Food Science of Animal Products

Review Article

Advances and challenges in loach (*Misgurnus anguillicaudatus*) peptides: preparation, biological activities and *in silico* research

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Abstract: Bioactive peptides derived from animal sources have attracted growing interest in functional food and pharmaceutical research due to their diverse physiological activities and high bioavailability. Loach (*Misgurnus anguillicaudatus*), a protein-rich freshwater fish with low fat content, has emerged as a promising source of bioactive peptides. Recent studies have found that loach peptides have many biological effects, such as anti-oxidation, anti-fatigue, anti-bacteria, immunomodulation, anti-cancer, anti-hypertension, and anti-osteoporosis activities. In this review, the preparation, separation, purification and characterization of loach peptides were summarized. The recent progress of biological functions of loach peptides was also discussed. At present, the research of loach peptide is still in the initial stage, the relationship between the structures and functions of loach peptides, the molecular mechanisms of the function of loach peptides are needed to be further studied. In addition, this review addresses the challenges associated with the application of loach peptides and summarizes recent advances in their deep-processing and high-value utilization, aiming to provide a reference for future studies and to facilitate the continued development and broader application of loach peptides.

Keywords: loach; peptide; preparation; biological activities; mechanisms; *in silico*

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1 Introduction

Peptides are short chains of amino acids linked by peptide bonds and are generally composed of fewer than 100 amino acid residues, with molecular weights typically below 10 kDa[1–3]. Peptides can exhibit diverse biological activities, such as antioxidant, antibacterial, antihypertensive, and anticancer-related activities, which may differ from those of free amino acids or intact proteins[4]. There are many sources of peptides in nature, such as animal peptides, plant peptides and microbial peptides[5–6]. Peptides can either be extracted from naturally occurring substances or synthesized chemically[7–8]. Animal-derived peptides are commonly obtained from protein-rich food materials, such as sea cucumber, egg, and milk proteins[9–11]. Animal-derived peptides have attracted increasing attention in food science because of their abundant sources, high nutritional value, and broad biological activities.

Loach (*Misgurnus anguillicaudatus*) is a common freshwater fish in East Asia with an early historical record of consumption in China. It is characterized by palatable flesh and high nutritional value and has long been regarded in China as a traditional "medicine-food homologous" resource. In traditional Chinese dietary and medicinal practices, loach has been used for the management of inflammation-related conditions, and modern studies have reported several potential bioactivities, including anti-inflammatory and anticancer-related effects[12–14]. Nutritionally, loach contains high-quality protein with a well-balanced amino acid profile. Gao et al.[15] reported that the whole-body crude protein content of loach ranges from 16.3% to 18.3% on a wet-weight basis, while crude fat content varies from 2.8% to 7.7% between wild and cultured populations. In addition, loach lipids are rich in health-beneficial *n*-3 polyunsaturated fatty acids (PUFAs), such as eicosapentaenoic acid and docosahexaenoic acid[16]. Compared with commonly consumed freshwater fish such as grass carp and tilapia, loach has been reported to show comparable protein levels and, in some cases, a more favorable *n*-3/*n*-6 PUFA ratio. Variability in nutritional composition arises from factors such as genetic background, diet, farming system (wild *vs.* cultured), seasonal variation, and tissue type, which should be considered when evaluating loach as a raw material for functional foods.

China is the world's largest producer of loach. According to the *China Fishery Statistical Yearbook*, national loach aquaculture production reached 374 981 t in 2022[17]. This substantial production volume reflects the broad market presence of loach and provides an abundant raw material base for the development of loach-derived bioactive peptides. Together, these nutritional and production advantages support the potential of loach as a raw material for bioactive peptide production. However, the direct utilization of native loach proteins in food systems remains limited. Raw loach protein exhibits poor solubility and weak emulsifying capacity, which makes it difficult to uniformly disperse in aqueous food systems and hinders compatible mixing with other food ingredients during processing, greatly restricting its application scope and finished product quality[18]. Therefore, systematic optimization of hydrolysis, separation, purification, and peptide identification strategies is needed to support the conversion of loach proteins into functional peptide ingredients suitable for food applications.

In response to these limitations, recent studies have focused on improving the preparation efficiency, functional properties, and application potential of loach proteins and peptides. Enzymatic hydrolysis, especially compound-enzyme hydrolysis and ultrasound-assisted enzymolysis, has been used to enhance peptide release, extraction yield, and bioactivity[8,19–20]. For example, Chu et al.[19] developed an ultrasound-assisted compound enzymolysis process that improved the recovery and bioactivity of loach peptides (LPs). In parallel, modification strategies have been developed to improve the solubility, emulsifying capacity, and processing adaptability of native loach protein[18,21]. Analytical approaches such as peptidomics, molecular docking, and molecular simulation have further enabled the identification of active peptide sequences and the interpretation of structure-activity relationships[20,22]. In addition, accumulating experimental evidence indicates that loach protein and its enzymatic hydrolysates possess multiple physiological functions, including antioxidant, anti-fatigue, antibacterial, immunomodulatory, and intestinal flora-regulating activities[23–25]. A series of supporting processing technologies covering loach deodorization and by-product collagen extraction have also been developed to realize comprehensive resource utilization[26–28]. Owing to their diverse bioactivities and improved processing properties, LPs have been increasingly investigated as functional food ingredients, aquaculture feed additives, and bioactive compound delivery carriers.

Although existing studies have laid a foundation for the high-value utilization of LPs, most available research has focused mainly on upstream preparation and *in vitro* activity evaluation, whereas downstream processing, industrial scale-up, and product development remain insufficiently discussed. To provide a clearer overview of the research landscape and identify major research focuses, this review adopted a bibliometric approach. Publications containing the term "loach" in their titles, abstracts, or keywords from 1996 to 2022 were retrieved from the Web of Science database, yielding a total of 1 341 records. These publications belonged to subject categories including "Zoology", "Marine Freshwater Biology", "Biochemistry Molecular Biology", "Food Science Technology", and "Nutrition Dietetics". The exported data were visualized using VOSviewer software (Figure 1A). The keyword co-occurrence network showed that previous studies on loach mainly focused on mitogenome, genome, evolution, and phylogenetic analysis. Although studies related to LPs accounted for a relatively small proportion, the network also indicated emerging connections between LPs and other research fields. In addition, retrieval of publications containing "loach peptide" in the Web of Science database showed that LP-related studies gradually emerged around 2000. Since then, the number of publications has generally increased, reaching a peak around 2020 (Figure 1B). Compared with previous studies that mainly focused on peptide preparation and *in vitro* bioactivity evaluation, this review summarizes current progress in the preparation, separation, purification, structural

