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**Review**

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# Food-derived bioactive peptides: health benefits, structure-activity relationships, and translational prospects

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**Abstract:** Food-derived bioactive peptides (FBPs), particularly those with 10 or fewer amino acid residues and a molecular weight below 1300 Da, have gained increasing attention for their safe, diverse structures and specific biological activities. The development of FBP-based functional foods and potential medications depends on understanding their structure-activity relationships (SARs), stability, and bioavailability properties. In this review, we provide an in-depth overview of the role of FBPs in treating various diseases, including Alzheimer's disease, hypertension, type 2 diabetes mellitus, liver diseases, and inflammatory bowel diseases, based on the literature from the last five years. Subsequently, attention is directed towards elucidating the associations between the bioactivities and structural characteristics (e.g. molecular weight, and the presence of specific amino acids within sequences and compositions) of FBPs. We also discuss in-silico approaches for FBP screening and their limitations. Finally, we summarize recent advancements in formulation techniques to improve the bioavailability of FBPs in the food industry, thereby contributing to healthcare applications.

**Key words:** Functional foods; Biological compound; In silico prediction; Bioavailability; Oral delivery; Absorption

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## 1 Introduction

Bioactive peptides (BPs) are short chains of 2-20 natural amino acids. Most BPs with specific physiological functions are typically low in molecular weight. These peptides are encrypted within food proteins. They are usually produced via protein hydrolysis, fermentation, or food processing (Kang et al., 2022). Peptides with low molecular weight are easier to digest and absorb than larger peptides and intact proteins (Wang and Li, 2017a). Moreover, they are frequently preferred in solid-phase synthesis (Hamley, 2017). Due to their diverse activities, small food-derived bioactive peptides (FBPs) are among the most popular research topics in the field. This review covers FBPs with a molecular weight of less than 1300 Da and no more than 10 amino acid residues.

While traditional absorption theory suggests complete degradation of proteins into free amino acids for absorption, discoveries since the 1960s have shown that proteins can be absorbed by intestinal mucosal cells in the form of peptides following digestion. Various BPs, notably hepatoprotective glutathione (Masubuchi et al., 2011), antioxidant carnosine (Vistoli et al., 2012), and anticancer tyroservatide (Jia et al., 2005), have been incorporated into nonpharmacological therapeutic approaches. Meanwhile, the Food and Drug Administration has approved numerous peptide drugs for medical purposes (Erak et al., 2018), demonstrating efficacy in treating metabolic disorders, such as obesity, diabetes, hypertension, infections, and inflammation (Fosgerau and Hoffmann, 2015).

FBPs exhibit high biological activity and minimal side effects beyond their nutritional value (Duffuler et al., 2022). However, the current methods for FBP discovery, which depend on structure-activity relationships (SARs) using *in silico* approaches, combined with bioinformatics and biomolecular simulations, are hindered by a limited understanding of the actual SARs of these peptides (Cu et al., 2011; Daliri et al., 2017a). Therefore, there is an urgent need to elucidate the explicit relationships between the biological activities and structural characteristics of FBPs.

Although FBPs have potential uses for disease prevention, their bioactivity *in vitro* does not easily translate into pharmacological effects *in vivo*, due to obstacles such as mucus and digestive enzymes in the gastrointestinal tract (GIT). These factors can disrupt the stability, bioavailability, and oral absorption of BPs (Sun and Udenigwe, 2020). As oral agents, the palatability of BPs with high oral bioavailability is essential to ensure patient compliance. Ensuring palatability is one of the biggest challenges to consumer acceptance (Jakubczyk et al., 2020b). This review focuses on the potential pharmacological intervention of FBPs in chronic diseases (Fig. 1) based on literature from 2017 to the present. To facilitate and broaden the possible applications of FBPs as functional foods and potential medications, we also introduce advanced formulation techniques of FBPs and their application prospects. An in-depth understanding of the biological activities, mechanisms, and obstacles of intestinal absorption will broaden the potential preventive applications of FBPs.

