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Recent advances in the effect of simulated gastrointestinal digestion and encapsulation on peptide bioactivity and stability

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Food-derived bioactive peptides have garnered significant attention from researchers due to their specific biological functions, including antihypertensive, antioxidant, antidiabetic, anticancer, anti-inflammatory, and anti-osteoporosis properties. Despite extensive *in vitro* research, the bioactivity of these peptides may be compromised in the gastrointestinal tract due to enzymatic hydrolysis before reaching the bloodstream or target cells. Therefore, understanding the fate of bioactive peptides during digestion is crucial before advancing to clinical trials and commercial applications. To exert their health-promoting effects, these peptides must maintain their bioactivity throughout digestion. Encapsulation has emerged as a promising strategy for protecting peptides in the gastrointestinal tract. This review examines the effects of *in vitro* simulated gastrointestinal digestion on peptide bioactivity and stability, highlighting recent research on encapsulation strategies designed to enhance their gastrointestinal stability. Furthermore, the review addresses existing research gaps and suggests future research directions to advance our understanding and the application of bioactive peptides.

1. Introduction

Food-derived bioactive peptides are fragments of 2–20 amino acid residues.[1] Beyond basic nutrition, these peptides exhibit various bioactivities, including antihypertensive,[2] antioxidant,[3] anticancer,[4] antidiabetic,[5] anti-inflammatory,[6] and anti-osteoporosis[7] properties (Fig. 1). They are released from parent proteins through protease hydrolysis, fermentation, or germination.[8] Parent proteins can be obtained from animals, plants, microorganisms, and their derivatives[9] (Fig. 1), providing abundant sources of peptides. Unlike synthetic drugs, which may lead to severe side effects,[10] bioactive peptides offer promising health benefits as natural alternatives, due to their low molecular weight, low toxicity, selectivity, and high bioactivity.[11,12]

Current research on bioactive peptides predominantly focuses on *in vitro* studies, where the efficacy observed does not always correlate with their *in vivo* functionality. This discrepancy is primarily due to the degradation of bioactive peptides by digestive enzymes in the gastrointestinal tract, resulting in a loss of bioactivity. Once ingested, bioactive peptides will encounter numerous digestive proteases and peptidases. These include pepsin, trypsin, chymotrypsin, elastase, carboxypeptidases A/B, and brush border peptidases such as several aminopeptidases and carboxypeptidase M.[13] Pepsin preferentially hydrolyzes peptide bonds at the carboxyl-terminal Phe, Leu, Tyr, and Trp residues.[14] Trypsin, an endopeptidase, tends to cleave peptides at the carboxyl-terminal Arg and Lys residues.[13] Chymotrypsin, a serine protease, cleaves aromatic amino acid residues like Trp, Tyr, and Phe, except when adjacent to Pro.[15] Elastase has an affinity for uncharged residues such as Ala, Gly, and Ser.[16] Carboxypeptidases are metalloproteinases with a zinc atom in their active site. Pancreatic carboxypeptidases include types A and B. Carboxypeptidase A hydrolyzes peptide bonds involving aromatic amino acids at the carboxyl-terminal end, whereas carboxypeptidase B cleaves Lys or Arg residues from the same terminus.[13] Fortunately, the stability of bioactive peptides can be improved through encapsulation. This method converts sensitive materials into stable components, effectively shielding them from gastrointestinal digestion.[17]

Human and animal *in vivo* digestion models generally involve *in vivo* imaging[18] and wireless telemetry techniques.[19] Although these models provide accurate physiological data on the digestion of bioactive peptides, they involve long research periods, high costs, and ethical concerns related to surgical

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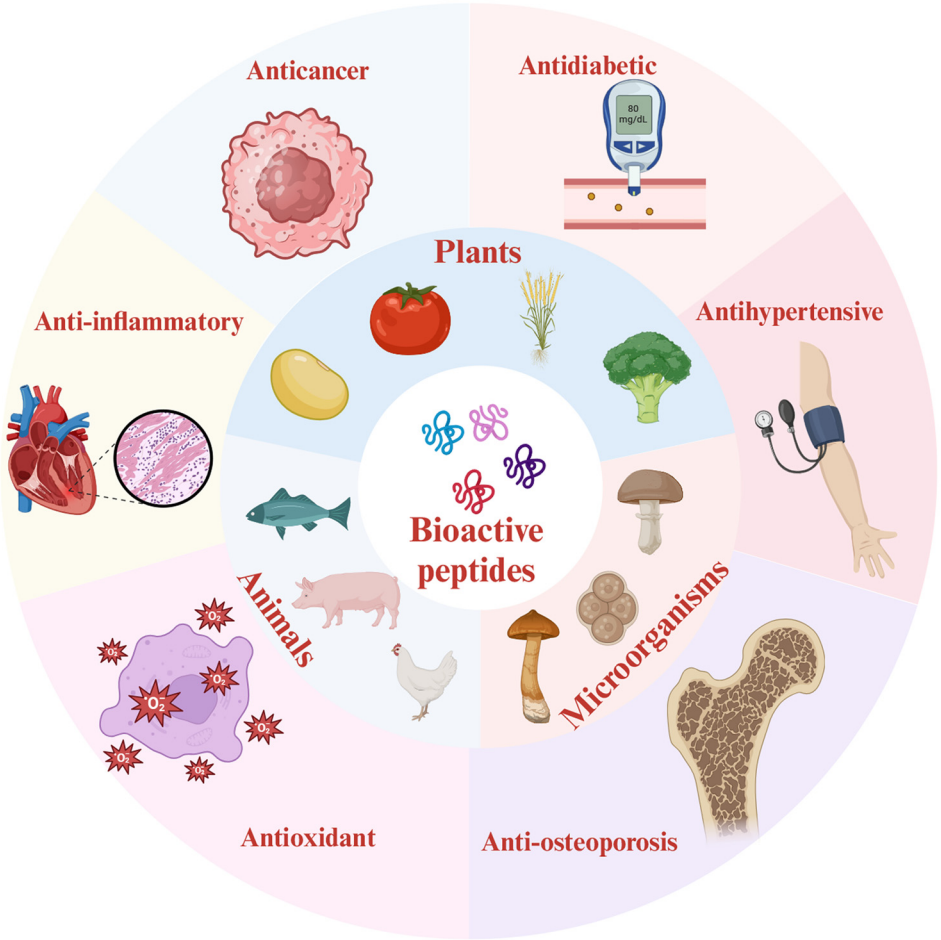

Fig. 1 The biological functions and sources of bioactive peptides.

approaches and animal mortality.[20] Consequently, *in vivo* digestion models present practical research challenges. To overcome these limitations, several *in vitro* static and dynamic digestion models have been developed and optimized to assess the effect of gastrointestinal digestion on peptide bioactivity and stability in recent years. Among these, static digestion models are the most widely used due to their ease of operation, lack of specific equipment requirements, and low cost.[20] INFOGEST is a standardized and practical static digestion method.[21] In this method, during the oral phase, solid foods are minced to simulate mastication. The oral digestion phase for liquid foods is optional. Subsequently, the mixture is prepared by combining simulated salivary fluid, (SSF) with solid or liquid food at a 50 : 50 (v/w and v/v) ratio. The mixture contains salivary alpha-amylase and $CaCl_2$ at concentrations of 75 U mL^{-1} and 0.75 mM, respectively, with water added as necessary to dilute the stock solution of SSF. The mixture is incubated at 37 °C for 2 min. During the gastric phase, under standard conditions, the final ratio of simulated gastric fluid (SGF) (containing 0.075 mM $CaCl_2$ and pepsin with an activity of 2000 U mL^{-1} in the final digestion mixture) to liquid food or oral bolus is 50 : 50 (v/v), at a digestion time of 2 h, a temperature of 37 °C, and a pH of 3. During the intestinal phase, the pH is adjusted to 7, the temperature remains at 37 °C, and the digestion time is also 2 h. The final ratio of simulated intestinal fluid (SIF) to gastric chyme is also 50 : 50 (v/v). The enzyme in SIF can be a porcine pancreatic enzyme or individual enzymes, with the final digestion mixture containing bile salts (10 mM), $CaCl_2$ (0.3 mM), chymotrypsin (25 U mL^{-1}), and trypsin (100 U mL^{-1}). On this basis, an enhanced version, INFOGEST 2.0, was developed and detailed in a 2019 article published in *Nature Protocols*. Unlike INFOGEST, INFOGEST 2.0 requires the inclusion of the oral digestion phase to ensure consistent dilution of food, regardless of whether the food is solid or liquid. Additionally, INFOGEST 2.0 introduces gastric lipase, significantly impacting fat digestion.[22] Although static digestion has many advantages, it still presents limitations such as maintaining constant pH and enzyme activity, a lack of gastric emptying, and non-gradual addition of digestive fluids. Therefore, static digestion is only suitable for assessing digestion endpoints rather than the digestion process itself. Hence, the recently developed semi-dynamic digestion protocol has enhanced the secretion of digestive fluids, pH control, and gastric emptying compared to the static method. In this

protocol, SGF is gradually added. The pH is adjusted by adding HCl, reaching pH 2 by the end of gastric digestion. Gastric emptying occurs at a linear rate, and this protocol recommends delivering calories to the intestinal phase at a rate of 2 kcal min^{-1}.[23] However, neither static nor semi-dynamic digestion methods can accurately simulate gastrointestinal peristalsis and the internal structure of the gastrointestinal tract. Therefore, in recent years, researchers have developed numerous dynamic digestion models that not only simulate the continuous secretion of digestive fluids, dynamically adjust pH, and facilitate gastric emptying, but also replicate the real environment and peristalsis of the gastrointestinal tract.[24] Nevertheless, due to cost and operational limitations, dynamic digestion models are not as widely applied as static digestion models.