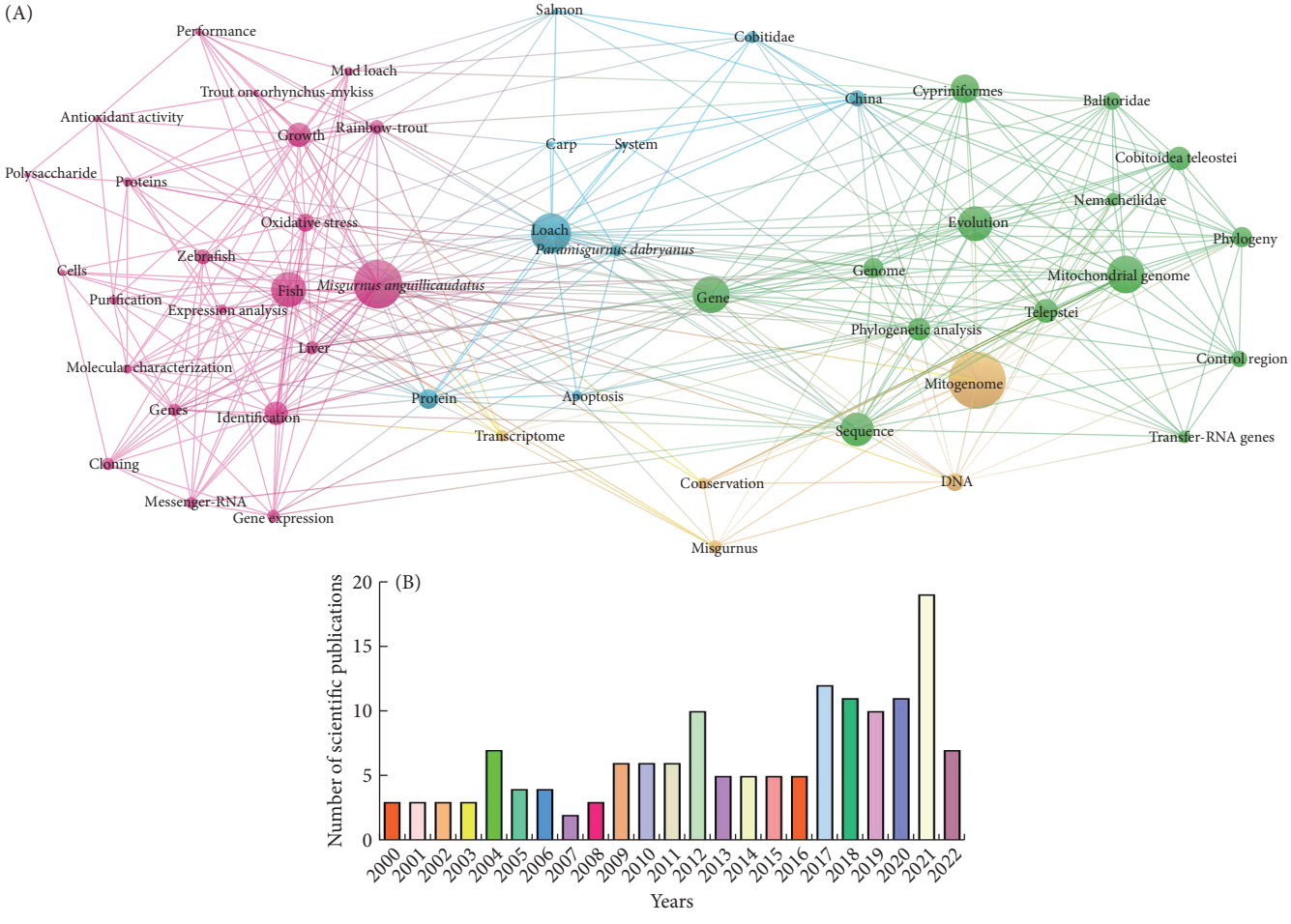

Figure 1 (A) Co-occurrence network of keywords in bibliographic data from 1996 to 2022 via VOSviewer software. (B) Number of scientific publications reported by Web of Science containing the term "loach peptide" in the title, abstract, or keywords within the years from 2000 to 2022.

identification, biological activities, potential applications, and *in silico* analysis of loach-derived bioactive peptides. It also discusses current limitations and future directions for their further development and utilization.

2 Preparation of LPs

The process of preparing peptides by microbial fermentation is relatively complicated. It mainly uses protease produced in microbial fermentation process to act on the protein in the substrate, and protease hydrolyzes the protein molecules into peptides or free amino acids. In addition to proteases, other types of enzymes are produced to degrade complex macromolecules of carbohydrates and lipids throughout the process of fermentation to prepare peptides[29–31]. Lactic acid bacteria, *Bacillus subtilis*, and so on are commonly used in the process of preparing peptides by microbial fermentation[32]. Chemical hydrolysis is using chemical reagents such as acid or base to break the peptide chain of protein molecules at a certain temperature to generate small molecule peptides[33]. Enzymatic hydrolysis is a method that uses specific enzymes to hydrolyze amide bonds in specific parts of proteins to generate different peptide fragments, so as to obtain the ideal peptide. The main enzymes of enzymolysis protein are trypsin, pepsin, chymosin and so on. However, mainly because of the specificity of enzymes, different proteases have different cleavage sites. The prepared peptides varied in type, size and biological activity[34].

Because of the uncontrollability of the fermentation process parameters, the yields and activities of the enzymes are also difficult to control, resulting in poor repeatability and quality control of the peptides. In addition, the chemical hydrolysis method also has some problems, such as chemical reagent contamination. The amount of reagent added will affect the denaturation of polypeptide or protein[30,35]. Therefore, there are few studies on the preparation of LPs by microbial fermentation and chemical hydrolysis based on factors such as the control of flavor quality changes and restrictions of relevant safety and environmental regulations. Due to mild enzymatic reaction conditions and good controllability, it is currently a common method for preparing LPs. Enzymatic preparation of LPs includes pretreatment, enzymatic hydrolysis, purification, identification and biological activities evaluation (Figure 2).

2.1 Pretreatment

Pretreatments are usually involved in the use of heat-induced protein denaturation, homogenization to stabilize the system, degreasing, detoxification and drying. The purpose of this pretreatment is to enhance the contact efficiency between the active sites of the hydrolytic protease and the specific cleavage sites within the protein sequence, thereby facilitating subsequent hydrolysis and processing steps[36]. In addition, thermal denaturation inactivates microorganisms present in the raw material and destroys endogenous tissue enzymes, thus preventing interference from non-specific enzymes that could compromise the yield and purity of the peptides produced. After the protein is denatured, the whole macromolecular protein structure will become loose, thus exposing more specific binding sites for enzyme binding[37]. Homogenization can increase the total surface area of proteins to increase the reaction contact area and improve the reaction efficiency. In addition, it can make the whole raw material system in a uniform and stable state, which is conducive to the follow-up reaction[38]. In addition, fat can be removed by using organic solvents such as ethanol to reduce the impact of lipolysis on peptides. The removal of toxic substances can reduce the denaturation and inactivation of hydrolase. Consequently, the enzyme activity can be increased and the efficiency of enzymatic hydrolysis can be improved[39]. Zhang et al.[40] pretreated bovine collagen by high pressure boiling before enzymatic hydrolysis, and found that the pretreated bovine collagen had better degree of hydrolysis, which was conducive to the subsequent enzymatic hydrolysis reaction.

LPs were mainly prepared by researchers themselves, and most researchers used pretreatment in the preparation process. After freshly slaughtered loaches were blanched in hot water, their skin and mucus could be easily removed[25,41–42]. Although studies have shown that the mucus on the surface of loach is rich in active ingredients such as glycoproteins[43], it is not suitable for extraction and identification because the mucus contains a lot of water and is not easy to handle[44]. Consequently, the mucus on the surface of loach will be removed during the pretreatment process. Protein is widely distributed in loach meat. In order to improve the efficiency of subsequent enzymatic hydrolysis reaction, the contact area between substrate and enzyme needs to be increased. As a result, loach meat is ground into mince by meat grinder, which is convenient for mixing with distilled water and conducive to homogenization and enzymatic hydrolysis treatment. In the early

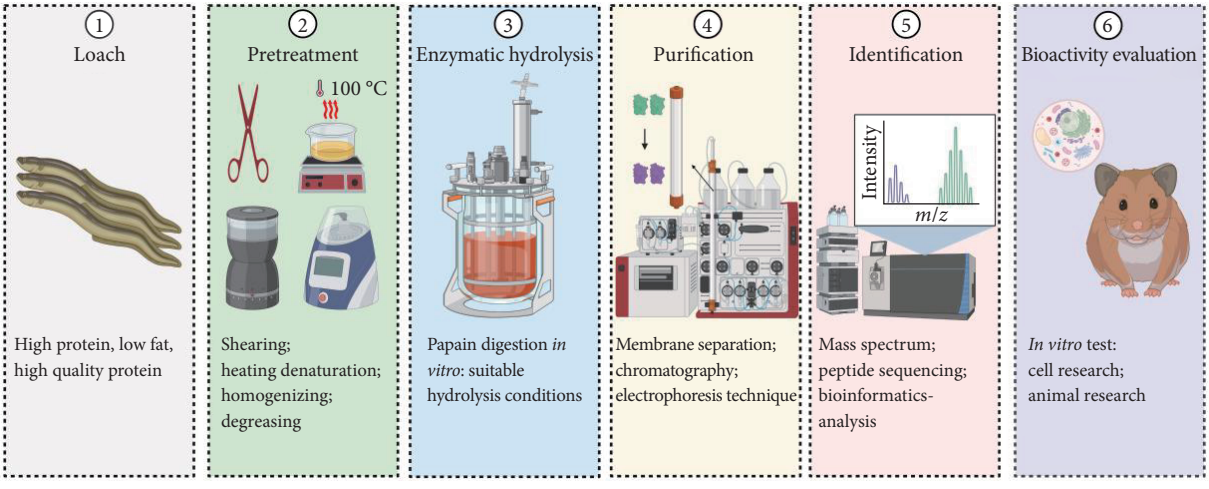

Figure 2 Schematic diagram for the production and purification of LPs and the commonly used methods.

preparation of LPs, homogenization technology was not used due to the limitation of experimental conditions[18]. However, the mixed system after homogenization treatment is in a stable state, which ensures the smooth progress of the experiment. Therefore, You et al.[25,42] used a homogenizer to homogenate LPs at 10 000 r/min for 1 min in the process of preparing LPs in recent years. In conclusion, pretreatment is an indispensable step in the study of other animal polypeptides including LPs in recent years. However, there are also defects in the current pretreatment operation, such as how to reduce raw material loss in the pretreatment process and how to restrain the decline of raw material quality[45–46]. These are still questions that need to be addressed in future studies to prepare peptides.