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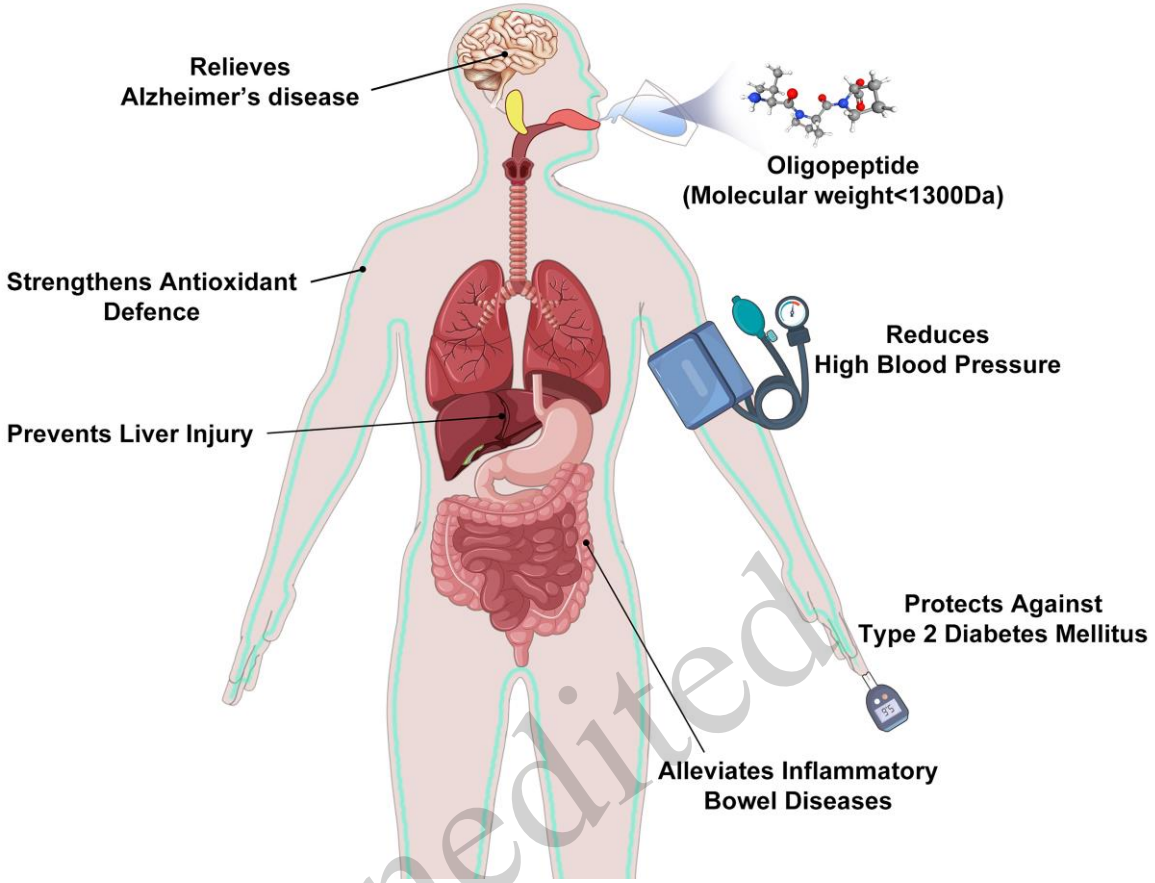


**Fig. 1 Illustration of the potential medical applications of food-derived bioactive peptides (FBPs) in various chronic diseases, highlighting their roles in antioxidant defense, oral route tolerance, and gastrointestinal absorption.**

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## 2 Bioactivity and preventive potential of FBPs in chronic diseases

Certain FBPs demonstrate antioxidant, antihypertensive, antidiabetic, hepatoprotective, and anti-inflammatory activities, contributing to the multi-preventive potential of FBPs in different chronic diseases. Antioxidant FBPs inhibit reactive oxygen species (ROS) or reactive nitrogen species (RNS). Suppression of activation of hepatic stellate cells (HSC) by FBPs is a key strategy in liver protection, as HSC activation leads to liver fibrosis and potentially to cirrhosis or even hepatocellular carcinoma. Antihypertensive peptides target mainly angiotensin-converting enzyme (ACE) and renin, while antidiabetic FBPs usually inhibit α-amylase, α-glucosidase, or DPP-IV. Furthermore, anti-inflammatory FBPs act predominantly by suppressing the NF-κB or MAPK pathways and anticancer FBPs work by promoting immune defense and apoptosis while inhibiting tumor cell proliferation (Fig. 2). Using FBPs for pharmacological intervention against chronic diseases offers significant advantages such as safe sources, low cost, and additional nutritional benefits. Generally, FBPs not only provide essential amino acids but also offer benefits for preventing and treating chronic diseases (Udenigwe and Aluko, 2012). As novel functional foods, FBPs have become a promising source for drug discovery (Pavlicevic et al., 2022). However, the preventive or therapeutic use of FBPs is still limited, possibly due to low bioavailability or indirect relationships between administered FBPs and their actual effects, leading to misconceptions and incorrect assumptions regarding their mechanisms of action. This section summarizes the bioactivities of some FBPs in specific chronic diseases, with a particular focus on disease-related mechanisms of action (Table 1).

**Table 1 Food-derived bioactive peptides with different activities and corresponding mechanisms**

| Sequence | Activity | Source | Function | Target | Reference |
|---|---|---|---|---|---|
| QQRQQQGL | Antioxidative | Defatted walnut meal | *In vitro*: Protection on $H_2O_2$-Injured SH-SY5Y cells | ROS | (Sheng et al., 2019) |
| YVLLPSPK | Antioxidative<br>Anti-Alzheimer | Walnut | *In vivo:* Alleviating learning and memory impairments in Scopolamine-treated mice | NRF2/KEAP1/HO-1 Pathway | (Zhao et al., 2021) |
| YW | Anti-Alzheimer | Soybean | *In vivo:* Increasing spatial working memory | Catecholamine synthesis and metabolism | (Ichinose et al., 2020) |
| YP | Anti-Alzheimer | Soybean | *In vivo:* Increasing working and fear conditioning memory; Enhancing choline acetyltransferase protein expression | Cholinergic neurotransmission pathway | (Tanaka et al., 2020) |
| KELEEK<br>RDPEER<br>LDDDGR<br>GFAGDDAPRA<br>DAAGRLQE | Antioxidative | Watermelon seed | *In vitro*: Cytoprotecting $H_2O_2$-Injured HepG2 cells | ROS | (Wen et al., 2020) |
| QMDDQ<br>KMDDK<br>KMDDQ<br>QMDDK<br>MTTNI | Antioxidative | Shrimp meat | *In vitro*: Increasing the viability of PC12; Inhibiting cell apoptosis | ROS | (Wu et al., 2019) |
| RDRHQKIG<br>TDRHQKLR<br>MNDRVNQGE<br>RENIDKPSRA<br>SYPTECRMR | Antioxidative | Sesame (*Sesamum indicum L.*) | *In vitro*: Radical scavenging effect | ROS | (Lu et al., 2019) |
| SF<br>QY | Antioxidative | *Moringa oleifera* seeds | *In vitro*: Increasing activities of superoxide dismutase and catalase | ROS | (Liang et al., 2020) |
| YLVN,<br>EEHLCFR<br>TFY | Antioxidative | Pea protein hydrolysates | *In vitro*: Radical scavenging effect;<br>*In silico*: Inhibiting the activation of the Keap1-Nrf2 pathway | ROS | (Zhao and Liu, 2023) |
| VYPFPGPIPN | Antioxidative<br>Anti-Alzheimer | Brazilian kefir | *In vitro*: Radical scavenging effect<br>*In silico*: Interacting with β-amyloid plaque; Neutralizing the negative effects of β-amyloid plaque aggregation | ROS<br>β-amyloid plaque | (Malta et al., 2022) |
| Se-MPS | Antioxidative | Se-rich brown rice | *In vitro*: Inhibiting lipid peroxidation; Transferring hydrogen atom | ROS | (Liu et al., 2019a) |
| LIVGIIRCV | Antihypertensive | Beef myofibrillar proteins | *In vitro*: ACE inhibitory activity | ACE | (Lee and Hur, 2019) |