Understanding the fate of bioactive peptides during digestion is crucial before proceeding to clinical trials and commercial applications. In brief, these peptides must maintain their bioactivity, determining whether they can be broadly used in functional foods or nutraceuticals. This review summarizes the effect of *in vitro* simulated gastrointestinal digestion on the bioactivity and stability of various bioactive peptides and explores recent research on encapsulation methods to enhance their gastrointestinal stability.

2. The effect of gastrointestinal digestion on peptide bioactivity and stability

Bioactive peptides, originating from various sources and having different functions and molecular sizes, may experience different bioactivity changes during gastrointestinal digestion. Table 1 shows the effect of *in vitro* simulated gastrointestinal digestion on peptide bioactivity and stability, emphasizing its role as the initial barrier to the *in vivo* effectiveness of biopeptides. The following review is organized according to the functional classification of bioactive peptides.

2.1 ACE inhibitory peptides

ACE is associated with two blood pressure systems: the renin–angiotensin system (RAS) and the nitric oxide system (NOS). In the RAS, angiotensinogen (AGT, DRVYIHPFHLVI) is cleaved by renin to form the inactive decapeptide angiotensin I (ANG I, DRVYIHPFHL). ANG I is then hydrolyzed by ACE to form the bioactive octapeptide angiotensin II (ANG II), a potent vasoconstrictor that causes blood vessel constriction, thereby increasing blood pressure.[57–59] This process is crucial for blood pressure regulation. In the NOS, bradykinin (BK) binds to β-receptors, increasing intracellular Ca^{2+} levels, which stimulates endothelial nitric oxide synthase (eNOS) to convert L-arginine into nitric oxide (NO).[60] NO is a vasodilator that aids in maintaining normal blood pressure.[61] However, ACE can deactivate BK, resulting in insufficient vasodilation and increased blood pressure.[62]

Although synthetic ACE inhibitors like lisinopril, enalapril, benzapril, fosinopril, and captopril[57] are effective at lowering blood pressure, their use as therapeutic agents is limited by side effects like taste disturbances, skin rashes, angioedema, hypotension, and dry cough.[15] Food-derived ACE inhibitory peptides are considered natural and safer alternatives to synthetic drugs. However, they must either maintain their intact structure or be hydrolyzed into active products during gastrointestinal digestion, then be absorbed by the intestines to reach the cardiovascular system and exhibit their bioactivity.[63]

Some ACE inhibitory peptides' bioactivity remains unchanged or increases after gastrointestinal digestion. Ma *et al.*[2] hydrolyzed *Moringa oleifera* (MO) leaf using Alcalase enzymes, obtaining two ACE inhibition peptides, LGF and GLFF, with IC_{50} values of 0.29 ± 0.13 mM and 0.31 ± 0.04 mM, respectively. Both LGF and GLFF resisted intact gastrointestinal digestion, indicating that their bioactivity remained unchanged after digestion. Their ACE inhibitory activity and gastrointestinal stability might be due to their relatively high content of hydrophobic amino acids, such as Phe and Leu. A novel ACE inhibitory peptide, LLLLPKP (IC_{50} = 10.22 μM), isolated from yeast extract, was hydrolyzed into smaller peptides, particularly LLLLPK, LLLL, and LPKP, which also showed potential ACE inhibitory activity, during simulated gastrointestinal digestion. Its overall activity was not significantly affected. The ACE inhibitory activity of LLLLPKP and its digestion products might be attributed to their short chain lengths and the hydrophobic amino acid Leu.[64] KYPHVF, an ACE inhibitory peptide, remained intact when exposed to gastrointestinal proteases. Its ACE inhibitory activity was unaffected during digestion, demonstrating its stability. Additionally, 44 potential antihypertensive targets were identified through network pharmacology, suggesting that KYPHVF was a multi-target, multi-pathway antihypertensive peptide.[65] A recently identified ACE inhibitory peptide (NDRP) from soybean protein exhibits high stability during digestion. The activity of the digested sample only decreased to 82.47 ± 1.07%, compared to 94% for the pure peptide.[66] Garcia-Mora *et al.*[67] discovered that pepsin and pancreatin digestion enhanced the ACE inhibitory activity of lentil-derived peptides by releasing smaller peptide fragments. For example, the IC_{50} of LLSGTQNQPSFLSGF decreased from 119.75 ± 3.04 to 21.25 ± 1.17 μM.

However, gastrointestinal digestion may decrease or even eliminate the ACE inhibitory activities of some peptides. A pea-derived tripeptide, LRW, could not lower the blood pressure of spontaneous hypertensive rats (SHRs) due to its low gastrointestinal stability.[68] The ACE inhibitory activities of two peptides from ovalbumin, YAEERYPIL and RADHPFL, decreased by approximately 100-fold after simulated digestion.[69] The ACE inhibitory peptides DKVGINY and KGYGGVSL were highly susceptible to gastrointestinal digestion, with their IC_{50} values significantly increasing after the action of pepsin and pancreatic extract. The IC_{50} of DKVGINY increased from 25.2 ± 1.0 to 254.5 ± 47.0 μg mL^{-1}, and the IC_{50} of KGYGGVSL increased from 0.8 ± 0.1 to 2.0 ± 0.1 μg mL^{-1}.[70]

Table 1 The effect of *in vitro* simulated gastrointestinal digestion on peptide bioactivity and stability

Bioactivity	Protein source	Enzyme used for hydrolysis	Hydrolysate name/peptide sequences	Results	Ref.
Angiotensin-converting enzyme (ACE) inhibitory	Tilapia (*Oreochromis niloticus*)	Properase E	LSGYGP	Maintain good digestive stability for 4 h	25
	Bitter almond albumin	Papain and thermolysin	RPPSEDEDQE	ACE-inhibitory capacity was not significantly reduced	26
	Walnut glutelin-1 hydrolysates	Pepsin	VERGRRITSV and FVIEPNITPA	ACE inhibitory rate was reduced to 76.9% and 70.3%, respectively	27
	Ginkgo seed globulin	Alcalase and thermolysin	EASPKPV	The IC_{50} (half maximal inhibitory concentration) of EASPKPV increased from 87.66 ± 2.96 mmol L^{-1} to 93.56 ± 7.38 mmol L^{-1}	28
	Oil palm kernel globulin	Papain	EVPQAYIP	71.23% of its activity was retained	29
	Oil palm kernel glutelin-2	Alcalase, flavourzyme, pepsin, and trypsin	VIEPR, LPILR, ADVFNPR and VVLYK	The activity of these peptides did not significantly decrease	30
	Naked oat bran albumin hydrolysates	Papain and flavourzyme	QYVPF and GYHGH	The IC_{50} values of digested QYVPF and GYHGH were 260.39 and 368.87 μmol L^{-1}, respectively, showing no significant difference from the untreated peptide values of 243.36 and 321.94 μmol L^{-1}	31
	Styela clava	Commercial proteases (flavourzyme, protamex, and kojizyme) and digestive proteases (pepsin and papain)	LWHTH	After digestion by pepsin, trypsin, and α-chymotrypsin, LWHTH maintained its ACE inhibitory effects with IC_{50} values of 22.31, 19.62, and 21.25 μM, respectively, compared to 16.42 ± 0.45 μM for undigested LWHTH, demonstrating high stability	32
	Spent hen protein	Protex 26L, pepsin, and thermoase	Spent hen muscle protein hydrolysate (SPH) prepared by protex 26L, pepsin, and thermoase (SPH-26L, SPH-P, and SPH-T)	The activity of SPH-T remained unchanged, whereas the activities of SPH-P and SPH-26L were reduced	33
	Foxtail millet protein (FMPH)	Alkaline	FPGVSPF, SPAQLLPF, LVPYRP, and WYWPQ	WYWPQ and LVPYRP exhibited superior gastrointestinal stability, maintaining 85.22 ± 2.06% and 83.22 ± 0.39% of their activity, respectively	34
	Larimichthys crocea protein (LCP)	Trypsin and papain	IPYADFK, FYEPFM, NWPWMK, LYDHLGK, INEMLDTK, IHFGTTGK	The activities of NWPWMK, FYEPFM, and IPYADFK remained nearly unchanged, whereas LYDHLGK, INEMLDTK, and IHFGTTGK showed a decrease in activity	35
Antioxidant	Millet bran glutelin-2	Papain	CFMTY, CTGTPYC, and RGLLLPSMSNAP	CFMTY (IC_{50} value of 2,2′-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) ($ABTS^{+}$) scavenging activity increased from 80.85 to 81.07 μmol L^{-1}) and CTGTPYC (IC_{50} rose from 87.78 to 92.73 μmol L^{-1}) remained stable, whereas RGLLLPSMSNAP (IC_{50} increased from 214.17 to 1236.01 μmol L^{-1}) was unstable	36
	Heads and bones of hybrid groupers	Alcalase	A fraction with a molecular weight of less than 1 kDa obtained from bone hydrolysate after ultrafiltration (HB-IV)	The hydroxyl radical ($HO^{\bullet}$) scavenging and reducing power were enhanced	37
	Carp (*Cyprinus carpio*) skin gelatin	Protamex® (novozymes) enzyme preparation	Unfractionated hydrolysate and AY	Retain the overall antioxidant effect	38
	Round scad	Protamex and pancreatin	WCPFSRSF	The content of WCPFSRSF dropped to less than 1% following gastrointestinal digestion, significantly impacting its antioxidant activity	39
	Coconut cake albumin	Trypsin, favourzyme, pepsin, and Alcalase	KAQYPYV, KIIIYN and KILIYG	The $HO^{\bullet}$ scavenging activities of KIIIYN and KILIYG decreased remarkably, while KAQYPYV could retain its antioxidant activity	40
	Spirulina sp. LEB 18	Protemax 580L	Protein hydrolysates obtained at the 90 and 120 min reaction time points (H90 and H120)	The activity of H90 remained unaffected, while the activity of H120 decreased	41
	Soybeans	Trypsin, papain, protamex, Alcalase 2.4L, and neutrase 0.8L	SFQSeM	SFQSeM remained mostly intact, with only 11.92% ± 2.76% hydrolysis observed	42
	Meat and visceral mass of marine snail *Neptunea arthritica cumingii*	—	YSQLENEFDR and YIAEDAER	The 2,2′-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical scavenging and ACE inhibitory activities of both peptides slightly increased	43
	Tomato seed proteins	Alcalase	The fraction of tomato seed protein hydrolysate (TSPH) (F2)	Peptides in fraction F2 had high gastrointestinal stability	44
	Wheat protein	Alkaline protease	Selenium-chelating polypeptides derived from wheat protein (WPH-Se)	WPH-Se showed resistance to gastrointestinal digestion and preserved its antioxidant effects	45

