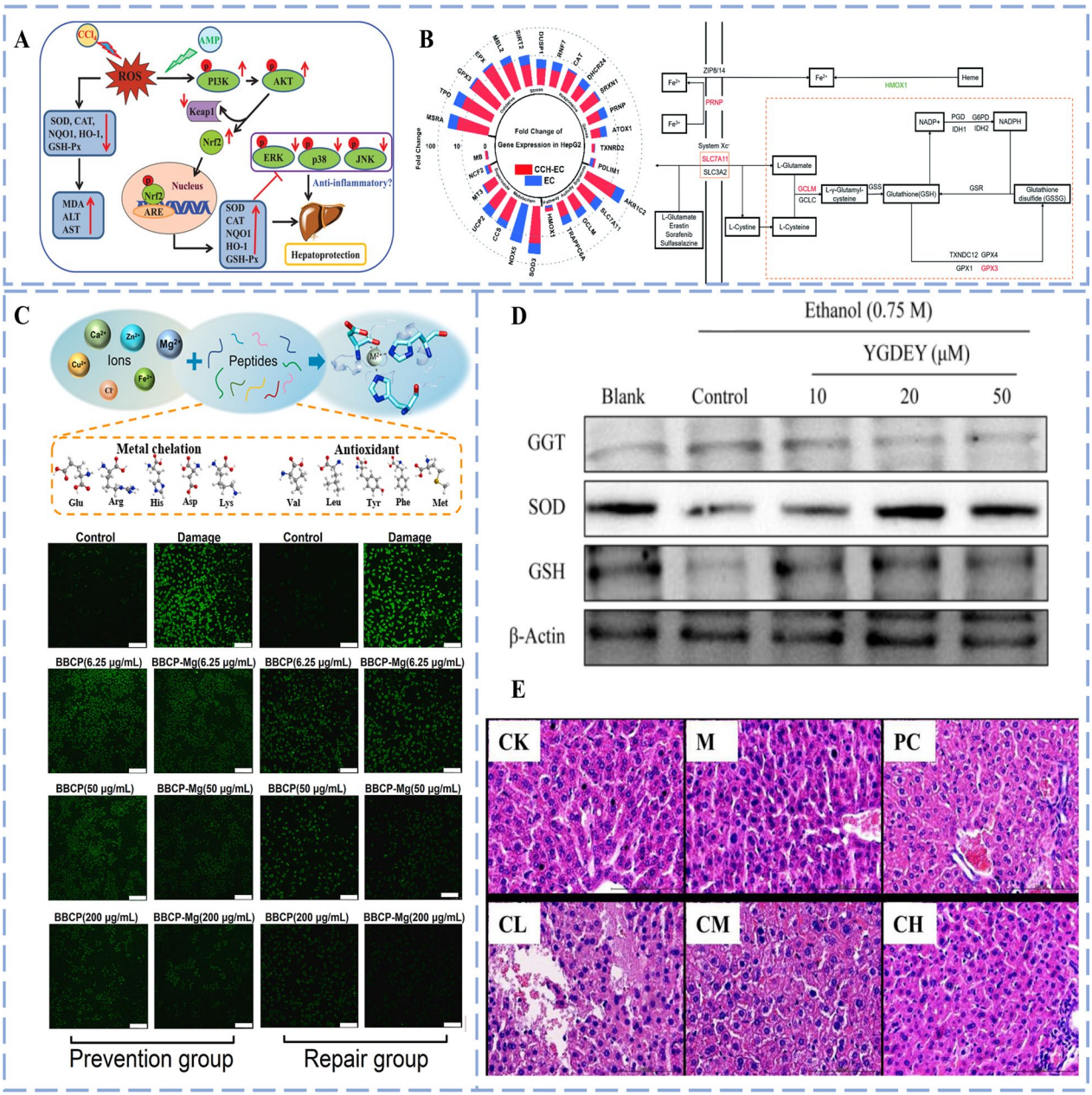


Fig. 4 Antioxidant collagen hydrolysates that prevents acute liver injury. The molecular mechanisms schematic of antioxidant collagen hydrolysates via, **A** the ferroptosis and glutathione metabolism pathway and **B** mediating the three classical signaling pathways [34]. Copyright 2021, The Author(s), under the terms of the Creative Commons CC-BY license. Reproduced with permission [59]. Copyright 2021, Wiley. **C** Schematic of metal ions chelating with antioxidant collagen hydrolysates. In vitro evaluation of different concentrations of antioxidant collagen hydrolysates and its magnesium chelates reduced intracellular ROS with DCFH-DA fluorescent probe images in different groups. Reproduced with permission [87]. Copyright 2022, Elsevier. **D** In vitro evaluation of intracellular endogenous antioxidants by Western Blot. [39] Copyright 2019, The Author(s), under the terms of the Creative Commons CC-BY license. **E** In vivo evaluation of antioxidant collagen hydrolysates against mice liver injuries induced by cyclophosphamide with H&E staining images. (CK: normal control group, M: diseased model group, PC: positive control (levamisole hydrochloride), CL: low concentration group, CM: medium concentration group, and CH: high concentration group) Histopathological changes in the liver (HE, 200×). The liver of mice in the CK group was intact, with uniformly distributed hepatic cords and uniformly colored and arranged hepatocytes. In the CH and PC groups, a small number of hepatocytes were swollen, and the hepatic cords were visible [115]. Copyright 2022, The Author(s), under the terms of the Creative Commons CC-BY license