2.2 Enzymatic hydrolysis

Enzymatic hydrolysis is a key step in the production of LPs. Efficiency of enzymolysis mainly depends on pH, temperature, enzyme-substrate mass ratio, enzymolysis time and other factors. Therefore, the preparation process can be controlled by changing the pH value, temperature or adding enzyme inhibitors at any time during the whole enzymatic hydrolysis process[47]. At present, the preparation of peptides mostly uses papain. Papain has extensive proteolytic function for amino acid peptides of various organisms. Researching on papain hydrolysis of freshwater fish collagen found that papain has good hydrolysis efficiency and can hydrolyze 63.06% of collagen, the peptides obtained by hydrolysis have good antioxidant and other biological activities, and the scavenging efficiency of free radicals (FRs) after hydrolysis can reach 71.55%[48]. Notably, loach can be hydrolyzed by papain enzymes to produce peptides.

Although papain enzymatic hydrolysis of loach minced meat is the most commonly used method to produce LPs, it has certain limitations. Using a particular type of enzyme could cause financial stress[49]. In addition, pre-tests are needed to determine the optimal conditions (enzyme to substrate ratio, temperature, time and pH value), which will affect the quality and efficiency of LPs preparation.

In addition to choosing papain as a tool for enzymatic hydrolysis of peptides, some researchers also chose to use alkaline enzymes and bromelain for enzymatic hydrolysis when preparing peptides[50]. In order to improve the efficiency of enzymatic hydrolysis, ultrasonic assisted enzymatic hydrolysis was used in the process[51]. Ultrasound-assisted enzymatic hydrolysis achieves protein hydrolysis by inducing protein structure to unfold and loosen and promoting the exposure of peptide bonds, which improves the efficiency of protein enzymatic hydrolysis to synthesize bioactive peptides. The results showed that compared with the traditional enzymatic hydrolysis process without ultrasonic pretreatment, the degree of protein hydrolysis and the conversion rate of ultrasound assisted enzymatic hydrolysis were increased by 5.42% and 11.27%, respectively[52]. Therefore, in order to better improve the efficiency of preparation of LPs, the previous preparation process can be optimized. It is necessary to improve the efficiency of the preparation of bioactive peptides while relieving the economic pressure.

2.3 Purification

The preparation of high purity bioactive peptides is the key to study their physicochemical properties and bioactivities. The hydrolysate is a mixture after enzymatic hydrolysis, which mainly contains some unhydrolyzed proteins, free amino acids and inorganic salts. In order to improve the efficiency of the experiment, it is necessary to purify the prepared peptides mixture[53]. By purifying sardine byproducts hydrolyzed by protease, researchers found that the higher the purity of the peptide, the stronger the antioxidant capacity, and the purified peptide increased by about 63%[54]. Therefore, the obtained hydrolyzed mixture of LPs also needs to be purified.

Before the purification of bioactive peptides, it is necessary to determine the separation and purification technology according to the physicochemical properties of the active peptides. The separation and purification methods mainly include membrane separation, chromatography and electrophoresis[55]. Membrane separation is usually the first step in the purification of a hydrolytic mixture. The hydrolytic solution is passed through a membrane separation system under pressure. The separation membrane is capable of intercepting peptides of different molecular weights. Considering economic and environmental concerns, ultrafiltration is preferred in membrane separation operations[56]. After ultrafiltrating α-chymotrypsin-produced animal protein hydrolysates (< 5, 5–10, and > 10 kDa), investigators found that the peptides with the lowest molecular weight (< 5 kDa) had the highest antioxidant protection[57]. Although the membrane separation method of ultrafiltration could improve the purity of peptides to some extent, ultrafiltration membranes can only retain peptides based on their molecular weight. Other physicochemical properties such as charge difference and hydrophobicity need to be considered in order to meet the requirements of subsequent tests. Chromatography mainly includes ion exchange chromatography, gel filtration chromatography, affinity chromatography and reversed phase high performance liquid chromatography (RP-HPLC)[58–60]. Further purification of peptide fractions requires chromatography. Based on charge difference and hydrophobic interaction, the commonly used methods for further purification of LPs are ion exchange chromatography, gel filtration chromatography and RP-HPLC[22]. The purity and activity of antiproliferative peptides of fish protein hydrolysates treated with ion-exchange chromatography and RP-HPLC were improved[61]. Electrophoresis is a separation and purification method with high resolution and selectivity. However, due to its low injection volume and low efficiency, it is not widely used in the separation and purification of LPs at present[62].

2.4 Characterization and identification

After the extraction, isolation and purification of LPs are completed, characterization and identification of LPs are required. Currently, there are two germplasm spectroscopic methods used in the characterization and identification of other peptides: 1) bottom-up analysis (i.e., peptide mapping); 2) mass measurement of the complete peptide and subsequent top-down analysis (i.e., mass spectrometry). In the analysis of the peptide map, the entire peptide is injected into the mass spectrometry and information about the peptide identity and sequence characterization is provided. Bottom-up analysis mainly involves enzymic hydrolysis of peptides and mass spectrometry prior to isolation to allow the peptide to be identified by characteristic correspondence to specially cut fragments[63]. The peptide sequence is determined by protonation chromatography through top-down analysis experiments. The peptide sequence is completed using bioanalysis software[54,64]. Bernay et al.[65] used a 5 μm particle size column instead of the 10 μm XTerra C_{18} packing column used in the isolation and purification phase to control the purity of the peptide at the later

stage of characterization. It allows for more efficient analysis of the amino acid sequence of the peptides. Of course, other less commonly used methods can also be used to characterize and identify peptides. Bioinformatics knowledge is also in the characterization and identification of food active peptides. In the database of bioactive peptides, researchers can query the sequence and identify the bioactivities of proteolytic products. Protein molecular docking method and quantitative structure-activity relationships are also used to predict and screen potential bioactive peptides[55]. Through a series of mass spectrometry, peptides sequencing technology, bioinformatics analysis and other methods, researchers could better characterize and identify the sequence of LPs. At present, matrix assisted laser desorption ionization-time of flight, electron spray ionization, fast atom bombardment and other mass spectrometry techniques are also commonly used to characterize and identify polypeptide sequences due to high sensitivity and high efficiency[66]. In summary, the combination of some methods in the characterization and identification process, relying on complementary advantages, overcomes the low efficiency of some traditional characterization and identification methods, which will be the development trend of peptide research in the future.

3 Biological activities of LPs

Because of its molecular weight of the constituent peptides and the structural properties of small amino acids, peptides have rich physiological activities. In this section, we highlight the underlying effects of LPs on a number of diseases, which include anti-oxidation, microbial infections, anti-fatigue, hypertension, anti-inflammation, immune regulation such as cancer, and anti-osteoporosis. By discussing and analyzing the biological activity and potential mechanism of LPs, we can clarify their important physiological functions, so as to better guide the research of modern medicine (Figure 3 and Table 1).