Table 1 *(Contd.)*

Bioactivity	Protein source	Enzyme used for hydrolysis	Hydrolysate name/peptide sequences	Results	Ref.
Antidiabetic	*Chlorella pyrenoidosa* protein	Trypsin and pepsin	FLKPLGSGK, QIYTMGK, FLFVAEAIYK and QHAGTKAK	QIYTMGK and QHAGTKAK resisted digestive enzymes, whereas FLKPLGSGK and FLFVAEAIYK were unstable	46
	Atlantic salmon (*Salmo salar*) co-product (trimmings and skin gelatin)	Flavourzyme 500L and Alcalase 2.4L	Salmon trimmings hydrolysate and gelatin hydrolysate	The salmon trimmings hydrolysate retained its dipeptidyl peptidase IV (DPP-IV) inhibitory and glucagon-like peptide-1 (GLP-1) secretory activities and improved its insulin secretory activity. In contrast, the gelatin hydrolysate lost its GLP-1 secretory activity	47
	Atlantic salmon (*Salmo salar*) trimmings	Corolase PP	Salmon trimmings protein hydrolysate (STP-C1)	The DPP-IV inhibitory activity was retained, and the peptide profiles remained similar	48
	Orange by-products	Pepsin	Fractions 45–49 and 50–54	The α-glucosidase inhibition and α-amylase inhibition activity increased or remained unchanged	49
	Rubing cheese by-product	Papain	YPVEPF	The α-glucosidase inhibitory activity retention rate was 19.64%	50
	Poultry by-product	FoodPro PNL	YA, IY, and VL	All three dipeptides were stable	51
	Quinoa	—	Malts	The DPP-IV inhibitory activity of most malts could be significantly increased	52
Anti-inflammatory	Dry-cured Xuanwei hams	—	Xuanwei ham peptides (XHPs)	Its anti-inflammatory effect was enhanced because of the release of novel peptides	53
Alcohol dehydrogenase (ADH) activating ability	Chickpea protein	Alcalase	Chickpea protein hydrolysates (CPHs-Pro-30)	The ADH activation rate could be maintained above 80%	54
Elastase inhibitory	Walnut (*Juglans regia* L.) meal	Alkaline protease	FFVPF	It could resist digestion in the gastric environment but was almost entirely hydrolyzed after gastrointestinal digestion	16
Antibacterial	Atlantic mackerel	Protamex	AMGAP	Only 2% of the intact peptide remained after digestion	55
Hepatocyte-protective	Jinhua ham	—	KRQKYD	36.60% of KRQKYD was split into smaller fragments after intestinal digestion, yet it still demonstrated strong hepatocyte-protective activity	56

2.2 Antioxidant peptides

Reactive oxygen species (ROS), such as hydrogen peroxide, hydroxy radicals, superoxide radicals, and peroxyl radicals,[71] are free radicals primarily generated by the mitochondrial respiratory chain and play a vital role in the body. However, excessive accumulation of ROS disrupts redox homeostasis, leading to oxidative stress, which is closely linked to various degenerative aging diseases, including cardiovascular diseases, Parkinson's, Alzheimer's, and cancer.[72–74] Under normal circumstances, the antioxidant system maintains the balance between oxidants and antioxidants in biological tissues, protecting the body from oxidative damage. However, as age advances, the body's antioxidant function gradually diminishes, necessitating supplementation with exogenous antioxidants. Several synthetic chemical antioxidants, such as propyl gallate, butylated hydroxyanisole, *tert*-butylhydroquinone, and butylated hydroxytoluene,[75,76] are widely used due to their excellent antioxidant properties. However, these antioxidants can have toxic side effects on human health during usage. Consequently, natural food-derived antioxidant peptides have attracted significant attention in recent years. The antioxidant potential of these peptides arises from their synergistic effects, including inhibiting lipid peroxidation, chelating transition metals, scavenging free radicals, and inducing gene expression to deactivate intracellular ROS. Peptide antioxidant activity is closely related to the peptide's composition, hydrophobicity, and conformation.[77] Histidine, tyrosine, cysteine, proline, lysine, tryptophan, methionine, and aromatic amino acids can effectively scavenge free radicals, enhancing the antioxidant activity of peptides.[78,79]

As natural alternatives to synthetic antioxidants, antioxidant peptides or hydrolysates must resist degradation by gastrointestinal enzymes, enter the portal circulation, and reach target organs and tissues in an active form to exert antioxidant effects. Some antioxidant peptides demonstrate robust functional stability during *in vitro* simulated gastrointestinal digestion. Antioxidant peptides GAC and SSC, purified from purple wheat bran protein hydrolysates, exhibited significant Trolox equivalent antioxidant capacity (TEAC) and demonstrated strong digestive stability.[3] Furthermore, the peptides PKK, YEGGD, and GPGLM demonstrated antioxidant activity by scavenging DPPH$^{\bullet}$ (EC_{50} = 0.978 ± 0.006, 1.062 ± 0.032, 1.149 ± 0.039 mg mL^{-1}), HO$^{\bullet}$ (EC_{50} = 1.158 ± 0.032, 1.234 ± 0.027, 1.285 ± 0.016 mg mL^{-1}), $ABTS^{+}$ (EC_{50} = 0.188 ± 0.002, 0.200 ± 0.002, 0.216 ± 0.007 mg mL^{-1}), and O_2^{-} (EC_{50} = 0.924 ± 0.003, 0.933 ± 0.011, 0.969 ± 0.014 mg mL^{-1}). These peptides remained highly stable under simulated gastrointestinal digestion for 240 min, with minimal decreases in DPPH$^{\bullet}$ scavenging activities of 3.25%, 3.87%, and 4.30%, respectively.[80] Chen

et al.[81] studied the effect of gastrointestinal digestion on the antioxidant activity of *L. plantarum* 60 fermented goat milk. Their findings revealed a slight decrease in DPPH$^{\bullet}$ radical scavenging activity (from 68.16% to 52.68%), demonstrating good stability during gastrointestinal digestion. Zhan *et al.*[82] isolated the peptide YDQLPEPRKPIE from porcine plasma hydrolysates; it exhibited significant antioxidant activity, especially after *in vitro* digestion. The antioxidant activity of YDQLPEPRKPIE was promoted by acidic amino acids (*e.g.*, D, E) and hydrophobic amino acids (*e.g.*, I, P, L). Additionally, amino acids and peptides (*e.g.*, R, K, P, KP, RK, PR, PIE, PE, YDQL) generated during gastrointestinal digestion could more effectively scavenge free radicals by the resonance of the imidazole ring and interacting with other peptides. Xiao *et al.*[83] found that chicken hydrolysates (CHs) exhibited significant antioxidant activity and resistance to simulated gastrointestinal digestion.

However, some antioxidant peptides are unstable during gastrointestinal digestion. The antioxidant peptide SNAAC, derived from Spanish dry-cured ham, is highly susceptible to gastrointestinal enzymes, resulting in over 90% decrease in activity compared to pre-digestion levels. MALDI-ToF MS analysis revealed the intact peptide's degradation and the SNAA fragment's formation during the gastric and gastrointestinal phases.[84] The peptide AEEEYPDL from Spanish dry-cured ham can be digested into AEEEY and PDL fragments. Its oxygen radical absorbance capacity (ORAC) decreased from nearly 900 nM TE mg^{-1} to 250 nM TE mg^{-1}, and its ABTS^{+} radical scavenging capacity exhibited 90% reduction after gastrointestinal digestion.[85] MDLFTE and WPPD, peptides isolated from blood cockle (*Tegillarca granosa*), demonstrated instability during simulated gastrointestinal digestion. Their initial EC_{50} values (MDLFTE: 0.47 ± 0.03 mg mL^{-1}; WPPD: 0.38 ± 0.04 mg mL^{-1}) were significantly lower than the values after digestion (MDLFTE: 5.52 ± 0.36 mg mL^{-1}; WPPD: 2.74 ± 0.42 mg mL^{-1}).[86]

2.3 DPP-IV inhibitory peptides

Approximately 90% of diagnosed diabetes cases are attributed to type-2 diabetes, which primarily arises from inadequate insulin secretion and utilization.[87] It is recognized as a multifaceted disease due to its association with several complications, including cardiovascular disease, lower limb amputation, kidney failure, and blindness.[88] Fortunately, several synthetic drugs (such as pyrimidinone, xanthine, and arylmethylamine analogs) have been developed to manage type-2 diabetes.[89] However, they often cause adverse side effects, such as hypertension and liver damage. Consequently, there is significant interest in natural alternatives like bioactive peptides. DPP-IV inhibitors, in particular, are emerging as promising therapeutic options due to their minimal risk of hypoglycemia.[90,91] The key hormones regulating blood sugar are the incretins, specifically glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), which promptly stimulate insulin synthesis and secretion. DPP-IV, an enzyme, is primarily recognized for its antagonism ability against incretin hormones.[92] Inhibiting DPP-IV prolongs the half-life of incretin hormones, thereby benefiting insulin secretion, glycemic control, and the treatment of type-2 diabetes.[93]