In vitro studies have proved the protective effect of collagen hydrolysates on hepatocytes from environmental pollutant-induced oxidative stress. Notably, Zhang et al. [87] showed that the chelates of bovine collagen hydrolysate with Mg(II) had higher radical scavenging activities than the peptides alone, enhancing the intracellular antioxidant enzyme activities and GSH levels. These metal-chelated collagen hydrolysates demonstrated higher protective and reparative effects against H_2O_2-induced oxidative damage in HepG2 cells (Fig. 4C). This is explained by the fact that metal chelation could change the spatial conformation via the coordination with amino acid residues and enhance the biological activity of peptides. Moreover, an increasing number of studies have identified the efficient collagen peptide from antioxidant collagen hydrolysates, which helps to explore the molecular mechanisms of antioxidant activities. Taking the experiment of Wang et al. [32] as an example, they confirmed that redlip croaker-derived collagen peptides (GPEGPMGLE, EGPFGPEG, and GFIGPTE) with strong radical scavenging activities, lipid peroxidation inhibiting ability, and oxidation-damaged DNA protective activities, could downregulate intracellular ROS accumulation and improve cellular antioxidant enzyme (SOD, CAT, and GSH-Px) defense system. Similarly, YGDEY from tilapia fish skin collagen hydrolysate with hydroxyl radical scavenging activity has significant protective effects against alcohol-induced hepatotoxicity in HepG2 cells by regulating key intracellular antioxidants (SOD and GSH) (Fig. 4D) [39].

Regarding in vivo studies, Li et al. [116] assessed that tilapia skin collagen hydrolysate can alleviate liver injuries in aging mice induced by D-gal. Its mechanism of action is, at least partially, associated with the attenuation of oxidative stress and enhancement of immune function. Subsequently, it was proved that oral collagen hydrolysate administration enhanced the activity levels of antioxidant defense enzymes (SOD and CAT) and reduced lipid peroxidation levels in hepatic tissues [51]. In a similar research, collagen peptides from Micropterus salmoides skin also enhanced the repair of liver function damage induced by cyclophosphamide by significantly increasing the activity of SOD and decreasing MDA content in mice (Fig. 4E) [115]. Further clinical trials are needed to further confirm that the collagen hydrolysates have similar beneficial effects in human like increasing the activity level of antioxidant defense enzymes in vivo and reducing lipid peroxidation in hepatic tissues.

4.4 Other diseases

In addition to skin, cardiovascular, and liver diseases, several other diseases are also associated with oxidative damage, such as type II diabetes, osteoarthritis, degenerative retina diseases, and acute kidney injury. The therapeutic potential of antioxidant collagen hydrolysates in these diseases is also explored using in vitro or in vivo models, as specified below.

Regarding type II diabetes, it has been proved that oxidative stress and inflammatory responses are well-known contributors to increased insulin resistance, which is characteristic of disease development. Sivaraman et al. [117], for the first time, demonstrated that antioxidant collagen hydrolysates possessed hypoglycaemic effects in mice. After treatment with antioxidant collagen hydrolysates, the blood glucose level in diabetic mice decreased, with an increase in antioxidant enzymes SOD and CAT and decrease in MDA. The therapeutic effect was similar to metformin, a drug on the market that lowers blood glucose levels. Subsequently, Woo et al. [100] further confirmed that skate skin collagen hydrolysate might alleviate hepatogenic insulin resistance by increasing the antioxidative activity and suppressing the inflammatory response in the liver.

Osteoarthritis (OA), the most common degenerative joint disease worldwide found in elderly individuals, is a leading cause of physical disability. Although the etiology and underlying mechanisms of OA are complicated, many evidence has suggested that oxidative stress due to the chronic production of ROS plays an important role in the physiology and pathophysiology of OA. Therefore, developing antioxidants to alleviate oxidative stress in the joint becomes a promising therapeutic strategy for the treatment of OA. In an in vitro case study, collagen hydrolysate from chicken sternal cartilage was proven to be effective against H_2O_2-induced cellular oxidative damage in the knee joint of rat cells due to their antioxidative capability and rebalancing the catabolism and anabolism processes in arthrodial cartilage [81].

Oxidative stress perpetuates the cycle of destruction at the root of retina diseases. Application of collagen mimetic peptides reduced production of ROS and improve retinal pigment epithelium cells adherence and survival by repairing collagen damaged [118]. In H_2O_2 simulated in vitro oxidative stress microenvironment, carboxymethyl cellulose modified with collagen peptide (CMCC) showed antioxidant capacity, which can effectively inhibit ROS production in rat retinal endothelial cells. In addition, CMCC, as a drug carrier, significantly reduced the retinal oxidative stress level and potently recovered the activities of typical antioxidant enzymes, SOD and CAT in the retina of mice after loading anti-inflammatory drugs [119].

Acute kidney injury (AKI) is common in critically ill patients and can lead to chronic kidney disease when left untreated. Inflammation and oxidative stress play the key role in the development of AKI. In the nucleus, the

expression of antioxidant enzymes such as SOD, heme oxygenase-1 (HO-1), CAT, and GSH-Px is stimulated by the binding of the activated Nrf2 and the antioxidant response element [120]. A case study has reported that collagen hydrolysate from Acaudina molpadioides could activate the NF-κB and Nrf2 pathway through the PI3K/AKT pathway to protect kidney from damage caused by oxidative stress, which laid a foundation for the application of collagen hydrolysate in the prevention of AKI [61].

5 Summary and outlook

In summary, the present review provided an update on antioxidant collagen hydrolysates development in recent years. As elaborated in this review, antioxidant collagen hydrolysates, as collagen derivatives from natural resources, shows great potential for current and future biomedical applications. Previous studies demonstrated an increasing number of antioxidant collagen hydrolysates investigations against several diseases and disorders. These studies advanced the development of extraction and isolation techniques, revealed the structure–activity relationship between peptide and target protein catalytic sites, and explored the mechanisms of antioxidant collagen hydrolysates treating different diseases. As a result, we believe that such efforts will promote further interests in versatile antioxidant collagen hydrolysates.