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|---|---|---|---|---|---|
| | | | *In vivo*: Antihypertensive effect in spontaneously hypertensive rats | | |
| LPGPGP EYFR | Antihypertensive | *Channa striatus* | *In vitro*: ACE inhibitory activity | ACE | (Ma et al., 2021) |
| IF | Antihypertensive Antioxidative | Potato | *In vitro*: ACE inhibitory activity *In vivo*: Renal-protective effects | ACE ROS | (Tsai et al., 2020) |
| LY RALP GHS | Antioxidative Antihypertensive Anti-inflammatory | Rapeseed | *In vivo*: Lipid peroxidation inhibition; Inhibiting the secretion of pro-inflammatory cytokines; Improving cell damage; Decreasing the systolic blood pressure | ROS ACE Renin | (He et al., 2019a; He et al., 2019b) |
| YV | Antihypertensive | Ostrich (*Struthio camelus*) egg white ovalbumin | *In vitro*: ACE inhibitory activity | ACE | (Khueychai et al., 2018) |
| YACSVR CAEAGH | Antidiabetic | *Sardine pilchardus* | *In vitro*: DPP-IV inhibitory activity | DPP-IV | (Rivero-Pino et al., 2020) |
| LPLLR | Antidiabetic | Walnut | *In vitro*: Promoting glycogen synthesis; Improving glucose uptake; Suppressing improved glucose uptake; Activating IRS-1/PI3K/Akt and AMPK signal pathway (in insulin resistance HepG2 cell) | α-glucosidase and α-amylase; IRS-1/PI3K/Akt and AMPK pathway | (Wang et al., 2020) |
| GSR EAK | Antidiabetic | Soybean | *In vitro*: α-glucosidase inhibitory activities *In vivo*: Hypoglycemic efficacy in mice with alloxan-induced diabetes | α-glucosidase | (Jiang et al., 2018) |
| GRVSNCAA TYLPVH | Antihypertensive Antidiabetic | *Ruditapes philippinarum* | *In vitro*: ACE and DPP-IV inhibitory activity *In vivo*: Promoting NO secretion and reducing ET-1 secretion to lower blood pressure; Exerting hypoglycemic activity by increasing the PK and HK levels | ACE DPP-IV | (Zhang et al., 2021a) |
| IPIPATKT | Antihypertensive Antidiabetic | Sanhuang Chicken | *In vitro*: ACE and DPP-IV inhibitory activity *In vivo*: Promoting NO secretion and reducing ET-1 secretion to lower blood pressure; Exerting hypoglycemic activity by increasing the PK and HK levels | ACE DPP-IV | (Chen et al., 2021) |

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|---|---|---|---|---|---|
| DIKTNKPVIF | Antidiabetic | Potato (*Solanum tuberosum L.*) | *In vivo*: Regulating blood glucose; Retaining insulin levels; Efficient immunomodulatory effects | NF-κB-associated iNOS | (Marthandam Asokan et al., 2019) |
| LPTGWLM, MFE, GPAHCLL HLPGRG QNVLPLH, PLMLP | Antidiabetic | Camel and bovine casein | *In vitro*: α-glucosidase and DPP-IV inhibitory activity | α -glucosidase DPP-IV | (Mudgil et al., 2021) |
| KDLWDDFKGL MPSKPPLL | Antidiabetic | Camel milk | *In vitro*: α-amylase inhibitory activity | α-amylase | (Mudgil et al. 2018) |
| FPVG; LPVL VPFP; IPL | Antidiabetic | *Oncorhynchus keta* (chum salmon) milt | *In vitro*: DPP-IV inhibitory activity | DPP-IV | (Takahashi et al., 2021) |
| YPLP LPYP | Hepatoprotective Antioxidant | *Corbicula fluminea* | *In vitro*: Radical scavenging effect; Protection on ethanol-injured LO2 cells | ROS CYP2E1 | (Ren et al., 2021) |
| DHNNPQIR | Hepatoprotective Antioxidant | Rapeseed | *In vivo*: Suppressing fibrosis-associated genes in models of high-fat diet-induced and $CCl_4$-induced liver injury | Cell cycle ROS | (Zhao et al., 2019) |
| LDAPGHR | Anti-inflammatory | Hazelnut | *In vitro*: Inhibiting release of pro-inflammatory cytokines; Inhibiting NF-κB and MAPK pathways activation | NF-κB and MAPK pathways | (Ren et al., 2018) |
| LSW | Anti-inflammatory | Soybean | *In vitro*: Decreasing phosphorylation of Src, ERK1/2, nuclear transcription factor p50 | NF-κB pathway | (Lin et al., 2017) |
| YFYPQL | Anti-inflammatory Antioxidative | Buffalo casein | *In vitro*: Suppressing the secretion of IFN-γ; Promoting the secretion of IL-10 | ROS | (Sowmya et al., 2019) |
| WFNNAGP | Anti-inflammatory Antioxidative | *Tricholoma matsutake* | *In vivo*: inhibiting myeloperoxidase and pro-inflammatory cytokine expression and protecting the barrier function by promoting the expression of occludin and ZO-1 in the colon | NF-κB pathway | (Li et al., 2021) |
| GPAGPL GPP-GAP | Anti-inflammatory | Dry-cured ham | *In vitro*: Inhibiting the secretion of NO, IL-6, and TNF-α in LPS-induced RAW264.7 cell | PI3K/AKT signal pathway | (Fu et al., 2021) |