3.1 Antioxidant activity

Oxidative metabolism is an indispensable physiological activity in the process of energy supplement. During energy metabolism, the body produces huge amounts of FRs and reactive oxygen species (ROS)[81]. FRs and ROS could affect biological processes, such as immune response to external antigens. However, once the level of FRs and ROS is in a state of unbalance, it will lead to metabolic disorder, which causes oxidative damage to lipids, proteins and even DNA, and harm the normal physiological functions of the body, or even more serious symptoms[55]. These diseases include diabetes, high blood pressure, liver and lung dysfunction, inflammation and Alzheimer's disease[82]. In order to reduce oxidative damage to the body, a certain amount of antioxidants need to be consumed in the daily diet. Antioxidants are effective at scavenging FRs and other ROS molecules, including superoxide anions and nitric oxide radicals, as well as hydroxyl radicals[83]. Common antioxidants in the daily diet are natural astaxanthin, tea polyphenols, carotenoids, vitamin C (VC), peptides, etc.[84–87]. Because other antioxidants are easily degraded or oxidized, peptides are increasingly potential candidates for removing ROS. Antioxidant peptides in the peptide family activate endogenous antioxidant defenses in cells. Keap1/Nrf2/ARE pathway is the center of cell's resistance to oxidative stress. This signaling pathway

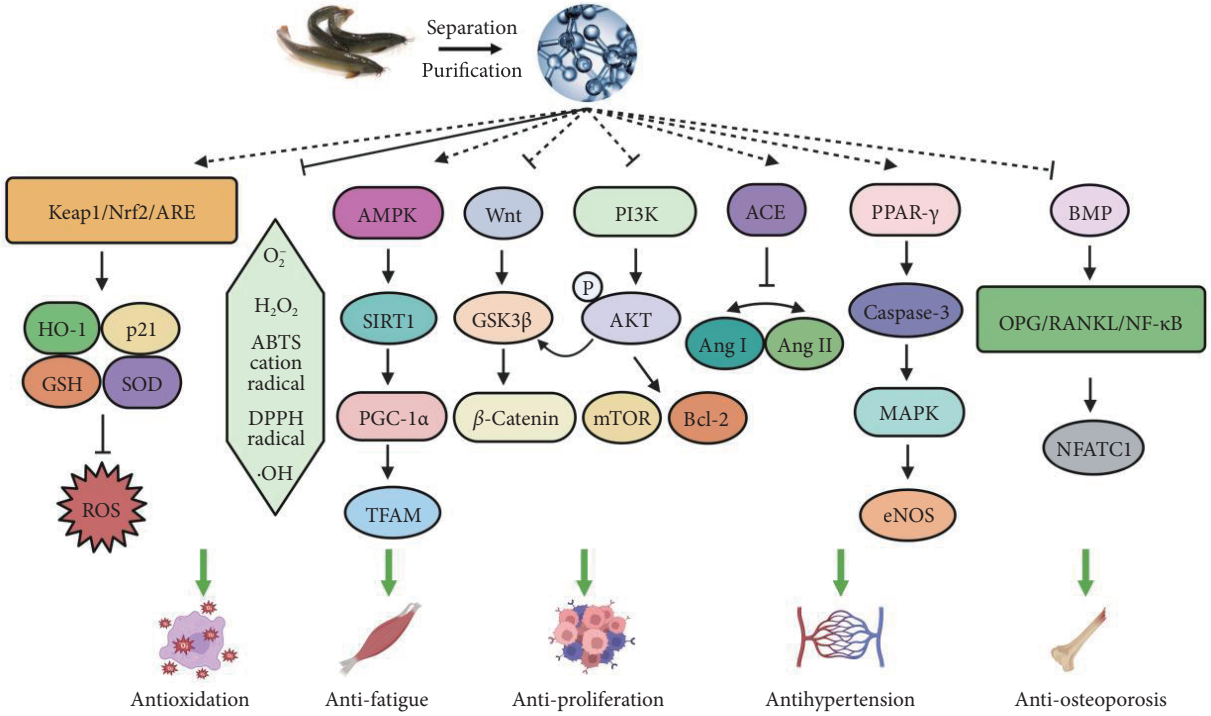

Figure 3 Biological activities of LPs. Keap1, Kelch-like ECH-associated protein 1; Nrf2, nuclear factor-erythroid 2 related factor 2; ARE, anti-oxidative response element; HO-1, heme oxygenase 1; GSH, glutathione; SOD, superoxide dismutase; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 1,1-diphenyl-2-picrylhydrazyl; AMPK, adenosine 5′-monophosphate activated protein kinase; SIRT1, sirtuin 1; PGC-1α, peroxisome proliferator-activated receptor γ coactivator 1α; TFAM, mitochondrial transcription factor; Wnt, wingless/integrated; GSK3β, glycogen synthase kinase 3β; PI3K, phosphoinositide 3 kinase; AKT, AKT8 virus oncogene cellular homolog; mTOR, mammalian target of rapamycin; Bcl-2, B-cell lymphoma 2; ACE, angiotensin I-converting enzyme; Ang, angiotensin; PPAR-γ, peroxisome proliferator-activated receptor γ; MAPK, mitogen-activated protein kinase; eNOS, endothelial nitric oxide synthase; BMP, bone morphogenetic protein; OPG, osteoprotegerin; RANKL, receptor activator of nuclear factor κB ligand; NF-κB, nuclear factor κB; NFATC1, nuclear factor of activated T cells 1.

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Table 1 Summary of bioactive properties of LPs.

Bioactivity	Preparation methods	Processing methods	Results	References
Antioxidation	Muscle tissue → homogenization →1 g homogenization adding 40 mL deionized water → mix → pH adjustment to 10.0 → add 1 500 U/g alkaline protease hydrolysis → 2 h → enzyme digestion → centrifugation (6 000 r/min; 15 min) → enzymolysis solution (< 10 kDa) → freeze drying for 24 h	Sixty mice were randomly divided into blank control group, model control group, high-dose, medium-dose and low-dose peptide groups, and VC control group. Under the condition that the variables were unique, different dose groups were fed with loach protein peptide with mass fraction of 200, 400 and 800 mg/(kg·day), respectively, while VC control group was fed with 300 mg/(kg·day). After 6 weeks, the relevant biochemical indexes of serum, liver and heart of mice were determined	SOD ↑, GSH-Px ↑, MDA ↓	[67]
	Fresh loach → pretreatment → 5% (*m*/*V*) suspension → thermal denaturation treatment (90 °C; 15 min) → cooling to room temperature → pH adjustment to 7.0 → 3 200 U/g neutral protease hydrolysis → 4 h → enzyme inactivation (90 °C; 15 min) → centrifugation (5 000 r/min; 20 min) → collect the supernatant → vacuum freeze drying	The DPPH radical scavenging ability of LPs prepared by neutral protease after plastein reaction was determined	DPPH radical ↓	[68]
		LP was added into linoleic acid emulsion system, and GSH was used as the control to determine the scavenging activity of DPPH and hydroxyl radical, chelating ability with Cu^{2+} and lipid peroxidation inhibition activity, and indirectly determine the antioxidant activity of LP *in vitro*	DPPH radical ↓, $O_2^{-}\cdot$↓, hydroxyl radical ↓, Cu^{2+} ↓	[41]
	Loach muscle tissue → grinding → 50 g loach meat mixed with 100 mL distilled water → homogenization for 1 min → pH adjustment to 7.0 → papain at 55 °C (3:1 000) → 4.5 h → enzyme digestion (100 °C, 15 min) → centrifugation (5 000 r/min; 20 min) → ultrafiltration membrane separation → < 5 kDa LPs solution	The ability of LP to scavenge hydroxyl radical and ABTS cation radical and chelate Cu^{2+} was determined before and after treatment with pepsin and trypsin *in vitro* digestion model system. To determine the changes in antioxidant activity of loaches prepared by papain digestion after *in vitro* digestion	ABTS cation radical ↓, hydroxyl radical ↓, Cu^{2+} ↓	[69]
		The LPH prepared by papain digestion was fractionated into four parts, and the hydroxyl radical scavenging and Cu^{2+} chelating abilities of each fraction were measured	Hydroxyl radical ↓, Cu^{2+} ↓	[25]
Antifatigue	Loach muscle tissue → grinding → 50 g loach meat mixed with 100 mL distilled water → homogenization for 1 min → pH adjustment to 7.0 → papain at 55 °C (3:1 000) → 4.5 h → enzyme digestion (100 °C; 15 min) → centrifugation (5 000 r/min; 20 min) → ultrafiltration membrane separation → < 5 kDa LPs	During the experiment, the mice were weighed every week and underwent adaptive swimming for 30 min every week. After four weeks of LP treatment, the mice were forced to undergo a full swimming test. Fatigue related parameters were determined after death	AMPK ↑, PGC-1α ↑, PI3K ↑, phosphorylated AKT ↑, glycogen ↑, exhausting swimming time ↑ BUN ↓, weight ↓	[41]
	Loach tissue → grinding → 1 g:25 mL mixed distilled water → homogenization → pH adjustment to 7.0 → 39.55 °C neutralization enzymolysis (1 726.85 U/g) → 5.5 h → enzymolysis (100 °C; 15 min) → centrifugation (8 000 r/min; 30 min) → ultrafiltration membrane separation → < 2.5 kDa LPs	By feeding different doses of LPs to mice for 4 weeks, the exhaustive swimming time, antioxidant enzymes (SOD, GSH-Px), LA, BUN and other biochemical indexes of mice were determined	SOD ↑, GSH-Px ↑ LA ↓, BUN ↓, MDA ↓	[70]
Antibacterial	Loach muscle tissue → pre-cooled 0.05 mol/L Tris-HCl buffer solution (adding ratio 1:1; pH 7.5) → grinding → homogenization → filtration → centrifugation (8 000 r/min; 30 min) → elution Sephadex G-50 column (1.6 cm × 60 cm) → freeze drying	The minimum inhibitory bacteria concentration of logitide against *Aeromonas hydrophila, E. coli* and *Staphylococcus aureus* was determined	*A. hydrophila* ↓, *E. coli* ↓, *S. aureus* ↓	[71]
	Loach skin mucus → homogenization (pH 6.0, 50 mm phosphate buffer) → centrifugation (15 000 r/min; 30 min) → filter (centriprep filter) → elution (RP-HPLC) → GKLNLFLSRLEILKLFVGAL	Determination of MIC of logitide against *S. aureus, E. coli, B. subtilis* and other microorganisms by microdilution method	*S. aureus* ↓, *E. coli* ↓, *B. subtilis* ↓	[72]
	Loach muscle tissue → grinding → adding ethyl acetate (20 °C; 1 000 mL) → centrifugation (15 000 r/min; 30 min) → filter (centriprep filter)→ elution (RP-HPLC) → CFGWN	LP was dissolved in the buffer solution and treated simultaneously at 4, 20, 37, 60, 80 and 100 °C for 30 min, respectively. The antibacterial activity was determined. Aging rats and amyloid precursor protein/presenilin 1 mice given blueberry extract	*B. subtilis* ↓, *E. coli* ↓, *S. aureus* ↓	[18]
	DNA fragment → cut → insert pET30a plasmid → *E. coli* BL21 (DE3) cells → expression → recombinant protein → denaturation → renaturation → purification → loach antimicrobial peptides	Bacterial suspension (180 μL) was mixed with 5 μg loach antibacterial peptide mixture and 20 μL phosphate-buffered saline, then added into microtitration plate hole and cultured at 30 °C for 24 h. The absorbance, or bacterial density, at 630 nm was recorded	*A. hydrophila* ↓, *Vibrio anguillarum* ↓, *E. coli* ↓, *S. aureus* ↓	[73]
	DNA fragment → cut → insert pET30a plasmid → *E. coli* BL21 (DE3) cells → expression → recombinant protein → denaturation → renaturation → purification → loach antimicrobial peptides	A medium-to-medium strain culture of 100 μL was mixed with 20 mL MHA and poured into a culture dish. After solidification, the freeze-dried recombinant loach antibacterial peptide was continuously diluted with sterile dH_2O twice the microtiter plate, stained on MHA for every 10 μL dilution, and incubated at 37 °C overnight. The inhibitory activity was determined	HAMP ↑, *V. anguillarum* ↓, *E. coli* ↓	[74]