Regarding future directions in this field, obtaining highly active antioxidant collagen hydrolysates is crucial to realize its therapeutic potential. This could be achieved by developing new enzymes for the hydrolysate preparation. By exposing key amino acid residues with antioxidant contribution in the final peptides, it is possible to enhance the antioxidation capability of the hydrolyzed collagen peptides obtained from the same raw material. Furthermore, we need a more comprehensive understanding of the structure–function relationship of collagen hydrolysates. In the future, the identification of antioxidant collagen hydrolysates should not only clarify the amino acid sequence, but also clarify the secondary structure. Due to the immense number of peptide combinations in antioxidant collagen hydrolysates, advances in the elaboration and constant update of databases regarding the peptides formed in proteolytic reactions are necessary. Prediction of possible products and the consequent biological activity is using computational simulation may improve the selection and production of new antioxidant collagen hydrolysates/peptides. In addition, it is highly recommended that researchers specify the species, and possibly the breed, of the selected collagen in their research, as this provides additional evidence for comparing the antioxidant potential of collagen hydrolysates from different animals.

For biomedical applications, the antioxidant effects reported in the in vivo and in vitro studies underscore the importance of collagen hydrolysates for the removal and defense against reactive substances such as ROS. Further research is needed to assess the efficacy and reproducibility in clinical trials in healthy subjects and patients with oxidative imbalance-related diseases, and to identify and develop preventive and therapeutic measures. Moreover, efforts should be dedicated to developing safety assessment methods to characterize the toxicological effects of antioxidant collagen hydrolysates before and during clinical trials to anticipate and prevent side effects.

Abbreviations

ROS	Reactive oxygen species
$O_2^{\bullet-}$	Superoxide radicals
H_2O_2	Hydrogen peroxide
SOD	Superoxide dismutase
CAT	Catalase
GSH-Px	Glutathione peroxidase
GSH	The glutathione
MMPs	Collagenase-type matrix metalloproteinases
DH	The degree of hydrolysis
GFC, IEC	Gel filtration chromatography, ion-exchange chromatography
RP-HPLC	Reverse-phase high-performance liquid chromatography
BLAST	Basic local alignment search tool
UV	Ultraviolet
MAPK	Mitogen-activated protein kinase
ECM	Extracellular matrix
TGF-β	Transforming growth factor β
HDF	Human dermal fibroblasts
MEFs	Mouse embryonic fibroblasts
HUVECs	Human umbilical vein endothelial cells
ACE	Angiotensin-I-converting enzyme
ANG II	Angiotensin II
eNOS	Endothelial nitric oxide synthase
AS	Atherosclerosis
HAECs	Human aortic endothelial cells
IL3RA	Interleukin-3 receptor subunit alpha
OA	Osteoarthritis
AKI	Acute kidney injury
HO-1	Heme oxygenase-1

Acknowledgements

Dr. S. Wang acknowledges the financial support from the Academy of Finland (grant no. 331106).

Author contributions

GD contributed to the conceptualization, investigation, formal analysis, visualization, and writing—original draft. KH contributed to investigation, formal analysis, and writing—original draft. XJ contributed to formal analysis and writing—original draft. KW contributed to the writing-original draft preparation and investigation. ZS contributed to the figure preparation. YS contributed to literature filtration. CL and SZ contributions to writing-review and editing of this review. SW, and YH made vital contributions to the conceptualization, methodology, and writing-review and editing of this review. All authors have read and agreed to the published version of the manuscript.

Funding

This work was supported by grants from the National Natural Science Foundation of China (No. 52242208).

Availability of data and materials

Not applicable.

Declarations

Competing interests

Yaqin Huang is a member of the editorial board of Collagen and Leather, and was not involved in the editorial review, or the decision to publish this article. All authors declare that there are no competing interests.

Author details

[1]Beijing Laboratory of Biomedical Materials, Key Laboratory of Biomedical Materials of Natural Macromolecules (Beijing University of Chemical Technology), Ministry of Education, Beijing University of Chemical Technology, Beijing 100029, People's Republic of China. [2]School of Pharmacy, Queen's University Belfast, Belfast BT9 7BL, UK. [3]Drug Research Program, Division of Pharmaceutical Chemistry and Technology, Faculty of Pharmacy, University of Helsinki, 00014 Helsinki, Finland.

Received: 1 July 2023 Revised: 14 August 2023 Accepted: 5 September 2023
Published online: 26 September 2023