Gastrointestinal digestive enzymes generally interact with DPP-IV inhibitory peptides, making them susceptible to degradation. As a result, their DPP-IV inhibitory activity often decreases or is lost after gastrointestinal digestion. For instance, the DPP-IV inhibitory activities of collagen peptides from sheep skin, GIOGVGPF, GPAGPIGPV, and GPAGPOGFPG, significantly decreased after digestion (IC_{50} of GIOGVGPF increased from 125.42 ± 0.81 μM to 214.12 ± 0.82 μM, IC_{50} of GPAGPIGPV increased from 84.19 ± 0.72 μM to 144.52 ± 0.23 μM, IC_{50} of GPAGPOGFPG increased from 67.12 ± 0.64 μM to 122.45 ± 0.54 μM).[94] Harnedy-Rothwell *et al.*[95] produced six blue whiting soluble protein hydrolysates (BW-SPHs). Among these, BW-SPH-B, BW-SPH-D, BW-SPH-E, and BW-SPH-F exhibited a significant reduction in DPP-IV inhibitory activity after simulated gastrointestinal digestion. Similarly, the activity of Bambara bean protein hydrolysates produced with Alcalase decreased from 44.253 ± 1.327% to 8.996 ± 0.270% after digestion.[96] Pérez-Gálvez *et al.*[97] employed the INFOGEST method to simulate the gastrointestinal digestion of lupin hydrolysates. The results demonstrated a significant reduction in the DPP-IV inhibitory activity of lupin hydrolysates. This reduction might be attributed to the digestion of bioactive peptides ranging from 1 to 3 kDa in lupin hydrolysates, releasing inactive low-molecular-weight peptide fragments. Conversely, the hydrolysis of digestive enzymes may enhance the activity of certain peptides by producing fragments with stronger DPP-IV inhibitory capacities. Rendon-Rosales *et al.*[98] identified twelve peptides in fermented milk. Among them, P1 (QEPVLGPVRGPFIIV), P2 (YIPIQYVLS), P8 (SLPQNIPPL), and P9 (NAVPITPTLN) exhibited increased DPP-IV inhibitory effects after digestion. Digested NAVPITPTLN and SLPQNIPPL exhibited the highest activity with IC_{50} values of 298.47 μM and 368.24 μM, respectively. Hydrophobic amino acids and Pro residues in the digestion products of peptides P8 (SL, PQNIPPL) and P9 (NA, PITPTL) could facilitate their binding to the DPP-IV catalytic site. Pei *et al.*[5] identified the DPP-IV inhibitory peptide VPLVM from broccoli. The hydrophobic Pro and Leu residues near the N-terminus conferred DPP-IV inhibitory activity to VPLVM. Most VPLVM remained undigested by gastrointestinal enzymes, demonstrating an overall stability of 84.58%. A portion of VPLVM was hydrolyzed into VPLV and PLV. Moreover, mice experienced a 20.7% reduction in blood glucose levels 30 min after VPLVM administration compared to the control group. The DPP-IV inhibitory peptides NLEIILR and TQMVDEEIMEKFR demonstrated good stability after simulated gastrointestinal digestion. The inhibitory ability of TQMVDEEIMEKFR increased by 21.00%, while the activity of NLEIILR remained unchanged.[99] Simulated gastrointestinal digestion *in vitro* of VPA, VPW, IPL, and IPR present in *Chlorella vulgaris* suggested that these peptides were likely resistant to gastrointestinal digestion and could retain their DPP-IV inhibitory activity. The hydrophobic Val residue at the

N-terminus and the Pro residue at the second position from the N-terminus of these peptides might play key roles in their activity and gastrointestinal stability.[100]

2.4 Anticancer peptides

Despite advancements in research and treatment methods, cancer remains increasingly prevalent worldwide and is a significant cause of morbidity and mortality. Most cancers, apart from chronic leukemia, are still regarded as chronic diseases that cannot be completely eradicated. Conventional cancer treatments, like chemotherapy and radiotherapy, often cause severe side effects due to damage to healthy cells and tissues.[101] The demand for innovative treatments has spotlighted peptides as potential drug candidates due to their several key advantages over conventional chemotherapy molecules. Peptides exhibit low toxicity, strong specificity, high affinity, and good tissue penetration.[102] Cancer cells can evade signals that inhibit growth, allowing them to resist cell death and multiply. Therefore, inducing apoptosis has emerged as a viable approach for managing cancer. Research has demonstrated that peptides are involved in multiple stages of carcinogenesis, such as angiogenesis, apoptosis, and metastasis.[103]

Similarly, gastrointestinal digestion may affect the activity of anticancer peptides. For instance, Sah *et al.*[104] discovered that simulating the gastrointestinal tract significantly enhanced the antiproliferative activities of the anticancer peptides from synbiotic yoghurt against HT-29 colon cancer cells; this was attributed to the generation of potent bioactive peptides. Specifically, the digestate of YQEPVLGPVRGPFPIIV (at 3 mg mL^{-1}) exhibited an activity of 86.63 ± 0.57%, while the digestate of SLPQNIPPLTQTPVVVPPF (at 3 mg mL^{-1}) showed an activity of 87.60 ± 0.89%. Both undigested peptides (H. Met-Gly-Pro-Pro-Gly-Leu-Ala-Gly-Ala-Pro-Gly-Glu-Ala-Gly-Arg. OH) isolated from flathead by-products and those resulting from gastric and gastrointestinal digestion possessed anticancer activity, inhibiting the proliferation of HT-29 cells by up to 28.89%, 29.68%, and 38.3%, respectively. The highest rate of cell death (3.54 ± 2.30%) was observed in HT-29 cells treated with peptides digested by pepsin and pancreatin.[105] Xue *et al.*[106] investigated the inhibitory activity of chickpea peptide CPe-III (RQSHFANAQP) on tumor proliferation *in vitro*. They conducted both *in vitro* simulated and *in vivo* digestion in mice, identifying Ser-His dipeptide as a common product in both processes. Further research revealed that this metabolite was a potential anticancer agent, inhibiting tumor cell proliferation.

2.5 Bioactive peptides with other functions

Besides investigating the ACE inhibitory peptides, antioxidant peptides, DPP-IV inhibitory peptides, and anticancer peptides, extensive research has also focused on the gastrointestinal stability of other bioactive peptides. For instance, Li *et al.*[107] suggested that peptides derived from *Staphylococcus simulans* QB7-fermented sausage, inhibiting cyclooxygenase 2 (COX2) or vascular cell adhesion molecule 1 (VCAM-1) expression, likely consisted of different sequences. Peptides inhibiting COX2 expression probably showed greater resistance to gastrointestinal digestion, whereas those targeting VCAM-1 expression might have been less resistant, degrading into smaller peptides while retaining remarkable VCAM-1 inhibitory capability. The peptide identification results indicated that gastrointestinal digestion led to the release of numerous peptide sequences containing positively charged and hydrophobic amino acids. This finding explained why the digested peptides exhibited high anti-inflammatory activity. The anti-inflammatory activity of IVYPWTQR from goose blood hydrolysate slightly decreased after pepsin digestion, as 45.19% of IVYPWTQR was cleaved into smaller fragments, including IV (20.00%) and YPWTQR (25.19%), which were resistant to trypsin.[108] Bovine-elastin-derived elastase inhibition peptides GAGQPFPI, FPGIG, and FFPGAG exhibit digestion resistances of 66.3%, 64.0%, and 61.3%, respectively.[109] Yang *et al.*[110] investigated the α-glucosidase inhibitory activity of hot-pressed peanut meal protein hydrolysates (PMHs) after *in vitro* gastrointestinal digestion. They discovered that this activity remained stable and even increased in the final stages. The ADH activation activity of chicken breast muscle hydrolysates (CBMHs) was reduced by 33.42% after simulated intestinal digestion compared to the untreated sample, due to the degradation of hydrophobic peptides into less active forms.[111] Liu *et al.*[112] identified cholesterol-lowering peptides ALPM, AVFK, and HTSGY from whey protein and found that their activity decreased by 5.43 ± 0.87%, 13.32 ± 1.30%, and 9.52 ± 0.89% after digestion, while their bioavailability was 68.19%, 72.16%, and 83.66%, respectively. The retention of cholesterol-lowering activity might stem from an elevated level of hydrophobic amino acids, enhancing their capacity to bind conjugated bile acids. The peptide β-casein (f193–209) (*m*/*z* 1881), derived from Prato cheese and exhibiting immunomodulatory, ACE inhibitory, antimicrobial, and antithrombotic activities, displayed a prominent peak after static digestion and was completely hydrolyzed during dynamic digestion. However, other studies demonstrated that this peptide could resist gastrointestinal digestion.[113] This suggests that different experimental protocols may lead to contradictory results, emphasizing the need for developing standardized experimental methods. Vivanco-Maroto *et al.*[114] employed a semi-dynamic protocol to investigate the effects of simulated gastrointestinal digestion on peptides in precursor micellar casein and casein hydrolysates that could induce the secretion of GLP-1 and cholecystokinin (CCK) from STC-1 cells. The results indicated that the peptides VYPFPGPIPN, YPFPGPI, and VYPFPGPI derived from β-casein consistently exhibited high intensity during the intestinal digestion process.