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Table 1 (*Continued*)

Bioactivity	Preparation methods	Processing methods	Results	References
	Loach muscle tissue → grinding → adding distilled water → homogenization → pH adjustment to 7.0 → enzymatic hydrolysis → enzyme inactivation (100 °C; 20 min) → centrifugation → ultrafiltration membrane separation → LPs	Sixty mice were randomly divided into blank control group, model control group, high-dose peptide group and low-dose peptide group. Under the condition that the variables were unique, the high and low doses were given intragastric administration of 500 and 100 mg/(kg·day), respectively. After 6 weeks, the relevant biochemical indexes in serum and other tissues of mice were determined	SOD ↑, CAT ↑, GSH-Px ↑, MDA ↓, TNF-α ↓, ROS ↓	[75]
Immunomodulation; anti-proliferation		The anticarcinogenic activity of LP was evaluated by measuring the biochemical indicators in the human hepatocarcinoma SMMC-7 721 cell line	Ca^{2+} ↑, ROS ↑, p53 ↑, Bcl-2-associated X protein ↑, Bcl-2 ↓, matrix metalloproteinase ↓	[76]
	Loach muscle tissue → grinding → 50 g loach meat mixed with 100 mL distilled water → homogenization for 1 min → pH adjustment to 7.0 → papain at 55 °C (3:1 000) → 4.5 h → enzyme digestion (100 °C; 15 min) → centrifugation (5 000 r/min; 20 min) → ultrafiltration membrane separation → < 5 kDa LP solution	The antiproliferative activity of LPH and its fractions was evaluated by measuring their inhibitory effects on the proliferation of HepG2, MCF-7, and Caco-2 human cancer cell lines. The CellTiter 96® AQueous One Solution Cell Proliferation Assay was used to assess cell viability and confirm the antiproliferative activity of the LP	The viability and proliferation of HepG2, MCF-7 and Caco-2 cancer cells ↓	[25]
		Using Caco-2 and HepG2 as model cells, the antiproliferative activity of loach hydrolysate was determined	The viability and proliferation of HepG2 and Caco-2 human cancer cells ↓	[77]
Anti-osteoporosis	Loach skin tissue → 1:10 distilled water bath → pH adjusted to 9.0 → alkaline protease hydrolysis (6 500 U/g) at 52.5 °C → 2 h → inactivation → centrifugation → filtration → spray drying	The mouse model of formic acid-induced osteoporosis was fed LPH. After a period of time, the femur was analyzed by micro-computed tomography, RNA extraction, and bone characteristics analysis	Quantity and quality of bone trabeculae ↑, blood calcium ↑, TNF receptor-associated factor 6 ↓, NF-κB ↓, NFATC1 ↓	[78]
Anti-hypertension	Loach muscle tissue → grinding → 100 g loach meat mixed with 200 mL distilled water → homogenization for 1 min → pH adjustment to 5.5 → bromelain hydrolysis at 55 °C (3:1 000) → 6 h → enzyme inactivation (100 °C; 10 min) → centrifugation (5 000 r/min; 20 min) → ultrafiltration membrane separation → 2.5 kDa LP solution	The ACE inhibition ability of synthetic peptides was determined by RP-HPLC	ACE ↓	[79]
		High-dose AHLL (Ala-His-Leu-Leu, 5 mg/kg body weight) and low-dose AHLL (1 mg/kg body weight) were administered orally for 8 weeks	Systolic blood pressure ↓, weight ↓	[80]

Note: GSH-Px, glutathione peroxidase; MDA, malondialdehyde; LPH, loach protein hydrolysate; BUN, blood urea nitrogen; LA, lactic acid; MIC, minimum inhibitory concentration; MHA, Mueller-Hinton agar; HAMP, hepcidin antimicrobial peptide; CAT, catalase; TNF-α, tumor necrosis factor-α.

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is activated by antioxidant peptides, which can effectively slow down the level of ROS in the body[88]. Keap1/Nrf2/ARE, a sensitive signaling pathway of oxidative stress response, plays a protective role by regulating the expression of genes related to apoptosis signaling factors and antioxidant enzymes[89]. Molecular docking studies have shown that peptides effectively bind to key proteins through hydrophobic and hydrogen bonding forces[90].

DPPH and ABTS are common methods to evaluate the FRs scavenging activities of peptides. While the reactions involved require specific pH values, durations, and buffer types[91], these methods are widely accepted and routinely applied for quantifying the antioxidant effects of natural foods or partially purified antioxidant peptides. Methods for assessing antioxidant capacity are often broadly categorized based on their underlying chemical reactions, such as hydrogen atom transfer (HAT) reactions and electron transfer (ET) reactions[92]. Oxygen radical absorbance capacity is an example of a HAT-based method, where antioxidants and substrates (fluorescein probes) dynamically compete with hydroxyl radicals. Trolox equivalent antioxidant capacity is commonly associated with ET reactions[93]. In the context of evaluating LPs and consistent with general practice in antioxidant peptide research, radical scavenging assays (including hydroxyl radical, DPPH radical, and ABTS cation radical scavenging activities) are frequently employed. Furthermore, metal ion chelating assays, particularly the iron ion chelating assay, are conventionally used to assess their secondary antioxidant potential.