References

1. Lennicke C, Cocheme HM. Redox metabolism: ROS as specific molecular regulators of cell signaling and function. Mol Cell. 2021;81(18):3691–707.
2. Senoner T, Dichtl W. Oxidative stress in cardiovascular diseases: still a therapeutic target? Nutrients. 2019;11(9):2090.
3. Chataigner M, Mortessagne P, Lucas C, Pallet V, Laye S, Mehaignerie A, et al. Dietary fish hydrolysate supplementation containing n-3 LC-PUFAs and peptides prevents short-term memory and stress response deficits in aged mice. Brain Behav Immun. 2021;91:716–30.
4. Andersen JK. Oxidative stress in neurodegeneration: cause or consequence? Nat Med. 2004;10(Suppl):S18-25.
5. Schwarz D, Lipoldova M, Reinecke H, Sohrabi Y. Targeting inflammation with collagen. Clin Transl Med. 2022;12(5):e831.
6. Liu T, Zou L, Ji X, Xiao G. Chicken skin-derived collagen peptides chelated zinc promotes zinc absorption and represses tumor growth and invasion in vivo by suppressing autophagy. Front Nutr. 2022;9:960926.
7. Niemann B, Rohrbach S, Miller MR, Newby DE, Fuster V, Kovacic JC. Oxidative stress and cardiovascular risk: obesity, diabetes, smoking, and pollution: part 3 of a 3-part series. J Am Coll Cardiol. 2017;70(2):230–51.
8. Forman HJ, Zhang H. Targeting oxidative stress in disease: promise and limitations of antioxidant therapy. Nat Rev Drug Discov. 2021;20(9):689–709.
9. Li H, Horke S, Forstermann U. Oxidative stress in vascular disease and its pharmacological prevention. Trends Pharmacol Sci. 2013;34(6):313–9.
10. Ramana KV, Reddy ABM, Majeti N, Singhal SS. Therapeutic potential of natural antioxidants. Oxid Med Cell Longev. 2018;2018:9471051.
11. Zheng M, Wang X, Chen Y, Yue O, Bai Z, Cui B, et al. A review of recent progress on collagen-based biomaterials. Adv Healthc Mater. 2022;12:e2202042.
12. Li C, Fu Y, Dai H, Wang Q, Gao R, Zhang Y. Recent progress in preventive effect of collagen peptides on photoaging skin and action mechanism. Food Sci Human Wellness. 2022;11(2):218–29.
13. Ramachandran GN. Structure of collagen. Nature. 1956;177:710–1.
14. Ricard-Blum S. The collagen family. Cold Spring Harb Perspect Biol. 2011;3(1):a004978.
15. Nuñez SM, Guzmán F, Valencia P, Almonacid S, Cárdenas C. Collagen as a source of bioactive peptides: a bioinformatics approach. Electron J Biotechnol. 2020;48(1):101–8.
16. Avila Rodríguez MI, Rodríguez Barroso LG, Sánchez ML. Collagen: a review on its sources and potential cosmetic applications. J Cosmet Dermatol. 2018;17(1):20–6.
17. Lin L, Regenstein JM, Lv S, Lu J, Jiang S. An overview of gelatin derived from aquatic animals: properties and modification. Trends Food Sci Technol. 2017;68:102–12.
18. Chen J, Li L, Yi R, Gao R, He J. Release kinetics of Tilapia scale collagen I peptides during tryptic hydrolysis. Food Hydrocolloids. 2018;77:931–6.
19. Himaya SW, Ryu B, Ngo DH, Kim SK. Peptide isolated from Japanese flounder skin gelatin protects against cellular oxidative damage. J Agric Food Chem. 2012;60(36):9112–9.
20. Zheng L, Wei H, Yu H, Xing Q, Zou Y, Zhou Y, et al. Fish skin gelatin hydrolysate production by ginger powder induces glutathione synthesis to prevent hydrogen peroxide induced intestinal oxidative stress via the Pept1-p62-Nrf2 cascade. J Agric Food Chem. 2018;66(44):11601–11.
21. Tang C, Zhou K, Zhu Y, Zhang W, Xie Y, Wang Z, et al. Collagen and its derivatives: from structure and properties to their applications in food industry. Food Hydrocolloids. 2022;131:107748.
22. Sila A, Bougatef A. Antioxidant peptides from marine by-products: isolation, identification and application in food systems. Rev J Funct Foods. 2016;21:10–26.
23. Hong H, Fan H, Chalamaiah M, Wu J. Preparation of low-molecular-weight, collagen hydrolysates (peptides): current progress, challenges, and future perspectives. Food Chem. 2019;301:125222.
24. Gauza-Wlodarczyk M, Kubisz L, Wlodarczyk D. Amino acid composition in determination of collagen origin and assessment of physical factors effects. Int J Biol Macromol. 2017;104(Pt A):987–91.
25. Chen J, Ahn T, Colon-Bernal ID, Kim J, Banaszak Holl MM. The relationship of collagen structural and compositional heterogeneity to tissue mechanical properties: a chemical perspective. ACS Nano. 2017;11(11):10665–71.
26. Huang YL, Li SH, Chuang TY, Chow CJ. In Vitro evaluation of antioxidant activities and inhibition of ACE activity by collagenase-treated hydrolysate derived from bullfrog skin. J Food Drug Anal. 2020. https://doi.org/10.38212/2224-6614.2191.
27. Cai S, Pan N, Xu M, Su Y, Qiao K, Chen B, et al. ACE inhibitory peptide from skin collagen hydrolysate of *Takifugu bimaculatus* as potential for protecting HUVECs injury. Mar Drugs. 2021;19(12):655.
28. Fu Y, Li C, Wang Q, Gao R, Cai X, Wang S, et al. The protective effect of collagen peptides from bigeye tuna (*Thunnus obesus*) skin and bone to attenuate UVB-induced photoaging via MAPK and TGF-β signaling pathways. J Funct Foods. 2022;93:105101.
29. Zhang S-Y, Zhao Y-Q, Wang Y-M, Yang X-R, Chi C-F, Wang B. Gelatins and antioxidant peptides from Skipjack tuna (*Katsuwonus pelamis*) skins: purification, characterization, and cytoprotection on ultraviolet-A injured human skin fibroblasts. Food Biosci. 2022;50:102138.
30. Cao C, Xiao Z, Tong H, Liu Y, Wu Y, Ge C. Oral intake of chicken bone collagen peptides anti-skin aging in mice by regulating collagen degradation and synthesis, inhibiting inflammation and activating lysosomes. Nutrients. 2022;14(8):1622.
31. Zhang H, Pan D, Dong Y, Su W, Su H, Wei X, et al. Transdermal permeation effect of collagen hydrolysates of deer sinew on mouse skin, ex vitro, and antioxidant activity, increased type I collagen secretion of percutaneous proteins in NIH/3T3 cells. J Cosmet Dermatol. 2020;19(2):519–28.
32. Wang WY, Zhao YQ, Zhao GX, Chi CF, Wang B. Antioxidant Peptides from collagen hydrolysate of redlip croaker (*Pseudosciaena polyactis*) scales: preparation, characterization, and cytoprotective effects on H_2O_2-damaged HepG2 cells. Mar Drugs. 2020;18(3):156.
33. Sheng Y, Wang WY, Wu MF, Wang YM, Zhu WY, Chi CF, et al. Eighteen novel bioactive peptides from monkfish (*Lophius litulon*) swim bladders: production, identification, antioxidant activity, and stability. Mar Drugs. 2023;21(3):169.
34. Du B, Deng G, Zaman F, Ma H, Li X, Chen J, et al. Antioxidant cuttlefish collagen hydrolysate against ethyl carbamate-induced oxidative damage. RSC Adv. 2021;11(4):2337–45.
35. Hori H, Hattori S, Inouye S, Kimura A, Irie S, Miyazawa H, et al. Analysis of the major epitope of the alpha2 chain of bovine type I collagen in children with bovine gelatin allergy. J Allergy Clin Immunol. 2002;110(4):652–7.
36. Sarbon NMHNK, Ahmad W. Angiotensin-I converting enzyme (ACE) inhibitory peptides from chicken skin gelatin hydrolysate and its antihypertensive effect in spontaneously hypertensive rats. Int Food Res J. 2019;26(3):903–11.
37. Chen PJ, Tseng JK, Lin YL, Wu YS, Hsiao YT, Chen JW, et al. Protective effects of functional chicken liver hydrolysates against liver fibrogenesis: antioxidation, anti-inflammation, and antifibrosis. J Agric Food Chem. 2017;65(24):4961–9.