The activity of bioactive peptides after digestion hinges on their stability and the bioactivity of their digestion products (Fig. 2). If the peptide remains relatively stable during gastrointestinal digestion or its hydrolysis products have high bioactivity, its overall activity will largely remain intact or even increase.[64–67,80,98] Conversely, if the peptide is highly susceptible to digestive enzymes and the resulting small peptides exhibit low bioactivity, the peptide's bioactivity may significantly

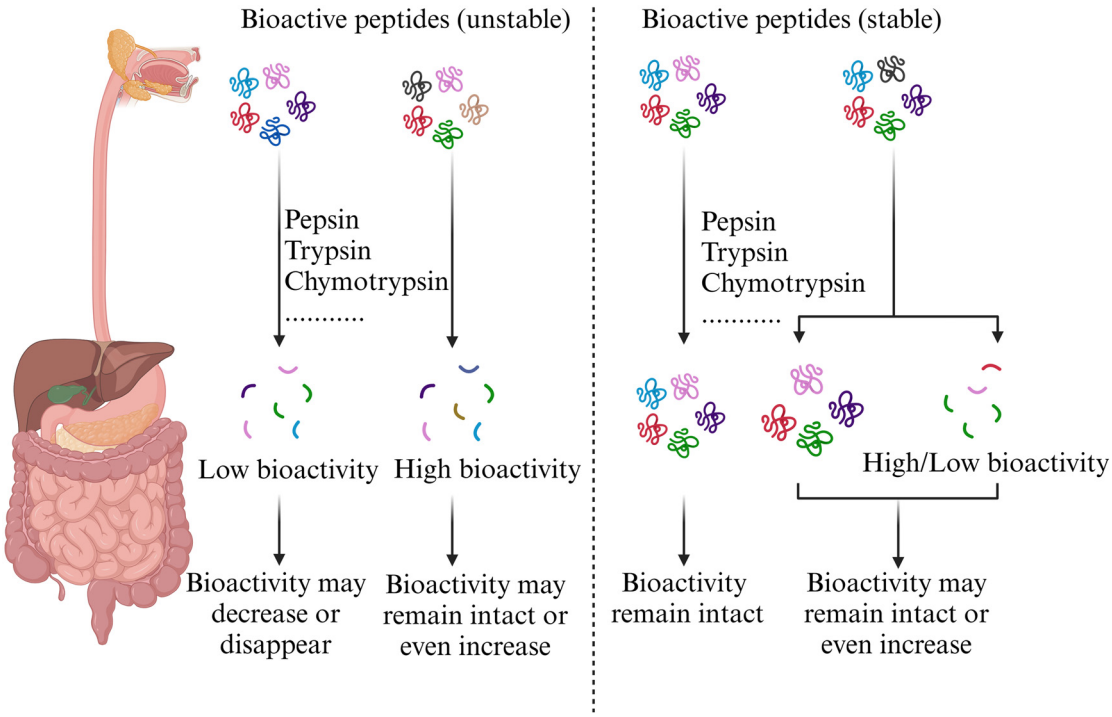

Fig. 2 The effect of gastrointestinal digestion on peptide bioactivity.

decrease or nearly disappear after digestion.[68,84,86,95] Since peptide bioactivity is easily affected by gastrointestinal digestion, several studies employ simulated gastrointestinal digestion to derive bioactive peptides. For instance, the novel ACE inhibitory peptide GHIITVAR (IC_{50} = 3.60 ± 0.10 μM) was identified from sesame (*Sesamum indicum* L.) protein using *in vitro* two-stage simulated gastrointestinal digestion.[115] Zhang *et al.*[116] identified five novel antioxidant peptides through simulated digestion. Among these, IREADIDGDGQVN (1.78 mM), PEILPDGDHD (1.18 mM), and ASDEQDSVRL (1.45 mM) exhibited the lowest $DPPH^{\bullet}$ radical scavenging IC_{50} values. Additionally, APLEEPSSPH demonstrated the most effective Fe^{2+} chelating capability with an IC_{50} of 0.09 mM. Suárez *et al.*[117] utilized an *in vitro* dynamic simulated digestion system to identify the peptide sequence IERGEGIMGV, which was likely to inhibit ACE, from the final digesta of an amaranth beverage (AB). Wu *et al.*[118] found that the digestive products of fermented soymilk contained bioactive peptides with α-glucosidase, DPP IV and ACE inhibitory and antioxidant activities. The highest relative quantity of bioactive peptides was released through the combined action of *Propionibacterium shermanii* and *Lactobacillus plantarum*. ACE inhibitory peptides LLR and AWR, derived from the gastrointestinal digestion products of sufu hydrolysates, exhibited IC_{50} values of 94.01 ± 5.07 μM and 98.04 ± 2.56 μM, respectively. *In vivo* experiments demonstrated that both peptides significantly reduced diastolic and systolic blood pressure in spontaneously hypertensive rats.[119] A recent review screened 17 675 articles and selected 31 that assessed the structural stability of bioactive peptides during *in vitro* simulated gastrointestinal digestion.[120] This research identified 93 unique peptide sequences and systematically elucidated the characteristics of stable peptides. The findings indicated that stable peptides had higher proportions of Pro and hydrophobic residues, shorter chain length and lower molecular weight, no C-terminal Leu, more basic amino acid residues, and branched-chain aliphatic N-terminal residues.

3. Encapsulation strategies to enhance the gastrointestinal stability of bioactive peptides

Due to the uncertain bioactivity of digestion products, directly preparing bioactive peptides with gastrointestinal enzymes or designing peptides to resist digestive enzymes offers benefits but reduces diversity. Therefore, developing strategies to enhance the gastrointestinal stability of bioactive peptides is crucial. Encapsulation, including microcapsules, gels, emulsions, liposomes, and nanofibers (Fig. 3), is a prevalent approach today, as illustrated in Table 2.

The digestive behavior of these wall materials is crucial for protecting bioactive peptides during digestion and ensuring their controlled release, thereby enhancing their bioavailability and therapeutic efficacy. Polysaccharides and proteins are the primary wall materials used to encapsulate bioactive peptides, including forms such as microcapsules, gels, and nanofibers. Digestion of polysaccharide wall materials, primarily starch, begins with salivary amylase in the oral cavity. Although the acidic environment of the stomach inhibits this process, pan-

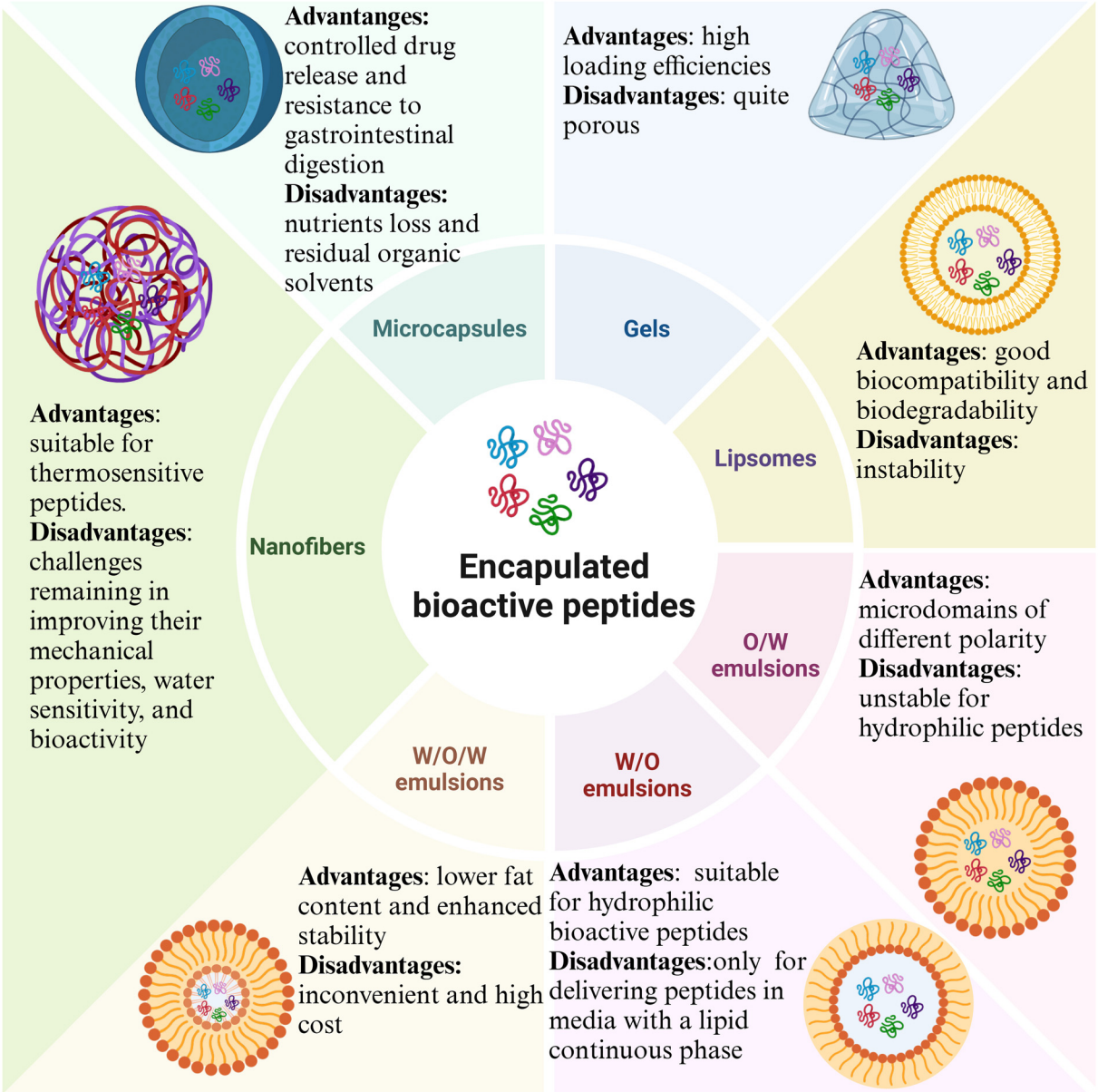

Fig. 3 Schematic diagrams of microcapsules, gels, emulsions, liposomes, and nanofibers for the encapsulation of bioactive peptides.