Abbreviations: ACE, Angiotensin converting enzyme; CYP2E1, Cytochrome P450 2E1; DPP-IV, Dipeptidyl peptidase IV;

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EPK1/2, Extracellular signal regulated kinases 1 and 2; ET-1, Endothelin-1; HK, Hexokinase; Hsp90α, Heat shock protein 90α; IFN, Interferon; IKK, Iκ$\kappa$B kinase; IL, Interleukin; iNOS, Inducible nitric oxide synthase ; I$\kappa$B$\alpha$, Inhibitor of NF kappa B alpha; JNK, c-Jun N-terminus kinase; MAPK, Mitogen-activated protein kinase; MMP, Matrix metalloproteinase; NF-κB, Nuclear factor-kappa light-chain-enhancer of activated B cells; NO, Nitric oxide; PI3K, Phosphoinositide 3-Kinase; PK, Pyruvate kinase; ROS, Reactive oxygen species

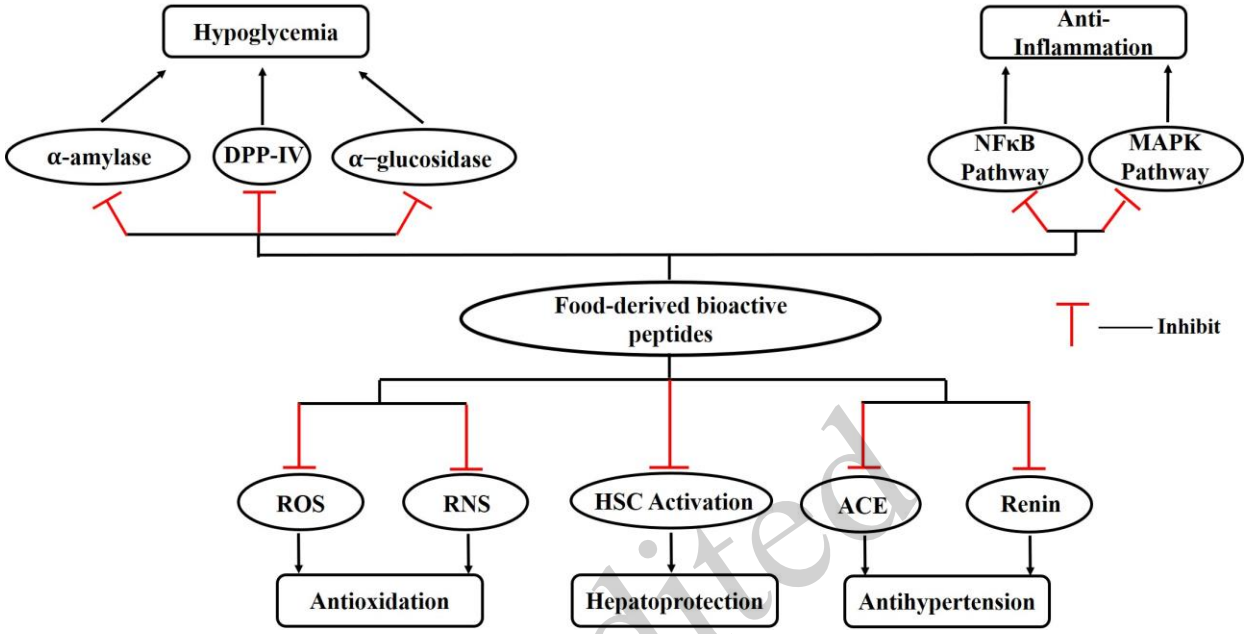


**Fig. 2 Diagram depicting the biological activities of food-derived bioactive peptides (FBPs) and their therapeutic targets, including their roles in combating oxidative stress, liver fibrosis, hypertension, diabetes, inflammation, and cancer.**

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## 2.1 Alzheimer's disease

Alzheimer's disease (AD) is characterized by the accumulation of amyloid-β peptides, dystrophic neurites, and neurofibrillary tangles (Martins et al., 2018), which are considered hallmarks of the disease. Despite uncertainties in the pathogenesis of AD, elevated levels of various biomarkers, including lipid peroxidation, 4-hydroxy-2-nonenal, 3-nitrotyrosine, and 8-hydroxy-deoxyguanosine, have been found in AD brains, indicating oxidative or nitrosative damage (Di Domenico et al., 2017). Research thus points to oxidative stress as being a crucial factor in the pathogenesis and development of AD (Butterfield and Boyd-Kimball, 2018; Butterfield and Halliwell, 2019).

Oxidative stress refers to the disequilibrium between the production of ROS/RNS and the efficacy of antioxidant defense. The ROS group includes superoxide, hydroxyl radicals, hydrogen peroxide ($H_2O_2$), and singlet oxygen, and consists of highly reactive molecules/radicals formed through $O_2$ oxidation. These species are integral to cellular metabolism in aerobic conditions and play a significant role in cellular signaling pathways (Jakubczyk et al., 2020). Therefore, antioxidant supplements have been suggested as potential pharmacological interventions to mitigate AD progression by neutralizing excess ROS and RNS (Butterfield and Halliwell, 2019).