LPs are foodborne animal peptides with strong antioxidant activity. Studies on antioxidant activity of LPs showed that LPs prepared by enzymatic hydrolysis of papain had good antioxidant activity *in vitro*. Using GSH as the control, the authors studied the ability of LPs to scavenge DPPH and hydroxyl radicals, chelate Cu^{2+} and inhibit lipid peroxidation in linoleic acid emulsion system. It was found that the scavenging capacities of hydroxyl group and DPPH radical of LPs had 2.6- and 2.4-times higher than those of GSH, respectively. Cu^{2+} chelating ability is 1.8 times that of GSH. Although there was no significant difference between LPs and GSH in lipid peroxidation inhibitory activity, the average molecular weight of LPs was 4 times that of GSH, that is to say, LPs had stronger lipid peroxidation inhibitory activity than GSH at the same molecular concentration[41]. Lipid peroxidation is generally regarded as an indirect reflection of FRs activities in the system, because lipid peroxidation is carried out by FRs mediated extraction of hydrogen atoms from methylene carbon in PUFAs[94]. Therefore, inhibiting the reaction process of lipid peroxidation can also play a role in removing FRs to a certain extent. In addition, the *in vitro* antioxidant test showed that loach had good antioxidant activity, but could it still obtain good effect *in vivo* antioxidant test? Does the gastrointestinal tract and other digestive system affect the antioxidant activities of LPs? This needs more in-depth research and discussion. In the *in vivo* antioxidant experiment, the high-dose LPs group showed increases of 36.6%, 28.1%, and 22.3% in SOD activities in the serum, liver, and heart, respectively, compared with the model control group. GSH-Px activities were increased by 43.2%, 132.2% and 92.5%. The contents of MDA were decreased by 16.2%, 18.3% and 26.2%, respectively[67]. This indicates that LPs can inhibit the damage caused by oxidation to a certain extent and improve the antioxidant capacity of the body.

Another study showed that loaches had good antioxidant capacity with or without gastrointestinal digestion. Interestingly, the antioxidant capacity of loaches simulated after gastrointestinal digestion *in vivo* increased[69]. It may be that the peptide configuration was changed after being treated by enzymes in the gastrointestinal tract, which makes it easier to participate in the reaction, or it may be that the treated peptide releases more free amino acids and other substances, which is more conducive to the full effect[95]. *In vivo* studies, LPs can increase the activities and content of GSH-Px, SOD and CAT in the body. It also reduces the contents of MDA. The accumulation of MDA causes certain damage to the cell membrane, resulting in membrane lipid peroxidation, damage to the structure of biofilm, damage to the structure and function of the cell membrane, change the permeability of the membrane, and thus affect the normal progress of a series of physiological and biochemical reactions. In addition, DNA is the main target of ROS-mediated oxidative damage. However, LPs can protect DNA from damage induced by hydroxyl radicals[96]. In spite of this, the animal studies on LPs antioxidant are limited. In addition, the lack of safety studies on LPs, high cost and low bioavailability of LPs are also existing challenges. However, given the good functional value of loaches, more research is needed to address the issues in LPs antioxidants and overcome the challenges that limit their transfer from the laboratory to practical applications.

3.2 Anti-fatigue activity

Fatigue is a term used to describe the general feeling of tiredness, decreased muscle strength, and even cognitive impairment caused by a variety of reasons, such as high intensity physical work or prolonged heavy mental work[97–98]. It was reported that most adults would experience a period of chronic fatigue. However, fatigue can lead to anxiety and depression and can manifest in a variety of symptoms such as cognitive impairment, poor sleep quality, and physical dysfunction[99]. In severe cases, fatigue may cause a variety of diseases related to physiological regulation and the immune system such as Alzheimer's disease, cancer, multiple sclerosis and Parkinson's disease, which can seriously affect the quality of life and productivity of patients. Research showed that high blood pressure, diabetes, anemia, liver disease and other diseases can also cause fatigue[100–101]. Fatigue is a physiological phenomenon with complex etiology, which requires long-term drug treatment. However, the chemicals currently used could have toxic and harmful side effects in the course of long-term treatment[102]. Some natural foods have been shown to have significant anti-fatigue effects, such as *Astragalus*[103], *Rhodiola*[104], corn[105], mackerel[70]. Due to the advantages of wide source, easy extraction and high safety, anti-fatigue peptides have become a research hotspot. Therefore, the search for safe and efficient natural drugs has become an important task of anti-fatigue research.

In the process of studying the causes of fatigue, exhaustion theory, blocking theory and radical theory have attracted much attention. According to the exhaustion theory, during prolonged exercise, glycogen, which is the source of energy, is constantly consumed, and the concentration of glucose decreases, leading to fatigue. Blocking theory believes that during strenuous exercise, LA and BUN and other energy metabolism products are not timely metabolic clearance, resulting in excessive accumulation of metabolites, muscle capacity decline, resulting in physical fatigue. Radical theory holds that there is a FRs control system in the body. If the body cannot maintain FRs at a certain level, it will cause damage to the body's vital activities. Many studies have shown that vigorous exercise leads to sharp increases in FRs levels. However, damage can occur if the body fails to remove excess FRs, which can

attack large molecules, cells, tissues and organs and cause peroxidation damage to the body[106–107]. As peptides have good antioxidant activity, Wang et al.[108] believe that peptides and other antioxidants can counteract exercise-induced oxidative stress and enhance the body's antioxidant defense. In addition, there are also theories that peptide targeting activates related signaling pathways p-AMPK/SIRT1/PGC1-α to achieve anti-fatigue effects[109–110]. There are different theories about how fatigue occurs, but researchers have found that active peptides have antioxidant properties that reduce ROS damage and reduce fatigue.

LPs have been shown to relieve exercise-induced fatigue. LPs delay fatigue by participating in regulating energy metabolism, reducing reactive oxygen content, reducing the level of FRs and regulating nerve signal transduction of neurotransmitters. Experiments have shown that LPs can extend the swimming time of mice under fatigue state by about 30%–50% compared with the control group. This may be related to the fact that LPs can improve the body's exercise metabolism and promote energy metabolism by increasing the body's glucose and liver glycogen levels. LPs can reduce the accumulation of metabolites and fatigue by increasing the metabolism of LA and BUN. In addition, LPs can improve the antioxidant capacity of mouse endogenous cells by increasing the activities of SOD, CAT, GSH-Px and other antioxidant enzymes. As a result, LPs can improve muscle capacity and endurance and promote fatigue recovery[41]. Another study on the relationship between the degree of hydrolysate of loach protein and its anti-fatigue activity suggested that: LPs with a hydrolysis degree of (21.00 ± 0.21)% mainly contained 65.41% of 1 000–3 000 Da peptides, while LPs with a hydrolysis degree of (35.00 ± 0.32)% mainly contained 58.27% of 500–1 000 Da peptides. Although LPs with different degrees of hydrolysis exhibited notable anti-fatigue effects, LPs with a lower degree of hydrolysis showed superior anti-fatigue activity, which was highly consistent with their antioxidant capacity, as supported by Pearson correlation analysis. This indicated that anti-fatigue activity is highly correlated with antioxidant activity[111]. These results provide an important basis for the development of LPs as an anti-fatigue functional food and drug. However, the evaluation of its anti-fatigue activity and anti-fatigue mechanism at cellular and molecular levels remains to be further investigated.

3.3 Antibacterial activity

The rising trend of global mortality in recent years is closely related to microbial infection. Although most bacterial infections are treated with antibiotics first, antimicrobial resistance affects the effectiveness of antibiotics to a certain extent. Therefore, it is urgent to seek new antimicrobials[112]. Antimicrobial peptides are a class of small molecular peptides with antimicrobial activity. Antimicrobial peptides present in the human body form part of the first line of defense for inactivated pathogens. Antimicrobial peptides play an antibacterial role by destroying the cell membrane structure of microorganisms and regulating the immune response of human body[113]. However, due to low levels of antimicrobial peptides in the body, it is necessary to get some natural food peptides in the diet that also have antibacterial properties[114].

Antimicrobial peptides usually contain less than 50 amino acids, nearly 50% of which are hydrophobic and have molecular weights below 10 kDa. On the one hand, antimicrobial peptides can kill bacterial pathogens by means of membrane penetration; on the other hand, they can also damage the structure and function of cell wall and other organelles by directly acting on bacterial cell wall or intracellular targets[115]. And in addition to killing pathogens directly, antimicrobial peptides also activate the body's immune system[116]. Antifungal peptides inhibit the synthesis of the main components of fungal cell walls. The biosynthesis of chitin, 1,3-β-glucan is important for the integrity of fungal cell walls. In addition, antifungal peptides can also disrupt cell structure and function by improving membrane permeability and pore formation, thus triggering signaling cascades that lead to cell death[117]. The mechanism of antibacterial peptides is relatively complex, and the research on antibacterial properties of peptides is still relatively shallow.