creatic amylase, lactase, sucrase, and maltase in the intestines further break down the polysaccharides. Conversely, if the wall material is a dietary fiber-type polysaccharide, such as pectin or alginate, its resistance to digestion may make it suitable for encapsulating bioactive peptides intended for absorption in the colon.[134] It is worth noting that when dietary fiber-type polysaccharides (*e.g.*, alginate) and starch are used together as wall materials, the release of the core materials from the system may be compromised.[135] Furthermore, when polysaccharides are used as encapsulating materials, swelling may occur during the digestion process. For example, during the intestinal phase, higher pH levels increase electrostatic repulsion between gel-based pectin polymer chains, resulting in swelling and the subsequent release of peptides.[134]

If the wall material is a protein, such as casein or gelatin, digestive enzymes initiate its degradation with pepsin in the stomach and subsequently break it down further with intestinal proteases like trypsin and chymotrypsin, leading to the controlled release of bioactive peptides. Additionally, the acidic pH of the stomach may induce the dissociation of protein-based delivery systems. As the gastric pH approaches the isoelectric point of proteins, they tend to aggregate and precipitate, thereby compromising the stability of the delivery system.[136] When proteins are combined with polysaccharides as wall materials, various electrostatic interactions between proteins and polysaccharides, as well as between core materials and polysaccharides, may affect the digestion of wall materials, potentially altering the release profile of bioactive peptides.[137]

Table 2 The encapsulation strategies designed to enhance the gastrointestinal stability of bioactive peptides

Encapsulation method	Material	Hydrolysate name/peptide sequences	Advantage	Ref.
Microcapsule	10% cluster dextrin	Fractionated riceberry bran protein hydrolysate obtained by ultrafiltration <3 kDa (URBPH < 3 kDa)	Encapsulated peptides retained their antioxidant activity better during digestion compared to the free form	121
	Agar (Agarforce®) and maltodextrin (15 DE)	Peptides obtained from brewer's spent grain (BSG-P)	Microencapsulation improved the hypolipidemic activity of BSG-P observed under simulated digestion	122
	Pyropia columbina phycocolloids	Brewers' spent grain ACE-I inhibitory peptides	Encapsulated peptides exhibited a lower IC_{50} value (2.4 ± 0.2 mg mL^{-1} protein) than unencapsulated peptides (7.2 ± 0.3 mg mL^{-1} protein)	123
	Alginate	Plantaricin EF (PlnEF)	Mixing PlnEF protected by crosslinked alginate microcapsules (CLAMs) into peanut butter (PB) safeguarded it from proteolysis and enhanced its intestinal release	124
Gel	Chitosan-tripolyphosphate (TPP)	Atlantic codfish skin-derived collagen hydrolysates	Encapsulated hydrolysates released approximately 58% of their content during digestion	125
	Casein and alginate	Protein nanoparticles consisting of a zein core and a whey protein shell	These microgels can potentially retain and protect protein nanoparticles in the gastric phase while facilitating their release in the intestinal phase	126
Emulsion	Amaranth proteins and sunflower oil	Amaranth protein hydrolysates (AH)	The ACE inhibitory activity was maintained after simulated gastrointestinal digestion	127
	Canola oil and tween 80	Collagen peptide (CP)	W1/O/W2 emulsions achieved the highest collagen peptide retention, with an encapsulation efficiency of over 90%	128
	Polyethylene glycol 40 stearate (PEG 40 St) and soybean oil	Bovine lactoferrin (LF)	After gastric digestion, 84% of the LF in these emulsions was protected. Under intestinal conditions, the PEG-LF emulsions demonstrated good stability and slower lipolysis	129
Liposome	CS-TPP/liposomes	Fish-purified antioxidant peptide fractionated by RP-HPLC (F5)	The antioxidant activity of F5 loaded into liposomes was retained after digestion	130
	Soy phosphatidylcholine liposomes with the addition of glycerol	Collagen hydrolysate	The liposome membrane effectively protected the active compounds from interactions within the gel matrix or degradation	131
Nanofiber	Bioactive electrospun fish sarcoplasmic proteins (FSP)	Insulin	Insulin was efficiently encapsulated, allowing for its controlled release in simulated intestinal fluid while protecting against chymotrypsin degradation	132
	Electrospun chitosan/poly (vinyl alcohol) (CS/PVA)	Fish-purified antioxidant peptide (AOP)	The encapsulated antioxidant peptide retained its activity and showed sustained release	133

Lipids are utilized as encapsulating materials in liposome and emulsion delivery systems. Lipid digestion is initiated by gastric lipase and continues in the intestinal phase, where pancreatic lipase plays a key role. Furthermore, bile salt aids in the removal of free fatty acids and proteins from the surface of oil droplets, thereby increasing the available surface area for enzymatic action.[138] In emulsions, emulsifiers, such as polysaccharides and proteins, significantly affect lipid digestion rates by determining the system stability and oil droplet size.[139] Liposomes, composed of one or more phospholipid bilayers, are unstable in the gastrointestinal tract. Their stability can be enhanced through modifications, as discussed in detail in subsequent sections.

3.1 Microcapsules

In recent years, the food industry has extensively employed microcapsules for the encapsulation of bioactive peptides.[140,141] It involves enclosing sensitive materials within a physical barrier, forming small particles. Various materials have been used as wall materials for preparing microcapsules, including maltodextrin, gum arabic, chitosan, casein, and sodium alginate. Spray-drying and freeze-drying are commonly used methods for microencapsulating various bioactive compounds.[142] Spray drying is a dehydration technique that converts a feed solution into powder. The process involves atomization of the feed solution, drying, and particle separation.[143] Freeze-drying creates microcapsules by sublimating frozen emulsion. This process includes freezing, ice sublimation, and secondary drying.[144] Encapsulating bioactive peptides in microcapsules provides several advantages, including controlled drug release rates, reduced toxicity, resistance to gastrointestinal digestion, protection against chemical and enzymatic degradation, and enhanced bioavailability.[145] Corrêa *et al.*[146] encapsulated bioactive sheep whey hydrolysates using prebiotic gum arabic as the wall material. The microcapsules demonstrated higher antioxidant and antihypertensive activities after simulated gastrointestinal digestion than non-encapsulated bioactive sheep whey hydrolysates. Cian *et al.*[147] used

agar and maltodextrin to prepare spray-dried microcapsules of brewers' spent grain protein hydrolysate (BSGH). During *in vitro* simulated gastrointestinal digestion, encapsulated BSGH had a cleavage rate of 39.2%, compared to 66.5% for non-encapsulated BSGH. Microencapsulation also preserved the *in vitro* antioxidant activity. Cian *et al.*[148] examined the bioactivity of *Phaseolus lunatus* peptides microencapsulated with maltodextrin and gum arabic as wall materials following digestion. The results indicated that microencapsulation preserved these peptides' α-glucosidase, α-amylase, and DPP-IV inhibition activity. Xun *et al.*[149] prepared caffeic acid grafted chitosan–sodium alginate microcapsules using microfluidics to efficiently encapsulate silkworm pupae bioactive peptide. Peptide release from the microcapsules was 9.76 ± 0.23% during simulated gastric digestion, and the microcapsules demonstrated good intestinal release. Additionally, the DPPH$^{\bullet}$ radical scavenging activity of the microcapsules was 31.15 ± 0.99%, compared to 13.31 ± 0.63% in the control group.

Current research on microcapsules primarily focuses on microparticle characteristics, encapsulation efficiency, processing parameters, and drug release duration. Utilizing non-toxic and safer food ingredients as wall materials opens new avenues for the development of microcapsules. Recently, plant proteins were employed as encapsulating agents, driven by the growing trend in specialized markets to restrict or limit animal products.[150] For instance, potato protein, with its functional properties and valuable nutritional content, is a potential alternative wall material. Furthermore, studies show that modifying plant proteins can significantly improve microparticle encapsulation efficiency through heat treatment, enzymatic hydrolysis, and acylation.[151] Future efforts should concentrate on devising strategies to enhance the preservation of peptide activity within microcapsules.[152]

3.2 Micro- and nanogels

Micro- and nanogels primarily consist of water and cross-linked polymer molecules, often polysaccharides and proteins.[153] Common polysaccharides include alginate, chitosan, methyl cellulose, and pectin, while common proteins are albumin, caseins, gelatin, and ovalbumin.[154] Their functional properties can be customized for specific applications by precisely controlling their dimensions, porosities, compositions, interfacial characteristics, and morphologies.[155] Various methods can be used to prepare these colloidal particles, such as emulsion templating, coacervation, antisolvent precipitation, and injection gelation.[153,156] Gels can achieve high loading efficiencies (LEs) due to favorable partitioning and have been extensively studied for encapsulating, protecting, and delivering bioactive agents.[155] Free-Manjarrez *et al.*[157] found that Greek-style yogurt with common bean protein hydrolysates encapsulated in a gel showed 44% reduction in bitterness and 52% decrease in astringency compared to control yogurt with unencapsulated hydrolysates. Additionally, yogurt containing encapsulated hydrolysates inhibited α-amylase (92.47%) and DPP-IV (75.24%) after digestion. Obuobi *et al.*[158] encapsulated antimicrobial peptides within DNA nanogels, preserving bioactivity and structural integrity. They demonstrated that the sustained release of these encapsulated peptides resulted in significant antimicrobial effectiveness against *S. aureus*.