38. Bourdon B, Casse F, Gruchy N, Cambier P, Leclercq S, Oddoux S, et al. Marine collagen hydrolysates promote collagen synthesis, viability and proliferation while downregulating the synthesis of pro-catabolic markers in human articular chondrocytes. Int J Mol Sci. 2021;22(7):3693.
39. Chen MF, Gong F, Zhang YY, Li C, Zhou C, Hong P, et al. Preventive effect of YGDEY from Tilapia fish skin gelatin hydrolysates against alcohol-induced damage in HepG2 cells through ROS-mediated signaling pathways. Nutrients. 2019;11(2):392.
40. Chen S, Yang Q, Chen X, Tian Y, Liu Z, Wang S. Bioactive peptides derived from crimson snapper and in vivo anti-aging effects on fat diet-induced high fat Drosophila melanogaster. Food Funct. 2020;11(1):524–33.
41. Zhang L, Zheng Y, Cheng X, Meng M, Luo Y, Li B. The anti-photoaging effect of antioxidant collagen peptides from silver carp (*Hypophthalmichthys molitrix*) skin is preferable to tea polyphenols and casein peptides. Food Funct. 2017;8(4):1698–707.
42. Sheng Y, Qiu YT, Wang YM, Chi CF, Wang B. Novel antioxidant collagen peptides of Siberian sturgeon (*Acipenserbaerii*) cartilages: the preparation, characterization, and cytoprotection of H2O2-damaged human umbilical vein endothelial cells (HUVECs). Mar Drugs. 2022;20(5):325.
43. Sivaraman K, Shanthi C. Role of fish collagen hydrolysate in attenuating inflammation-—an in vitro study. J Food Biochem. 2021;45(9):e13876.
44. Zheng J, Tian X, Xu B, Yuan F, Gong J, Yang Z. Collagen peptides from swim bladders of giant croaker (*Nibea japonica*) and their protective effects against H_2O_2-induced oxidative damage toward human umbilical vein endothelial cells. Mar Drugs. 2020;18(8):430.
45. Zhang K, Li B, Chen Q, Zhang Z, Zhao X, Hou H. Functional calcium binding peptides from pacific cod (*Gadus macrocephalus*) bone: calcium bioavailability enhancing activity and anti-osteoporosis effects in the ovariectomy-induced osteoporosis rat model. Nutrients. 2018;10(9):1325.
46. Ishak NH, Sarbon NM. A Review of protein hydrolysates and bioactive peptides deriving from wastes generated by fish processing. Food Bioprocess Technol. 2017;11(1):2–16.
47. Vijayan DK, Sreerekha PR, Dara PK, Ganesan B, Mathew S, Anandan R, et al. Antioxidant defense of fish collagen peptides attenuates oxidative stress in gastric mucosa of experimentally ulcer-induced rats. Cell Stress Chaperones. 2022;27(1):45–54.
48. Alemán A, Giménez B, Pérez-Santin E, Gómez-Guillén MC, Montero P. Contribution of Leu and Hyp residues to antioxidant and ACE-inhibitory activities of peptide sequences isolated from squid gelatin hydrolysate. Food Chem. 2011;125(2):334–41.
49. Ab Aziz NA, Salim N, Zarei M, Saari N, Yusoff FM. Extraction, anti-tyrosinase, and antioxidant activities of the collagen hydrolysate derived from Rhopilema hispidum. Prep Biochem Biotechnol. 2021;51(1):44–53.
50. Abedin MZ, Karim AA, Latiff AA, Gan CY, Ghazali FC, Barzideh Z, et al. Biochemical and radical-scavenging properties of sea cucumber (*Stichopus vastus*) collagen hydrolysates. Nat Prod Res. 2014;28(16):1302–5.
51. Vijayan DK, Perumcherry Raman S, Dara PK, Jacob RM, Mathew S, Rangasamy A, et al. In vivo anti-lipidemic and antioxidant potential of collagen peptides obtained from great hammerhead shark skin waste. J Food Sci Technol. 2022;59(3):1140–51.
52. Liu H, Yang Y, Liu Y, Cui L, Fu L, Li B. Various bioactive peptides in collagen hydrolysate from salmo salar skin and the combined inhibitory effects on atherosclerosis in vitro and in vivo. Food Res Int. 2022;157:111281.
53. Nalinanon S, Benjakul S, Kishimura H. Collagens from the skin of arabesque greenling (*Pleurogrammus azonus*) solubilized with the aid of acetic acid and pepsin from albacore tuna (*Thunnus alalunga*) stomach. J Sci Food Agric. 2010;90(9):1492–500.
54. Hong H, Roy BC, Chalamaiah M, Bruce HL, Wu J. Pretreatment with formic acid enhances the production of small peptides from highly cross-linked collagen of spent hens. Food Chem. 2018;258:174–80.
55. Danila E, Stan R, Kaya MA, Voicu G, Marin MM, MoroŞAn A, et al. Valorization of *Cyprinus Carpio* skin for biocompatible collagen hydrolysates with potential application in foods, cosmetics and pharmaceuticals. Waste Biomass Valoriz. 2021;13:917–28.