LPs have antibacterial activity. The main component of the mucus on the surface of loach is collagen, which has a short molecular structure and relatively small molecular weight. Mucus is a product of the organism's stress response to external stimuli on the body surface. It's an act of self-preservation. The mucus has certain antibacterial and immune-enhancing effects[118]. Similar to loach mucus, Su[72] isolated and identified a novel antimicrobial peptide containing 20 residues from the skin mucus of yellow catfish, with the amino acid sequence GKLNLFLSRLEILKLFVGAL. This antimicrobial peptide has broad-spectrum inhibitory activity against a variety of bacteria and has no hemolytic activity. Studies have shown that LPs have obvious antibacterial activity against *B. subtilis*, *E. coli*, *S. aureus* and other bacteria[18]. Interestingly, in the process of aquaculture, aquatic products are easily contaminated by bacteria and fungi, resulting in a decline in the output and quality of aquatic products. However, according to relevant studies, loach could exert antibacterial effects even if it is not treated with antibacterial manual intervention in the breeding process. Hepcidin protein encoded by HAMP and C-type lectins exhibit antibacterial activity[73]. Loach possesses at least two allelic forms of HAMP, which are expected to encode the same antimicrobial peptide, hepcidin protein. Generally, hepcidin content is higher in liver, spleen and kidney. *In vitro* antibacterial experiments showed that hepcidin had good antibacterial activity. In addition, hepcidin also has a regulatory effect on intestinal flora, which can maintain the diversity of intestinal flora and regulate the composition changes[119]. In summary, LPs have good antibacterial properties, which may help in the development of new treatments for infections associated with pathogens such as bacteria and fungi in humans and animals.

3.4 Immunomodulatory and antiproliferative activities

The body's innate immune system is the host's defense network system, which is composed of many immune cells, tissues and organs. The immune system gives priority to fighting potentially harmful substances and pathological or dead cells. The immune system is stimulated to trigger an immune response and build up an immune memory against dangerous foreign invading antigens. The immune regulation of the body is related to the effect of physiological health care and disease prevention[120–122]. In general, cancer is closely related to an imbalance in the immune system. In short, the immune system is insensitive to abnormal cells or unable to eliminate abnormal cancer cells, allowing them to grow and proliferate uncontrollably and further spread to surrounding tissues, causing great harm to the body. At present, some anticancer drugs used in clinical practice, as well as radiotherapy and chemotherapy treatment, will cause toxic side effects on the body, but also produce high-cost pressure[123–124]. In recent years, targeting related signaling pathways to regulate the expression of

cancer-related genes has become a hot topic in cancer prevention and treatment. By targeting the PI3K/AKT/mTOR and Wnt/GSK3β/*β*-catenin signaling pathways, therapeutic goals can be achieved and problems of tumor drug resistance can be resolved, thus exerting synergistic therapeutic effects with traditional therapies[125–127]. Therefore, how to regulate human immune system more safely and effectively and prevent and treat cancer is a hot topic in today's research. Foodborne peptides have attracted much attention because of their safety and gentleness.

Loach is a popular tonic food. LPs have rich functions of regulating immune system. Therefore, lipopolysaccharide-induced RAW264.7 was used to evaluate the immunomodulatory capacity of LPs. It was found that loach hydrolysate obtained by protease hydrolysis significantly inhibited the production of NO and the expression of inducible nitric oxide synthase mRNA[118,128]. LPs can activate NF-κB and MAPKs signaling pathways, reduce the release of inflammatory cytokines TNF-α, interleukin (IL)-1β and IL-6, and improve the immune response mediated by T lymphocytes in the body[129]. The immunomodulatory peptides of loach have potential development prospect. However, there is still much work to be done, such as the exploration of immunomodulatory peptide abundance, the construction of the relationship between structure and function, and the research of immunoregulatory peptide targeting enzymatic hydrolysis technology[130]. However, studies on immunomodulatory peptides in protein-rich foods such as sea cucumber and egg have been relatively mature, which can provide new ideas and reference value for LPs research.

The anti-tumor mechanism of peptides mainly includes: peptides promote the lysis of cancer cell membrane, peptides can cleave mitochondrial membrane, and peptides achieve non-membrane anticancer effect by regulating immune response and basic metabolic process of the body[131–132]. You et al.[25] reported that LPs, molecular mass < 3 kDa (40 mg/mL) prepared by papain digestion had a significant inhibitory effect on the *in vitro* growth of HepG2, MCF-7 and Caco-2 cells, and the cancer cell proliferation rate could be stabilized at about 4%–7%. This suggests that LPs are potential treatments for liver, breast and colon tumors. In addition to its anti-cancer activity, LPs can also prevent the spread of cancer cells. Colorectal cancer is the most common malignant tumor in the gastrointestinal tract. The effective prevention and treatment of colorectal cancer has become the focus of preclinical and even clinical research. LPs can promote the apoptosis of human colon cancer cell HCT116 and increase the oxidative damage of cancer cell DNA, thereby preventing cancer cell proliferation[120]. There are relatively few studies on LPs anti-cancer. Some amino acids also play a crucial role in anti-cancer activities[133]. However, the molecular mechanism, specific action and biosafety of LPs anticancer are still the key challenges for future research.

3.5 Other activities

In addition to the above bioactive functions, LPs have also achieved certain effects in the study of other functional properties, such as inhibiting hypertension and preventing and treating osteoporosis. ACE inhibitory pathway, renin inhibitory pathway and calcium channel blocking system are the main mechanisms of antihypertensive regulation[134]. In recent years, researchers have carried out research on LPs which inhibits ACE. LPs, which inhibit ACE, block the conversion of Ang I to Ang II, thereby preventing vasoconstriction in arterial walls and arterioles[135]. Elevated levels of Ang II can lead to symptoms of hypertension, inflammation and oxidative stress. However, polypeptide-mediated PPAR-γ, caspase-3 and MAPK signaling pathways promotes the production of NO by up-regulating the activity of eNOS, which can play a role in alleviating hypertension[136]. Loach protein had higher ACE inhibition activity after hydrolysis. The half maximal inhibitory concentration (IC_{50}) of molecular mass < 2.5 kDa LPs was (231.2 ± 3.8) μg/mL after ultrafiltration. Then, the purified LPs were separated and purified by gel filtration chromatography and RP-HPLC, and the IC_{50} was (18.2 ± 0.9) μg/mL. The ACE inhibitory activity was 33.7 times higher than that of crude hydrolysate. The peptide was identified as Ala-His-Leu-Leu (452 Da) by uadrupole time-of-flight mass spectrometry and amino acid analyzer. Oral administration of high purity LPs can significantly reduce systolic blood pressure in rats with spontaneous hypertension[137].

Osteoporosis is characterized by low mineral content in bone and degeneration of bone tissue, which can be caused by aging, poor nutrition, endocrine disorders and other causes that lead to weak bones. Studies have found that the causes of osteoporosis are also related to the disorder of related signaling pathways. Bone morphogenetic protein 3 and other negative regulatory factors related to bone activate the OPG/RANKL/NF-κB signaling pathway, and the downstream osteoclast gene *NFATC1* of this pathway is activated correspondingly[138]. However, peptides achieve bone health benefits by regulating the function of bone density and microstructure[139]. Liu et al.[80] studied the effects of collagen peptides in loach skin on osteoporosis in mice. The results showed that LPs alleviated osteoporosis in mice. LPs can improve bone microstructure, increase the quantity and quality of trabeculae bone, increase bone strength by 40%–50%, and maintain blood calcium and phosphorus balance, which is about 30% higher than that of the model group. This study provides a good direction for the prevention and treatment of osteoporosis.

4 Bioinformation technology in peptides research

The study of bioactive peptides usually includes extraction or hydrolysis of proteins, separation and purification of peptides, characterization and identification of peptides, and assessment of bioactive functions. Recently, however, bioinformatics approaches have been increasingly employed in peptide research, offering a more economical and time-saving alternative to traditional experimental methods[140–141]. With the development of protein and peptide research, there are many related databases such as National Center for Biotechnology Information and UniProt knowledge base for *in silico* analysis[142]. Protein sequence information can be obtained through computational analysis, and online bioinformatics tools are also available to simulate protein hydrolysis and predict potential bioactive fragments[143–144]. Several web-based tools, including ProtParam, SwissADME, ToxinPred and AllerTOP, are commonly used to predict the physicochemical properties, allergenicity, and toxicity of peptides[145]. For peptides with unknown biological activities, PeptideRanker is generally used to predict the potential biological activities possessed by peptides[146]. Macromolecular docking, one of the most important molecular simulation techniques, is a computational method that simulates the recognition process between two or more molecules[147]. Tools or programs such as AutoDock, SP-PEP, Glide and HPEPDOCK have been widely used to screen peptides and explore the molecular mechanisms underlying their biological functions[148]. In summary, the extensive application of bioinformatics technology in protein and peptides research is an unprecedented progress in the history of

biological research, which can achieve valuable research results while saving human and material resources.