FBPs exhibit antioxidant characteristics (He, et al., 2019a; Lu, et al., 2019; Qu et al., 2020), including radical scavenging effects, metal chelation, inhibition of lipid oxidation, and ferric-reducing capability, thereby effectively scavenging ROS. Some enzymatic hydrolysates obtained from walnut and soybean proteins exhibited memory-enhancing effects in animal models (Katayama et al., 2021). YVLLPSPK derived from walnut, increased spatial learning memory ability and suppressed oxidative stress and mitochondrial damage, activating PINK1 via the NRF2/KEAP1/HO-1 Pathway (Zhao, et al., 2021). The brain-transportable soy dipeptides, YW (Ichinose, et al., 2020) and YP (Tanaka, et al., 2020), alleviated amyloid beta peptide (25-35)-induced memory impairment in mice. In cellular model tests, recent research has indicated that the defatted walnut meal hydrolysate-derived peptide QQRQQQGL exhibits potent antioxidant activity by neutralizing hydroxyl radicals

and eliminating ROS to protect SH-SY5Y cells from $H_2O_2$-induced oxidative stress-induced harm (Sheng, et al., 2019). Purified watermelon seed protein hydrolysates DAAGRLQE, RDPEER, GFAGDDAPRA, LDDDGR, and KELEEK also protected from $H_2O_2$-induced cell damage (Wen, et al., 2020). Molecular docking results demonstrated that YLVN, EEHLCFR, and TFY derived from pea protein hydrolysates might inhibit the activation of the Keap1-Nrf2 pathway by occupying the Keap1-Nrf2 binding site (Zhao and Liu, 2023). Another major source, animal meat, contains peptides like QMDDQ, KMDDK, KMDDQ, QMDDK, MTTNI, and MTTNL found in shrimp meat which shield PC12 cells from oxidative damage through inhibition of the apoptotic pathway (Wu, et al., 2019). VYPFPGPIPN, screened in the "milk and bovine" database, was observed to interact with amyloid plaques based on molecular docking analysis (Malta, et al., 2022). The consequent conformational change might reduce the toxicity of β-amyloid plaques and therefore attenuate the effects of AD. Overall, ROS scavengers demonstrate the potential for augmenting antioxidant defense.

### 2.2 Hypertension

Hypertension is a prominent risk factor for heart disease and stroke, with its prevalence escalating rapidly, especially in East Asia, Southeast Asia, South Asia, and Oceania (Wedderkopp, 2020). Several physiological systems regulate blood pressure, including the renin-angiotensin system (RAS), renin-angiotensin-aldosterone system (RAAS), kallikrein-kinin system (KKS), and nitric oxide system (NOS), with renin and angiotensin-converting enzyme (ACE) playing critical roles (Daliri et al., 2017b). ACE promotes the conversion of angiotensin I (Ang-I) to angiotensin II (Ang-II), which negatively impacts vasodilation leading to sustained vasoconstriction, limiting blood pressure reduction by degrading the antihypertensive agent, bradykinin (Wu et al., 2017). Further, an excessive accumulation of Ang-II retards vasodilation and contributes to increased blood pressure. Renin initiates the conversion of angiotensinogen to Ang-I, marking the first and rate-limiting step in the RAS. Chymotrypsin can bypass the typical ACE pathway by facilitating the conversion of Ang-I to Ang-II, thus directly inhibiting renin, offering a greater potent antihypertensive effect than ACE inhibition (Wu, et al., 2017).

Some FBPs with antihypertensive potential have greater tissue affinity and fewer side effects than chemically synthesized antihypertensive drugs (Daliri, et al., 2017b; Duffuler, et al., 2022). Milk is the most abundant source of antihypertensive BPs (Kaur et al., 2021), such as the well-known antihypertensive peptides VPP and IPP (Li et al., 2019). Seafood, eggs, and certain plants also serve as rich FBP sources. The hydrolysis of ostrich egg white protein using pepsin, pancreatin, or alkaline hydrolysis yielded WESLSRLLG and YV, which exhibit ACE (angiotensin-converting enzyme) inhibition (Khueychai, et al., 2018; Kaur, et al., 2021). *In silico* analysis determined that DNRYY, a low molecular weight pentapeptide purified from hydrolysate of velvet antler, can interact with ACE (Im and Lee, 2023). *In vivo* analysis also demonstrated that DNRYY significantly reduced blood pressure in spontaneously hypertensive rats following oral administration. FBP inhibition mechanisms, particularly for ACE inhibitors, are categorized mainly into competitive, non-competitive, and anticompetitive types. For example, VY competitively inhibits ACE; VP, IY, and LW non-competitively inhibit ACE; AW, FY, and IW reduce enzyme activity by binding to the intermediate formed by ACE and its substrate (Sato et al., 2002). Lastly, LY, RALP, and GHS, which are purified from rapeseed, exhibit strong antihypertensive effects by inhibiting renin (He, et al., 2019b).