56. Song Y, Fu Y, Huang S, Liao L, Wu Q, Wang Y, et al. Identification and antioxidant activity of bovine bone collagen-derived novel peptides prepared by recombinant collagenase from *Bacillus cereus*. Food Chem. 2021;349:129143.
57. Kim JE, Kwon EY, Han Y. A collagen hydrolysate containing tripeptides ameliorates sarcopenia in middle-aged mice. Molecules. 2022;27(9):2718.
58. Wang Y, Sun Y, Wang X, Wang Y, Liao L, Zhang Y, et al. Novel antioxidant peptides from Yak bones collagen enhanced the capacities of antiaging and antioxidant in *Caenorhabditis elegans*. J Funct Foods. 2022;89:104933.
59. Li J, Li Y, Lin S, Zhao W, Chen Y, Jin H. Collagen peptides from Acaudina molpadioides prevent CCl4 -induced liver injury via Keap1/Nrf2-ARE, PI3K/AKT, and MAPKs pathways. J Food Sci. 2022;87(5):2185–96.
60. He L, Gao Y, Wang X, Han L, Yu Q, Shi H, et al. Ultrasonication promotes extraction of antioxidant peptides from oxhide gelatin by modifying collagen molecule structure. Ultrason Sonochem. 2021;78:105738.
61. Zhao W, Li J, Li Y, Chen Y, Jin H. Preventive effect of collagen peptides from Acaudina molpadioides on acute kidney injury through attenuation of oxidative stress and inflammation. Oxid Med Cell Longev. 2022;2022:8186838.
62. Li Y, Li J, Lin SJ, Yang ZS, Jin HX. Preparation of antioxidant peptide by microwave-assisted hydrolysis of collagen and its protective effect against H_2O_2-induced damage of RAW2647 cells. Mar Drugs. 2019;17(11):642.
63. Doyen A, Udenigwe CC, Mitchell PL, Marette A, Aluko RE, Bazinet L. Anti-diabetic and antihypertensive activities of two flaxseed protein hydrolysate fractions revealed following their simultaneous separation by electrodialysis with ultrafiltration membranes. Food Chem. 2014;145(feb. 15):66–76.
64. Mhd SN, Farah B, Howell NK. Purification and characterization of antioxidative peptides derived from chicken skin gelatin hydrolysate. Food Hydrocolloids. 2018;85:311–20.
65. Hong GP, Min SG, Jo YJ. Anti-oxidative and anti-aging activities of porcine by-product collagen hydrolysates produced by commercial proteases: effect of hydrolysis and ultrafiltration. Molecules. 2019;24(6):1104.
66. Ahmed R, Chun B-S. Subcritical water hydrolysis for the production of bioactive peptides from tuna skin collagen. J Supercrit Fluids. 2018;141:88–96.
67. Offengenden M, Chakrabarti S, Wu J. Chicken collagen hydrolysates differentially mediate anti-inflammatory activity and type I collagen synthesis on human dermal fibroblasts. Food Sci Human Wellness. 2018;7(2):138–47.
68. Cai WW, Hu XM, Wang YM, Chi CF, Wang B. Bioactive peptides from skipjack tuna cardiac arterial bulbs: preparation, identification, antioxidant activity, and stability against thermal, pH, and simulated gastrointestinal digestion treatments. Mar Drugs. 2022;20(10):626.
69. Agrawal H, Joshi R, Gupta M. Purification, identification and characterization of two novel antioxidant peptides from finger millet (*Eleusine coracana*) protein hydrolysate. Food Res Int. 2019;120:697–707.
70. Feng Y-X, Ruan G-R, Jin F, Xu J, Wang F-J. Purification, identification, and synthesis of five novel antioxidant peptides from Chinese chestnut (*Castanea mollissima* Blume) protein hydrolysates. Lwt. 2018;92:40–6.
71. Ma Y, Zhang D, Liu M, Li Y, Lv R, Li X, et al. Identification of antioxidant peptides derived from tilapia (*Oreochromis niloticus*) skin and their mechanism of action by molecular docking. Foods. 2022;11(17):2576.
72. Nurilmala M, Pertiwi RM, Nurhayati T, Fauzi S, Batubara I, Ochiai Y. Characterization of collagen and its hydrolysate from yellowfin tuna *Thunnus albacares* skin and their potencies as antioxidant and antiglycation agents. Fish Sci. 2019;85(3):591–9.
73. Banerjee P, Suseela G, Shanthi C. Isolation and identification of cryptic bioactive regions in bovine achilles tendon collagen. Protein J. 2012;31(5):374–86.
74. Ren Y, Wu H, Chi Y, Deng R, He Q. Structural characterization, erythrocyte protection, and antifatigue effect of antioxidant collagen peptides from tilapia (*Oreochromis nilotica* L.) skin. Food Funct. 2020;11(11):10149–60.
75. Qi L, Zhang H, Guo Y, Zhang C, Xu Y. A novel calcium-binding peptide from bovine bone collagen hydrolysate and chelation mechanism and calcium absorption activity of peptide-calcium chelate. Food Chem. 2023;410:135387.