Although *in silico* analysis has only recently begun to be applied to the study of LPs, its application to other animal-derived peptides has been extensively reported, particularly in predicting and analyzing the structure, functional activities and interactions of protein precursors and peptides, and even in identifying proteolytic enzymes and active sites to explore the feasibility of peptide preparation or synthesis[142]. Based on network pharmacology, molecular docking, and bioinformatics analysis, Ge et al.[149] investigated the potential multi-component synergistic pharmacological mechanism of egg white peptides. Through *in silico* analysis, four bioactive peptides were identified, which had 52 potential interaction targets related to acute colitis. Further analysis revealed that these targets were involved in more than 30 molecular signaling pathways closely related to life activities. Yathisha et al.[150] used molecular docking technology to obtain a non-toxic ACE-inhibitory peptide with favorable binding affinity to target proteases from the hydrolysate of ribbon fish protein, and further employed molecular dynamics simulation to explore the structural stability and enzyme inhibition properties of the peptide-ligand complexes. Gomez et al.[151] applied *in silico* analysis to predict possible biological functions of Portuguese oyster (*Crassostrea angulata*) protein hydrolysate prior to the preparation of bioactive peptides. According to the BIOPEP-UWM database, *C. angulata* protein hydrolysates contained precursors of bioactive peptides with dipeptidyl peptidase IV and ACE inhibitory activities. Notably, the predicted bioactivities were largely consistent with subsequent *in vitro* validation results, although *in vivo* or clinical studies are still required to confirm their safety. In addition to the above applications, *in silico* analysis has also been used in the research of other foodborne animal peptides, such as the prediction and analysis of antimicrobial peptides in goat milk protein by computer bioinformatics technology[152], and antioxidant peptides in chicken protein hydrolysate[153]. Computer integrated analysis was used to predict and analyze peptide sensitization in milk[154]. In conclusion, the application of *in silico* analysis to the study of bioactive peptides is not new, and computer analysis has been recognized to play an important role in the process of generation and identification of bioactive peptides.

Nevertheless, although the application of *in silico* approaches to LPs is still in its infancy, their value in bioactive peptide research has been well established in recent years, as comprehensively reviewed by Rivero-Pino et al.[155]. These computational approaches offer rapid and cost-effective screening of a large number of peptide sequences, providing a useful complement to time-consuming and expensive experimental assays. Beyond screening, computational design strategies have also been developed to generate novel peptide sequences *de novo* with desired properties, as demonstrated by Bergman et al.[156] who employed a Monte Carlo-based algorithm (PepBD) to design peptides with high predicted affinity for common plastics. For LPs, *in silico* tools could play multiple roles in addressing current research bottlenecks. First, online proteolysis simulation platforms can predict potential peptide fragments released from loach proteins under specific enzymatic conditions[143–144]. Second, structure prediction tools such as PEP-FOLD3, combined with molecular docking, can model peptide-target interactions, helping elucidate the structure-activity relationships that remain unclear for most LPs. Third, bioactivity prediction servers such as PeptideRanker can prioritize candidate peptides with high predicted activity for subsequent experimental validation, thereby reducing the cost and uncertainty of wet-lab screening[146]. It should be noted, however, that *in silico* predictions cannot replace *in vitro* or *in vivo* validation; rather, they should be considered as a guiding tool for initial screening[155]. These computational strategies have recently shown initial success in LPs research; for instance, Chu et al.[19] employed bioinformatics screening and molecular docking to identify 8 candidate antioxidant peptides from 2 289 sequences derived from ultrasound-assisted enzymatic hydrolysis of loach protein, with FG-14 exhibiting the highest binding affinity to Keap1-Nrf2 (−86.43 kJ/mol). In a subsequent study, the same research group integrated peptidomics and molecular dynamics simulation to identify and validate a novel antioxidant peptide Tn from loach[20]. Collectively, these examples demonstrate that *in silico* approaches offer a promising avenue for accelerating the discovery and functional characterization of novel LPs.

5 Deep-processing and high-value utilization of LPs

While the aforementioned *in silico* approaches facilitate the rational screening of bioactive peptide candidates, the transition from laboratory-scale discovery to industrial application still faces several challenges, including process scalability, product stability, and the validation of practical efficacy. The high-value utilization of LPs therefore needs to be considered across multiple dimensions. Beyond antioxidant properties, Dou et al.[157] demonstrated that LPs exhibit multi-target bioactivities, including ACE inhibition (D-4, IC_{50} = 95.07 μg/mL) and pancreatic CE inhibition (D-2, IC_{50} = 3.19 mg/mL), endowing them with great potential for the development of functional foods targeting hypertension and hypercholesterolemia. Apart from human health-oriented food products, loach-derived antimicrobial peptides also show great commercial value in the aquaculture industry with clear industrialization paths. Jin et al.[21] developed a synthetic hepcidin peptide (Ma-sHep) expressed in *Pichia pastoris* as a feed additive; this synthetic hepcidin peptide exhibited potent broad-spectrum antibacterial activity (MIC = 6 μg/mL), excellent thermal and pH stability. When supplemented in feed, it reduced loach mortality upon *A. hydrophila* challenge from 100% to below 50% and optimized intestinal microbiota composition, verifying its capacity as a safe and effective substitute for traditional antibiotics. The comprehensive utilization of loach processing by-products is another promising high-value development route. Furthermore, Mao et al.[158] systematically proposed the application of LPs as multifunctional raw materials, including functional food ingredients, dietary nutritional regulators and natural food preservatives. Importantly, the integration of peptidomics, bioinformatics screening and molecular simulation, as reported by Chu et al.[20] for the identification of antioxidant peptide Tn, establishes an efficient technical framework for high-throughput mining of bioactive peptides and lays solid technical groundwork for linking laboratory exploration with the follow-up industrial development of loach-derived peptides. Collectively, the diversified high-value application scenarios summarized in this section make up for the deficiency of previous reviews that merely focused on upstream preparation and *in vitro* activity evaluation, and systematically sort out feasible research directions for the deep processing and industrial transformation of LPs.

6 Conclusions and challenges

The pretreatment, enzymatic digestion, purification, identification and bioactivity evaluation of LPs, as well as the current challenges are discussed in this review. LPs are commonly prepared by enzymatic hydrolysis, particularly using papain, and purified LPs have been reported to exhibit various bioactivities, including antioxidant, anti-fatigue, antibacterial, immunomodulatory, anticancer, antihypertension and anti-osteoporotic activity. The bioactivity of LPs is a subject of significant interest in the study of medicine-food homology. Loach-derived products and by-products, including LPs, show potential applications in functional foods, pharmaceutical supplements, and aquaculture-related products. Although considerable progress has been made in LPs research, there are still some challenges to be solved.

LPs face inherent limitations in their preparation, bioactivity research, and commercial application, which critically shape their future development trajectory. Specifically, optimizing the hydrolysis efficiency and ensuring the production safety of LPs are paramount during the preparation process. Moreover, intensified research into potential toxic and allergenic components within LPs is imperative for comprehensively addressing safety concerns. Furthermore, in the evaluation of biological functional activity, a focused investigation into the precise structure and amino acid sequence of LPs is crucial for accurate and reliable assessment.

The relationships among LPs structure, amino acid sequence, and biological function, rather than simply focusing on the protein mixture used for digestion, need to be explored more deeply in the future. The molecular mechanisms of LPs functional activity in the process of absorption and metabolism in humans need to be further elucidated. Looking forward, the successful translation of LPs into high-value products requires coordinated efforts in several key areas. These include the optimization of cost-effective and scalable preparation processes that maintain bioactivity, the elucidation of structure-activity relationships through integrated experimental and *in silico* approaches, rigorous safety and efficacy evaluation through *in vivo* and clinical studies, and the development of formulation strategies that ensure stability and bioavailability in end-use applications. Addressing these challenges will be essential to realize the full potential of LPs as next-generation functional ingredients in the food, pharmaceutical, and aquaculture industries.

Conflict of interest

There are no conflicts of interest.

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