### 2.3 Type 2 diabetes mellitus

The latest report by the International Diabetes Federation indicates that in 2019 about 463 million adults worldwide aged 20–79 years had diabetes. It was predicted that the number of people with diabetes will rise to 578.4 million by 2030 and to 700.2 million by 2045 (IDA, 2019), which shows that diabetes is reaching epidemic levels. (Ida, 2019). Diabetes mellitus (DM) is a chronic, progressive, metabolic disease whose pathogenesis remains partially understood. Hyperglycemia results from an imbalance between glucose absorption and insulin secretion (Chaudhury et al., 2017). DM manifests in two forms: Type 1 (T1DM) and Type 2 (T2DM). T1DM is triggered by an autoimmune reaction leading to a pancreatic attack, which reduces or halts insulin production. T2DM is closely linked to obesity, age, and genetic factors, defined by insufficient

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insulin production or insulin resistance (Yan et al., 2019). The pathogenesis of T2DM is more complex than that of T1DM (Ida, 2019).

For these reasons, the development of hypoglycemic drugs for the treatment of T2DM is a major focus of clinical research. Normally, chemically synthesized hypoglycemic drugs are associated with pancreatitis, hypoglycemia, weight gain, and other unfavorable effects (Daliri, et al., 2017a). Strategies to design hypoglycemic drugs aim to prevent excessive blood glucose levels and improve insulin sensitivity in patients. One approach involves identifying FBPs for compounds that could inhibit the activity of enzymes such as α-amylase and/or α-glucosidase which are responsible for digesting polysaccharides into glucose (Yan, et al., 2019). Additionally, food digestion releases insulin secretion hormones called incretins, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP), which act to maintain stable blood glucose levels (Campbell and Drucker, 2013). The inhibition of dipeptidyl peptidase IV (DPP-IV), which rapidly hydrolyzes GLP-1 and GIP, is another strategy to reduce blood glucose levels.

Vegetables (Chan-Zapata et al., 2020), dairy products, and marine sources are excellent sources of antidiabetic peptides. DIKTNKPVIF (derived from potato protein hydrolysate) (Marthandam Asokan, et al., 2019), LPTGWLM, MFE, GPAHCLL, HLPGRG, QNVLPLH, PLMLP (derived from camel and bovine casein hydrolysates) (Mudgil, et al., 2021), KDLWDDFKGL, MPSKPPLL (derived from camel milk protein hydrolysates) (Mudgil et al., 2018), FPVG, LPVL, VPFP, IPL (derived from salmon milt) (Takahashi, et al., 2021), CAEAGH, and YACSVR (derived from discarded *Sardine pilchardus* protein) (Rivero-Pino, et al., 2020), have shown potential antidiabetic activities. Zhang *et al*. (Zhang, et al., 2021a) found that GRVSNCAA and TYLPVH, which are derived from *Ruditapes philippinarum*, have both antihypertensive and antidiabetic activities by promoting nitric oxide (NO) secretion, reducing endothelin-1 secretion, and increasing hexokinase and pyruvate kinase levels. Another umami peptide, IPIPATKT, exhibits strong dual hypertensive and antidiabetic effects both *in vitro* and *in vivo* (Chen, et al., 2021). Furthermore, peptides GSR and EAK, obtained after hydrolyzing soybean protein with trypsin, effectively suppress α-glucosidase activity (Jiang, et al., 2018), while the walnut peptide LPLLR inhibits α-amylase and α-glucosidase (Wang, et al., 2020). YACSVR and CAEAGH, recognized for their potent DPP-IV inhibitory activity, appear to be viable alternatives to traditional antidiabetic medications (Rivero-Pino, et al., 2020). Although most studies have demonstrated the antidiabetic properties of FBPs *in vitro*, their efficacy *in vivo* remains less clear (Liu et al., 2019b), which has limited the translation of many antidiabetic FBPs into commercial antidiabetic medications (Buckley et al., 2018).

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### 2.4 Liver diseases

In recent decades, non-alcoholic steatohepatitis has emerged as the most prevalent chronic liver disease in developed regions. It is often accompanied by progressive collagen deposition and vascular remodeling, resulting in liver fibrosis and cirrhosis (Abdelmalek, 2021). In addition, acute hepatitis can be caused by excessive alcohol intake (Xiao et al., 2019), leading to ongoing liver damage and eventually liver fibrosis. Liver fibrosis, a precursor to cirrhosis and potentially hepatocellular carcinoma, results from an imbalance between hepatic parenchymal cells and the accumulation of extracellular matrix (ECM) (Tsochatzis, 2022). The regulation of ECM generation and degradation is generally accomplished by matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) (Yoshiji et al., 2002; Atta et al., 2014), while HSCs are crucial to the development of liver fibrosis and ECM-remodeling. Unfortunately, there is a lack of specific antifibrotic drugs that could effectively control HSC activation or liver fibrosis, with existing drugs showing limited efficacy and adverse effects (Bataller and Brenner, 2005; Tsuchida and Friedman, 2017). Active ingredients extracted from food may have significant potential in developing healthcare supplements to address liver diseases worldwide.

Several food-derived substances that exhibit hepatoprotective effects, including FBPs and polysaccharides, have been found in a variety of sources, such as corn, soybean, wheat, fruit, and marine animals (Yamaguchi et al., 1997; Liu et al., 2018; Jiang et al., 2020; Qu, et al., 2020). For instance, Ren *et al*. (Ren, et al., 2021) found that YPLP and LPYP derived from *Corbicula fluminea* provide strong protection for LO2 cells against

ethanol-induced damage, attributed to their antioxidant activity and inhibition of cytochrome P450 2E1. DHNNPQIR, a natural FBP derived from rapeseed, has been shown to improve insulin resistance and directly suppress fibrosis-associated genes in models of high-fat diet-induced and $CCl_4$-induced liver injury (Zhao, et al., 2019). Note that leucine and alanine alone do not have any hepatoprotective properties, but corn peptides consisting of these amino acids (either alone or in combination) aid in alcohol metabolism (Yamaguchi, et al., 1997).