However, gels enable bioactive peptides to diffuse out easily due to their porous nature. This issue can be mitigated by reducing the pore size of the gel or enhancing the interactions between the peptides and the biopolymer.[156]

3.3 Liposomes

Liposomes are colloidal structures composed of one or more phospholipid bilayers, with the non-polar tails of the molecules facing inward due to hydrophobic interactions.[159,160] Liposomes can encapsulate hydrophilic bioactive peptides between the polar head groups or within their aqueous core. Additionally, hydrophobic bioactive peptides can be embedded within the bilayers. Due to their phospholipid composition, liposomes may exhibit good biocompatibility, biodegradability, cell membrane permeability, and low toxicity and immunogenicity, making them widely used for encapsulating, protecting, and delivering bioactive peptides.[160] Pavlovic *et al.*[161] encapsulated soy protein hydrolysate in liposomes and found that, under simulated gastrointestinal conditions, liposomes provided the sustained release of the encapsulated hydrolysates. In the first 75 min, only 20% of the encapsulated soy peptides diffused out. The osteogenic activity of osteogenic peptides (OPs) encapsulated in nanoliposomes remains unaffected, with their cumulative release in phosphate buffer solution (PBS) within 8 h (58.2%) being significantly slower than that of non-encapsulated OPs (68.5%).[162]

However, a major drawback of liposomes as delivery systems is their instability during digestion.[163] Therefore, it is essential to consider their digestibility and optimize various factors to achieve ideal liposome digestion performance. For instance, improving liposome stability during digestion can be achieved by altering the type of phospholipid used. Huang *et al.*[164] developed liposomes modified with stearic acid (Lipo-SA) and decanoic acid (Lipo-DA) to encapsulate the peptide LKPNM. After 180 min of incubation in simulated gastric fluid, the peptide release percentages for Lipo-Pep, Lipo-SA-Pep, and Lipo-DA-Pep were 77.73 ± 2.03%, 67.01 ± 0.61%, and 68.51 ± 0.77%, respectively. In simulated intestinal fluid, the corresponding release percentages were 85.58 ± 2.01%, 72.4 ± 3.79%, and 78.24 ± 3.99%, indicating that modified liposomes exhibited better peptide preservation. Niu *et al.*[165] discovered that liposomes containing bile salts (BS-liposomes) demonstrated enhanced gastrointestinal stability and prolonged residence time compared to conventional liposomes.

3.4 Emulsions, nanoemulsions, and multiple emulsions

Emulsions are colloidal suspensions formed by blending water and oil, with one liquid forming small droplets within the other.[166] These emulsions can be categorized as water-in-oil (W/O) or oil-in-water (O/W) based on the arrangement of the water and oil phases. Furthermore, they can be classified as either emulsions (d > 100 nm) or nanoemulsions (d < 100 nm)

based on the mean droplet diameter.[167] According to McClements,[17] since both emulsions and nanoemulsions are thermodynamically unstable systems with similar structural and physicochemical properties, the term "emulsion" is used in this article to describe both types.

From a drug delivery perspective, emulsions are appealing because they contain microdomains of different polarity within a single-phase solution, enabling the solubilization of hydrophilic or hydrophobic materials. However, significant challenges exist in encapsulating hydrophilic peptides into the oily core of O/W emulsions. Due to their polarity, peptides may remain at the oil–water interface.[168] Additionally, bioactive peptides can adsorb onto the surfaces of emulsifier-coated oil droplets because of electrostatic interactions, compromising protection.[169] Current strategies to better protect them include converting peptides into a hydrophobic form for incorporation into O/W emulsions and encapsulating protein-coated droplets within other protective systems, such as microgels.[17,155] In contrast, W/O emulsions are more suitable for encapsulating hydrophilic bioactive peptides because their hydrophilic interior better accommodates these peptides.[170] For example, Gao *et al.*[171] effectively encapsulated bitter peptides in W/O emulsions, significantly mitigating their bitterness. Additionally, encapsulation improved the gastric stability of the peptides, shielded them from pepsin in the stomach, and facilitated the delivery of more intact peptides to the intestines. Nevertheless, W/O emulsions are only effective at delivering peptides in drugs, supplements, or functional foods with a lipid continuous phase, such as oil-filled capsules.[166] Fortunately, W/O/W emulsions can overcome this drawback. They are created by dispersing a W/O primary emulsion into an external aqueous phase containing an emulsifier.[172] There are two main reasons for the steady increase in researchers' interest in W/O/W systems. Firstly, W/O/W emulsions have a lower fat content than traditional O/W emulsions, while maintaining a similar mouthfeel. Secondly, they offer enhanced stability and more effectively protect sensitive water-soluble components. However, W/O/W systems also present challenges, including difficult and time-consuming preparation, high costs, and susceptibility to decomposition during storage.[172] Giroux *et al.*[173] investigated the impact of the oil phase composition (butter, linseed oil, or mineral oil) and the degree of hydrolysis (0%, 3.5%, or 7%) on the release kinetics of casein-derived peptides encapsulated in W/O/W double emulsions during digestion. Peptides with 3.5% and 7% hydrolysis showed faster release rates compared to the unhydrolyzed group in both digestion phases. After the gastric phase, peptide release ranged from 4% to 42%. During the intestinal phase, peptide release in double emulsions with linseed oil and butter exceeded 80%, whereas release in emulsions with mineral oil was below 18% due to its indigestibility.

3.5 Nanofibers

Electrospinning is a facile, innovative, and promising technique for preparing nanofibers with porosity and a high surface-to-volume ratio.[174] Bioactive peptides can be encapsulated into nanofibers by mixing them with a biopolymer solution and electrospinning. Commonly used biopolymers include proteins such as whey protein concentrate (WPC), collagen, and zein, as well as polysaccharides like chitosan, pullulan, dextrans, and starch.[175] Since nanofibers are prepared under ambient conditions, they are more suitable for encapsulating thermosensitive peptides compared to techniques such as spray drying.[174] Additionally, the room temperature processing route enables efficient encapsulation by enhancing the stability of bioactive peptides. Recently, nanofibers produced *via* electrospinning attracted significant attention. Rajanna *et al.*[176] synthesized casein-derived peptide-loaded pullulan nanofibers *via* electrospinning at pullulan concentrations of 120 and 140 g kg^{-1}, achieving mean diameters ranging from 60.45 to 133.05 nm and encapsulation efficiencies between 72.95% and 86.04%. Peptides from electrospun nanofibers exhibited sustained release under gastrointestinal pH conditions. Using electrospinning, Kirbas and Altay[177] prepared pullulan–sodium alginate nanofiber carriers loaded with L-carnosine (Car). The electrospinning process preserved Car antioxidant activity and enabled its sustained release during digestion.

Although nanofibers hold significant promise for various applications, challenges remain in improving their mechanical properties, water sensitivity, and bioactivity. Methods such as modifying biopolymers, and adding nanoparticles and bioactive components can improve the properties of nanofibers. For instance, antimicrobial aromatic compounds (AACs) are extensively used as property modifiers and bioactive components in various biopolymer nanofibers to improve their thermal properties, hydrophobicity, functionality, and mechanical properties.[178] Future research should delve deeper into the interactions between small molecules and biopolymers, elucidating the effects of these molecules on biopolymer properties.

4. Conclusion and future research perspectives

Bioactive peptides isolated from food proteins exhibit ACE inhibitory, antioxidant, DPP-IV inhibitory, anticancer, and anti-inflammatory activities. However, their *in vitro* activity does not always correlate with *in vivo* functionality due to hydrolysis by digestive enzymes. The activity of bioactive peptides after exposure to digestive enzymes can remain unchanged, decrease, or increase, depending on the stability of the peptides and the bioactivity of their hydrolysis products. If a bioactive peptide exhibits good gastrointestinal stability or its hydrolysis products retain high activity during transit, its overall activity may be maintained or even enhanced. Conversely, if a peptide is highly susceptible to gastrointestinal enzymes and its hydrolysis products have low activity, digestion will significantly reduce the activity. Therefore, the effect of gastrointestinal digestion on peptide bioactivity is uncertain. To address this uncertainty, some studies directly prepare

bioactive peptides through simulated gastrointestinal digestion, but this method is time-consuming and less effective. Therefore, enhancing the gastrointestinal stability of bioactive peptides is a more reliable approach to maintaining their activity. Encapsulation is widely used to improve bioactive peptides' gastrointestinal stability and bioavailability.

The ability of bioactive peptides to reach target organs and cells in their active form depends on their gastrointestinal stability and stability during transepithelial transport, blood metabolism, and kidney and liver metabolism (Fig. 4). To save resources, reduce costs, and protect experimental subjects, preliminary *in vitro* pharmacokinetic studies are necessary before clinical trials. Bioactive peptides must retain their activity after being processed by brush border peptidases and passing through intestinal epithelial cells. The primary mechanisms for bioactive peptide transport across intestinal epithelial cells include peptide transportable 1 (PepT1), the paracellular route, and transcytosis. Once bioactive peptides enter the bloodstream, they encounter various peptidases and proteases and must resist their action. Common peptidases in the blood include aminopeptidase N, carboxypeptidase N, plasmin, DPP4, furin, and neprilysin.[179] Additionally, bioactive peptides are easily filtered and excreted from the body by the kidneys and metabolized by the liver.