76. Ryu B, Shin KH, Kim SK. Muscle protein hydrolysates and amino acid composition in fish. Mar Drugs. 2021;19(7):377.
77. Wang X, Yu H, Xing R, Li P. Characterization, preparation, and purification of marine bioactive peptides. Biomed Res Int. 2017;2017:9746720.
78. Wong F-C, Xiao J, Wang S, Ee K-Y, Chai T-T. Advances on the antioxidant peptides from edible plant sources. Trends Food Sci Technol. 2020;99:44–57.
79. Ding D, Du B, Zhang C, Zaman F, Huang Y. Isolation and identification of an antioxidant collagen peptide from skipjack tuna (*Katsuwonus pelamis*) bone. RSC Adv. 2019;9(46):27032–41.
80. Li B, Chen F, Wang X, Ji B, Wu Y. Isolation and identification of antioxidative peptides from porcine collagen hydrolysate by consecutive chromatography and electrospray ionization–mass spectrometry. Food Chem. 2007;102(4):1135–43.
81. Wang J, Luo D, Liang M, Zhang T, Yin X, Zhang Y, et al. Spectrum-effect relationships between high-performance liquid chromatography (HPLC) fingerprints and the antioxidant and anti-inflammatory activities of collagen peptides. Molecules. 2018;23(12):3257.
82. Indriani S, Sae-Leaw T, Benjakul S, Hong Quan T, Karnjanapratum S, Nalinanon S. Impact of different ultrasound-assisted processes for preparation of collagen hydrolysates from Asian bullfrog skin on characteristics and antioxidative properties. Ultrason Sonochem. 2022;89:106163.
83. Shi C, Liu M, Zhao H, Lv Z, Liang L, Zhang B. A novel insight into screening for antioxidant peptides from hazelnut protein: based on the properties of amino acid residues. Antioxidants. 2022;11(1):127.
84. Larder CE, Iskandar MM, Sabally K, Kubow S. Complementary and efficient methods for di- and tri-peptide analysis and amino acid quantification from simulated gastrointestinal digestion of collagen hydrolysate. LWT. 2022;155:112880.
85. Islam MR, Li W, Ogata Y, Yoshioka T, Ura K, Yasuaki T. Production and antioxidant activity of peptides from sturgeon head. Sustain Chem Pharmacy. 2023;31:100944.
86. Gaspardi ALA, da Silva DC, Ponte LGS, Galland F, da Silva VSN, Simabuco FM, et al. In vitro inhibition of glucose gastro-intestinal enzymes and antioxidant activity of hydrolyzed collagen peptides from different species. J Food Biochem. 2022;46:e14383.
87. Zhang C, Du B, Song Z, Deng G, Shi Y, Li T, et al. Antioxidant activity analysis of collagen peptide-magnesium chelate. Polym Testing. 2023;117:107822.
88. Luo J, Yao X, Soladoye OP, Zhang Y, Fu Y. Phosphorylation modification of collagen peptides from fish bone enhances their calcium-chelating and antioxidant activity. LWT. 2022;155:112978.
89. Deng B, Long H, Tang T, Ni X, Chen J, Yang G, et al. Quantitative structure-activity relationship study of antioxidant tripeptides based on model population analysis. Int J Mol Sci. 2019;20(4):995.
90. Uno S, Kodama D, Yukawa H, Shidara H, Akamatsu M. Quantitative analysis of the relationship between structure and antioxidant activity of tripeptides. J Pept Sci. 2020;26(3):e3238.
91. Liping S, Qiuming L, Jian F, Xiao L, Yongliang Z. Purification and characterization of peptides inhibiting MMP-1 activity with C terminate of Gly-Leu from simulated gastrointestinal digestion hydrolysates of tilapia (*Oreochromis niloticus*) skin gelatin. J Agric Food Chem. 2018;66(3):593–601.
92. Babini E, Tagliazucchi D, Martini S, Dei Piu L, Gianotti A. LC-ESI-QTOF-MS identification of novel antioxidant peptides obtained by enzymatic and microbial hydrolysis of vegetable proteins. Food Chem. 2017;228:186–96.
93. Guo H, Kouzuma Y, Yonekura M. Structures and properties of antioxidative peptides derived from royal jelly protein. Food Chem. 2009;113(1):238–45.
94. Shi J, Zhou X, Zhao Y, Tang X, Feng L, Wang B, et al. The three-spot seahorse-derived peptide PAGPRGPA attenuates ethanol-induced oxidative stress in LO2 cells through MAPKs, the Keap1/Nrf2 signalling pathway and amino acid metabolism. Food Funct. 2021;12(4):1672–87.
95. Hu Z, Sha X, Zhang L, Huang S, Tu Z. Effect of grass carp scale collagen peptide FTGML on cAMP-PI3K/Akt and MAPK signaling pathways in B16F10 melanoma cells and correlation between anti-melanin and antioxidant properties. Foods. 2022;11(3):391.
96. Ma Y, Wu Y, Li L. Relationship between primary structure or spatial conformation and functional activity of antioxidant peptides from Pinctada fucata. Food Chem. 2018;264:108–17.
97. Mirzaei M, Mirdamadi S, Safavi M, Soleymanzadeh N. The stability of antioxidant and ACE-inhibitory peptides as influenced by peptide sequences. LWT Food Sci Technol. 2020;130:109710.
98. Yuan H, Lv J, Gong J, Xiao G, Zhu R, Li L, et al. Secondary structures and their effects on antioxidant capacity of antioxidant peptides in yogurt. Int J Food Prop. 2018;21(1):2167–80.