### 2.5 Inflammatory bowel diseases

Effective immunomodulation is essential for maintaining immune function and promoting human health through the suppression or stimulation of immune responses. When immune responses become dysregulated or excessively active, both acute and chronic inflammation can occur (Majumder et al., 2016). Inflammatory bowel diseases (IBDs) are chronic inflammatory diseases that pose a significant health burden and are becoming increasingly prevalent in many developing countries (Hoivik et al., 2013). They are characterized by gastrointestinal disorders and the extravasation of numerous inflammatory cells in patients with IBDs is caused by complex interactions. Numerous studies suggest that oxidative stress increases during chronic intestinal inflammation (Pereira et al., 2015). However, the mechanisms by which factors initiate and perpetuate inflammation and disrupt intestinal homeostasis remain unclear. Certain FBPs, including YFYPQL, exhibit both anti-inflammatory and antioxidant properties and may serve as effective interventions for IBDs (Sowmya, et al., 2019).

Anti-inflammatory FBPs exert their effects mainly by modulating the mitogen-activated protein kinase (MAPK) and the nuclear factor-kappa light-chain-enhancer of activated B cells (NF-κB) pathways. Additionally, down-regulation of cyclooxygenase-2 (COX-2), reduction of tumor necrosis factor-α (TNF-α), histamine release, and nitric oxide production all play essential roles in the anti-inflammatory activities (Chakrabarti et al., 2014; Majumder, et al., 2016; Guha and Majumder, 2019). Overall, most modulation targets the pro-inflammatory or inflammatory cytokines.

LDAPGHR, a peptide derived from hazelnut protein, can regulate both the NF-κB and MAPK pathways by inhibiting phosphorylation of IκBα, ERK1/2, p38, JNK, and nuclear translocation of p65. This action results in suppression of COX-2, NO, and inflammatory cytokines (TNF-α, IL-1β, IL-6). Thus, LDAPGHR produces a strong anti-inflammatory effect (Ren, et al., 2018). The *Tricholoma matsutake*-derived peptide WFNNAGP significantly ameliorates dextran sodium sulfate-induced oxidative damage by downregulating NF-κB expression and inhibiting the formation and activation of NLRP3 and caspase-1 (Li, et al., 2021). GPAGPL and GPPGAP derived from dry-cured ham showed anti-inflammatory capacity by decreasing the secretion of NO and IL-6 (Fu, et al., 2021). Moreover, some peptides modulate cytokines to exert anti-inflammatory effects. For instance, YFYPQL, an anti-inflammatory hexapeptide isolated from buffalo casein, suppressed the secretion of the pro-inflammatory cytokine IFN-γ and induced the secretion of the anti-inflammatory cytokine IL-10 in a Caco-2 cell model under attack by $H_2O_2$ (Sowmya, et al., 2019).

Natural antimicrobial peptides consist of 10-100, but usually fewer than 50, amino acid residues (Maroti et al., 2011), and have significant potential value in medicine. Some antimicrobial peptides also have anti-inflammatory bioactivity (Luo and Song, 2021), such as LIKKIYRKWKRW and LWKKIYRKWKRW, which are derived from duck cathelicidin (Kumar and Shin, 2020). The antimicrobial activity of these peptides is positively correlated with chain length (Liu et al., 2007). However, most antimicrobial peptides do not belong to FBPs because of their excessive number of amino acid residues. Screening approaches and the SARs of antimicrobial peptides have been summarized in recent reviews (Bin Hafeez et al., 2021; Luo and Song, 2021).

## 3 Relationships between the bioactivity and structure of FBPs

All the functions of FBPs are tightly related to their structure. The structural characteristics of FBPs found

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both in silico and in trials are summarized in Table 2. The following section highlights the molecular weight, specific amino acid sequences, and spatial structure of different FBPs to summarize the SARs of FBPs.

**Table 2 Structural characteristics of food-derived bioactive peptides**

| Activity | Structure characteristics | Examples | Reference |
|---|---|---|---|
| Antioxidative | Low molecular weight; Hydrophobic amino acids; Aromatic amino acids; Basic amino acids; Arg at C-terminal; Cys, Tyr, and Trp at N-terminal | IY, QY | (Nwachukwu et al., 2019; Wu, et al., 2019; Jakubczyk et al., 2020a; Liang, et al., 2020) |
| Antihypertensive | Hydrophobic amino acids, aliphatic or aromatic amino acids at the C-terminal; Hydrophobic amino acids at the N-terminal and bulky amino acids at the C-terminal | IW | (Udenigwe et al., 2012; Lee and Hur, 2019) |
| Antidiabetic | Tri- to hexa- peptides containing hydroxyl or other basic side chains at the N-terminal, Pro at the penultimate C-terminal, and Ala or Met as the C-terminal residue | LPLLR | (Ibrahim et al., 2018; Wang, et al., 2020) |
| Hepatoprotective | Hydrophobic amino acids | YPLP, LPYP | (Ren, et al., 2021) |
| Anti-inflammatory | Hydrophobic amino acids at N-terminal; Charged amino acids; Low molecular weight with Glycine | LDAPGHR | (Tang and Skibsted, 2016) (Ren, et al., 2018; Guha and Majumder, 2019) |

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### 3.1 Molecular weight

Although the relationships between FBP structure and the related bioactivity are still not fully elucidated, considerable evidence has highlighted that molecular weight plays a crucial role in antioxidant, antihypertensive, anti-diabetic, and anti-inflammatory effects (Guha and Majumder, 2019; Nwachukwu, et al., 2019; Yan, et al., 2019; Jakubczyk, et al., 2020a; Hu et al., 2023). Compared to other bio-functional peptides, FBPs have improved molecular mobility and diffusivity, due to their low molecular weight. Therefore, FBPs could more easily penetrate the intestinal barrier than traditional large peptides and terminate the free radical chain reactions to exhibit antioxidant activities (Chi et al., 2015; Pan et al., 2019). Meanwhile, crystallographic studies of ACE-inhibitory peptides have shown that large peptides cannot bind to active sites of ACE, which limits their antihypertensive activity (Natesh et al., 2003). However, these findings do not imply that low molecular weight FBPs exert better bioactivity. Jahanbani et al. (Jahanbani et al., 2016) found the antioxidant activities of smaller molecular weight fractions were not superior to those of whole hydrolysates.