Furthermore, bioactive peptides' allergenicity, toxicity, and bitterness must be considered before clinical trials and commercial application. Proteins are the primary allergens in food, and the development of some novel food processing technologies may help avoid or control these allergens.[180] *In vitro* tests, such as the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, are commonly used to assess the potential toxicity of proteins.[181] The taste of bioactive peptides is a crucial factor affecting their commercial application. Current techniques to reduce peptide bitterness include encapsulation, enzymatic treatment, and removal of hydrophobic residues.[182]

If *in vitro* experiments confirm that bioactive peptides can maintain their activity under various conditions and are non-allergenic and non-toxic, further *in vivo* experiments are necessary to support their beneficial impact. Martin *et al.*[183] investigated the *in vivo* antihypertensive effects of a whey protein hydrolysate containing peptides WL and IW. Although the hydrolysate significantly reduced plasma ACE activity (to 86.4 ± 5.9% and 75.1 ± 6.9% of baseline activity after ingestion of 5 g and 50 g hydrolysate, respectively), it did not lead to a reduction in blood pressure over the study period. Recently, the *in vivo* antihypertensive effects of processed rice bran, containing the antihypertensive peptide LRA, were evaluated over

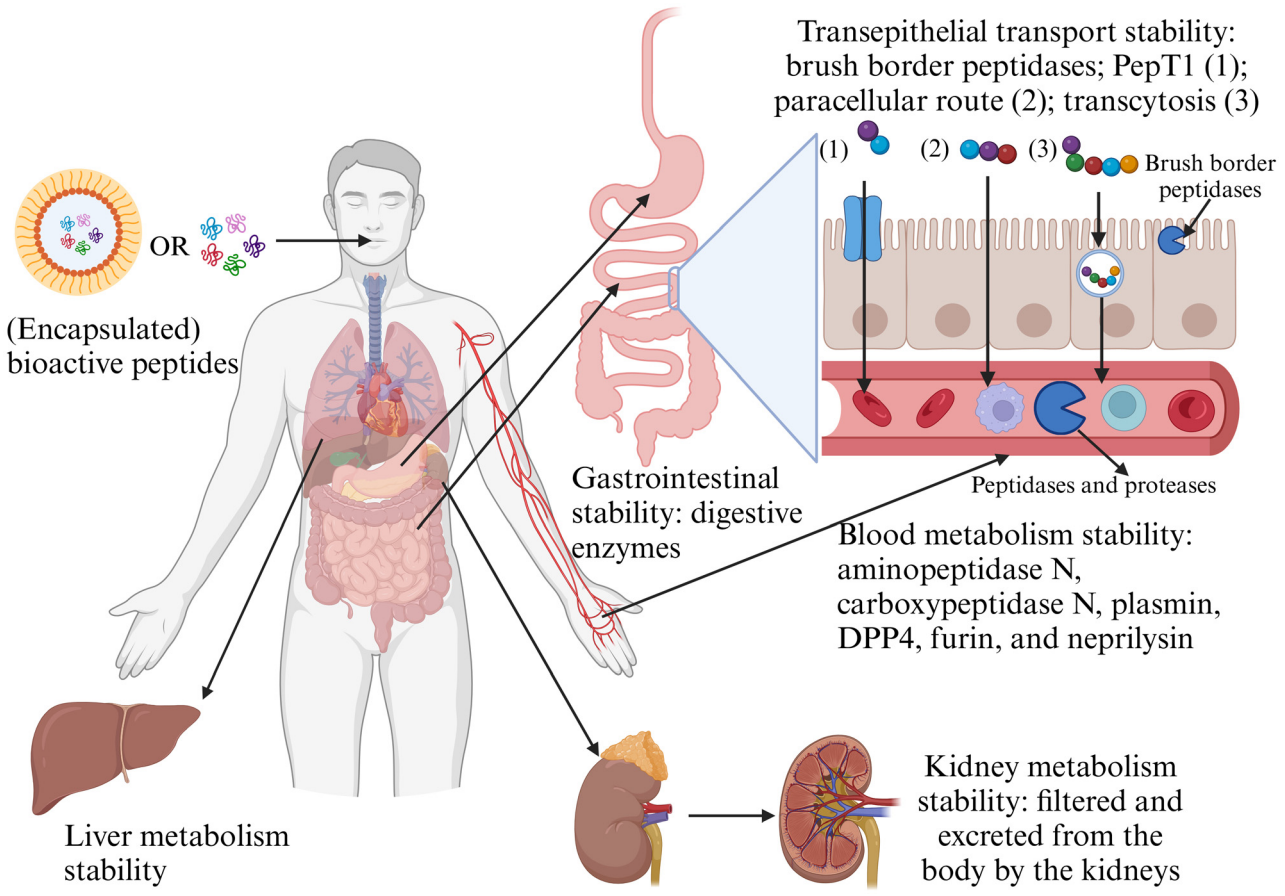

Fig. 4 Gastrointestinal, transepithelial transport, blood metabolism, liver metabolism, and kidney metabolism stability challenges encountered by bioactive peptides.

a 12-week trial. The results indicated that a daily dosage of 43 μg LRA significantly reduced the blood pressure of volunteers with mild hypertension compared to the placebo group ($p < 0.05$).[184] A milk protein hydrolysate with α-glucosidase inhibitory activity was shown to exert a postprandial glycemic effect. Low doses of the hydrolysate significantly reduced postprandial plasma glucose concentrations in subjects compared to the placebo ($p < 0.05$) and mildly regulated insulin secretion.[185] Similarly, the *in vivo* activities of certain encapsulated bioactive peptides have been validated. A 35-day study demonstrated that encapsulating carp skin gelatin hydrolysate in furcellaran-based microcapsules reduced its *in vivo* antioxidant activity. This outcome may be attributed to the incomplete digestion of the wall materials in the small intestine, preventing the hydrolysate from being released. Therefore, selecting an appropriate wall material is crucial for ensuring that bioactive peptides can exert their effects *in vivo*.[186] During a 100-day administration period, encapsulated brewers' spent grain peptides effectively lowered blood glucose and blood pressure in an insulin-resistant and hypertensive rat model induced by a sucrose-rich diet.[187] Clinical trials are essential for bridging *in vitro* activity with *in vivo* function and represent a crucial stage for the commercial application of bioactive peptides. However, few animal models and even fewer clinical trials have been conducted, with the existing ones primarily focusing on antihypertensive peptides. Future research must provide robust evidence for the health claims surrounding bioactive peptides through well-designed clinical trials.

Regulatory approvals are essential for protecting consumers from fraudulent claims, deceptive practices, and risks related to bioactive peptides.[188] First, it is necessary to determine whether bioactive peptides should be classified as food or drugs. Generally, they are classified based on their intended use. If a product is consumed for sustenance and nourishment, it should be considered a food; if it is designed to treat a disease, it should be regarded as a drug.[189] Bioactive peptides are usually classified as food for human consumption, complying with the same safety regulations as traditional food items.[190] Although researchers have isolated numerous food-derived bioactive peptides, only a few have reached commercial applications. Additional animal studies, clinical trials, and regulatory approvals are needed to advance the commercial application of bioactive peptides.

Due to factors such as low cost, minimal equipment requirements, and ease of operation, most current studies use static methods to investigate the effects of simulated gastrointestinal digestion on bioactive peptides, with few employing semi-dynamic or dynamic approaches. Although static methods are convenient, they are primarily suited to evaluating digestion endpoints rather than the digestion process itself. Moreover, simple mixed solutions cannot fully replicate actual body fluids, potentially leading to inaccurate results. Another limitation is that different studies may employ various static methods to simulate gastrointestinal digestion. For example, Ma *et al.*[2] added 1% pepsin to the solution and incubated it for 2 h at a pH of 2.0. Then, they adjusted the pH to 7.5, added 1% trypsin, and incubated the mixture for another 4 h. However, Gallego *et al.*[85] added pepsin (2000 U mL^{-1}) to the solution, digesting the mixture at a pH of 3.0 for 3 h. Then, they adjusted the pH to 7.0 and added pancreatic lipase (2000 U mL^{-1}), pancreatic α-amylase (200 U mL^{-1}), chymotrypsin (25 U mL^{-1}), and trypsin (100 U mL^{-1}) for another 3 h of incubation. Variations in enzyme amounts and reaction conditions, like solution composition, reaction time, and pH, may result in different outcomes. The enzyme source is also crucial but different studies may use trypsin from bovine or porcine origins, affecting the results. Therefore, future studies should concentrate on developing more standardized and realistic digestion simulation methods that closely mimic human conditions.

In recent years, bioinformatics has been utilized to predict peptides' allergenicity, toxicity, taste-evoking properties, potential bioactivity, and the hydrolytic actions of gastrointestinal proteases, enhancing efficiency and reducing costs.[191] For example, Nongonierma and FitzGerald[192] performed *in silico* gastrointestinal digestion on eight casein-derived DPP-IV inhibitory peptides and found that NP and IP were resistant to cleavage by pepsin, trypsin, and chymotrypsin. However, *in silico* simulated digestion differs from actual digestion. *In silico* gastrointestinal digestion is a database-based simulation assuming that all peptide bonds theoretically susceptible to a given digestive enzyme are hydrolyzed. However, peptide hydrolysis is often incomplete under actual conditions due to factors such as temperature, pH, duration, the availability of bonds, and the complexity of the gastrointestinal environment. As bioinformatics advances, exploring more realistic *in silico* gastrointestinal digestion simulations becomes increasingly valuable.

Bioactive peptides demonstrate significant potential for disease prevention and treatment. However, challenges such as poor gastrointestinal stability must be addressed before commercial application, necessitating further attention and research for their development.

Author contributions

Chenlong Chen: conceptualization, formal analysis, investigation, methodology, project administration, software, visualization, writing – original draft, writing – review & editing. Wancong Yu: formal analysis, investigation, methodology, writing-review & editing. Xiaohong Kou: conceptualization, visualization, writing – original draft. Yujia Niu: software, visualization. Jiaxin Ji: project administration. Ying Shao: writing – original draft. Shuqi Wu: visualization. Mengyi Liu: project administration. Zhaohui Xue: conceptualization, formal analysis, methodology, funding acquisition, writing – review & editing.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

Conflicts of interest

There are no conflicts to declare.

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