99. Ma Q, Liu Q, Yuan L, Zhuang Y. Protective effects of LSGYGP from fish skin gelatin hydrolysates on UVB-induced MEFs by regulation of oxidative stress and matrix metalloproteinase activity. Nutrients. 2018;10(4):420.
100. Woo M, Seol BG, Kang KH, Choi YH, Cho EJ, Noh JS. Effects of collagen peptides from skate (*Raja kenojei*) skin on improvements of the insulin signaling pathway via attenuation of oxidative stress and inflammation. Food Funct. 2020;11(3):2017–25.
101. Kong J, Hu XM, Cai WW, Wang YM, Chi CF, Wang B. Bioactive peptides from skipjack tuna cardiac arterial bulbs (II): protective function on UVB-irradiated HaCaT cells through antioxidant and anti-apoptotic mechanisms. Mar Drugs. 2023;21(2):105.
102. Kim J-S, Kim D, Kim H-J, Jang A. Protection effect of donkey hide gelatin hydrolysates on UVB-induced photoaging of human skin fibroblasts. Process Biochem. 2018;67:118–26.
103. Sivaraman K, Shanthi C. Purified fish skin collagen hydrolysate attenuates TNF-alpha induced barrier dysfunction in-vitro and DSS induced colitis in-vivo model. Int J Biol Macromol. 2022;222(Pt A):448–61.
104. Chen T, Hou H, Fan Y, Wang S, Chen Q, Si L, et al. Protective effect of gelatin peptides from pacific cod skin against photoaging by inhibiting the expression of MMPs via MAPK signaling pathway. J Photochem Photobiol B. 2016;165:34–41.
105. Zhang L, Zhang S, Song H, Li B. Ingestion of collagen hydrolysates alleviates skin chronological aging in an aged mouse model by increasing collagen synthesis. Food Funct. 2020;11(6):5573–80.
106. Chen T, Hou H. Protective effect of gelatin polypeptides from Pacific cod (*Gadus macrocephalus*) against UV irradiation-induced damages by inhibiting inflammation and improving transforming growth factor-beta/Smad signaling pathway. J Photochem Photobiol B. 2016;162:633–40.
107. Wang X, Hong H, Wu J. Hen collagen hydrolysate alleviates UVA-induced damage in human dermal fibroblasts. J Funct Foods. 2019;63:103574.
108. Chen J, Liang P, Xiao Z, Chen MF, Gong F, Li C, et al. Antiphotoaging effect of boiled abalone residual peptide ATPGDEG on UVB-induced keratinocyte HaCaT cells. Food Nutr Res. 2019. https://doi.org/10.29219/fnr.v63.3508.
109. Griendling KK, Camargo LL, Rios FJ, Alves-Lopes R, Montezano AC, Touyz RM. Oxidative stress and hypertension. Circ Res. 2021;128(7):993–1020.
110. Paravicini TM, Touyz RM. Redox signaling in hypertension. Cardiovasc Res. 2006;71(2):247–58.
111. CunhaNeves A, Harnedy-Rothwell PA, FitzGerald RJ. In vitro angiotensin-converting enzyme and dipeptidyl peptidase-IV inhibitory, and antioxidant activity of blue mussel (*Mytilus edulis*) byssus collagen hydrolysates. Eur Food Res Technol. 2022;248(7):1721–32.
112. Dinh QN, Chrissobolis S, Diep H, Chan CT, Ferens D, Drummond GR, et al. Advanced atherosclerosis is associated with inflammation, vascular dysfunction and oxidative stress, but not hypertension. Pharmacol Res. 2017;116:70–6.
113. Davignon J, Ganz P. Role of endothelial dysfunction in atherosclerosis. Circulation. 2004;109(23 Suppl 1):III27-32.
114. Saito-Takatsuji H, Yoshitomi Y, Ishigaki Y, Yamamoto S, Numata N, Sakai Y, et al. Protective Effects of collagen tripeptides in human aortic endothelial cells by restoring ROS-induced transcriptional repression. Nutrients. 2021;13(7):2226.
115. Han M, Zhang Z, Li X, Tong H, Xu Z, Ding Z, et al. Effects of collagen peptides from Micropterus salmoides skin on oxidative damage induced by cyclophosphamide in mice. Front Nutr. 2022;9:1037212.
116. Li DD, Li WJ, Kong SZ, Li SD, Guo JQ, Guo MH, et al. Protective effects of collagen polypeptide from tilapia skin against injuries to the liver and kidneys of mice induced by d-galactose. Biomed Pharmacother. 2019;117:109204.
117. Zhang R, Chen J, Jiang X, Yin L, Zhang X. Antioxidant and hypoglycaemic effects of tilapia skin collagen peptide in mice. Int J Food Sci Technol. 2016;51(10):2157–63.

118. Ribeiro M, Pasini S, Baratta RO, Del Buono BJ, Schlumpf E, Calkins DJ. Collagen mimetic peptides promote adherence and migration of ARPE-19 cells while reducing inflammatory and oxidative stress. Int J Mol Sci. 2022;23(13):7004.
119. Mu H, Wang Y, Wei H, Lu H, Feng Z, Yu H, et al. Collagen peptide modified carboxymethyl cellulose as both antioxidant drug and carrier for drug delivery against retinal ischaemia/reperfusion injury. J Cell Mol Med. 2018;22(10):5008–19.
120. Miao B, Zheng J, Zheng G, Tian X, Zhang W, Yuan F, et al. Using collagen peptides from the skin of monkfish (*Lophius litulon*) to ameliorate kidney damage in high-fat diet fed mice by regulating the Nrf2 pathway and NLRP3 signaling. Front Nutr. 2022;9:798708.

Publisher's Note