### 3.2 Specific amino acid sequence and composition

The bioactivities of most FBPs may hinge more on the presence of specific amino acids rather than peptide length. Recent reviews have summarized the specific amino acid residues that contribute to effective antioxidant activities, including hydrophobic amino acids (Ala, Ile, Leu, Val, Met, and Pro), aromatic amino acids (Phe, Trp, and Tyr), acidic amino acids (Asp and Glu) and basic amino acids (His) (Nwachukwu, et al., 2019; Jakubczyk, et al., 2020a). Metal-chelating amino acid residues (e.g. Gln and Lys) within FBPs lead to a strong radical scavenging potential by inhibiting the prooxidant activity of superior $Fe^{2+}$ and/or charged groups (Xia et al., 2012). Most hydrophobic amino acid residues can interact with free radicals (Sila and Bougatef, 2016), whereas the enrichment of hydrophobic residues in FBPs does not directly correlate with antioxidant activities. For example, IY with two hydrophobic amino acid residues has a weaker free radical scavenging

ability than QY which contains only one (Liang, et al., 2020). The location and repetitive units of specific amino acids can be critical factors for antioxidant capacity as in LDDDGR and YPQLLPNE (Siow and Gan, 2013; Du-Xin et al., 2016; Wen, et al., 2020). Moreover, amino acids involved in both terminals of FBPs are also vital to antioxidant activities, such as the C- (Arg) and the N- (Cys, Tyr, and Trp) terminals, which may be attributed to carboxyl- and amino-active hydrogen atoms serving as active sites (Wu, et al., 2019).

Hydrophobic amino acids are similarly crucial in antihypertensive activity and comprise a high proportion of the amino acids in antihypertensive FBPs. Meanwhile, acidic amino acids (Asp and Gln) in the sequence of FBPs may chelate zinc atoms essential for enzyme activity (e.g. ACE) (Aluko, 2015). Additionally, Wu et al. (Wu et al., 2006a; Wu et al., 2006b) suggested SARs for antihypertensive dipeptides, tripeptides, and tetrapeptides, identifying specific structural prerequisites for each peptide type that contribute to their antihypertensive efficacy. Such dipeptides contained amino acids with hydrophobic and bulky side chains; tripeptides had an aromatic amino acid at the C-terminal, a hydrophobic residue at the N-terminal and a positively charged amino acid in the middle; in the case of tetrapeptides, the preferred amino acids at the C-terminal were Cys and Tyr, with Trp, His or Met in the second position, Met, Val, Leu or Ile in the third position, and Trp at the N-terminal. Notably, the modulation of hypertension by identified antihypertensive FBPs encompasses ACE inhibition and renin suppression, with the amino acid composition at the C-terminal significantly influencing ACE-inhibitory activity (Daliri, et al., 2017b). Higher ACE-binding affinity is observed when the C-terminal of antihypertension FBPs is composed of aliphatic (Gly, Ala, Val, Leu, Ile) or aromatic amino acids (Trp, Tyr, Phe) or other hydrophobic residues rather than hydrophilic groups (Lee and Hur, 2019). Likewise, for renin inhibition, dipeptides exhibiting bulky side chains and hydrophobic amino acids at the C- and N-terminal, respectively, demonstrate potent renin suppression. For instance, enzymatic inhibition assays show that dipeptides IW and LW provide effective renin inhibition, whereas AW and VW are inactive under the same conditions (Udenigwe, et al., 2012).

In anti-diabetic FBPs, Pro at the penultimate C-terminal and Ala or Met at the C-terminal enhance α-glucosidase inhibitory activity, whereas a negative charge diminishes it (Ibrahim, et al., 2018). The hydrophobicity and isoelectric points are less predictive of α-glucosidase inhibition.

Anti-inflammatory FBPs that contain glycine were found to have a high affinity for calcium binding and to disrupt $Ca^{2+}$ signaling through glycine-gated chloride channels (Tang and Skibsted, 2016). Charged amino acids and hydrophobic amino acids also participate in hepatoprotective and anti-inflammatory FBPs. FBPs containing either positive or negative charge amino acids, such as Lys, Arg, and Gln, also exhibit strong anti-inflammatory effects (Ren, et al., 2018). These effects can be further enhanced by hydrophobic amino acids clustering at the N-terminal (Lin, et al., 2017), probably due to enhanced peptide-cell membrane interactions. A high proportion of hydrophobic amino acids is usually found in hepatoprotective food hydrolysates (Yu et al., 2017; Chen et al., 2020; Ren, et al., 2021).

Existing findings offer plenty of clues for screening appropriate FBPs. However, these scattered clues still need systematic organization, which depends on *in silico* tools.

## 4 *In silico* methods used in FBP screening

To identify specific biological activities of FBPs, it is necessary to promote the development of suitable screening methods. Methods commonly used to screen for FBP activity include bioassays, kinetic analysis, and computational docking simulation (Nong and Hsu, 2022). Bioassays and kinetic analysis use a variety of instruments, including spectrophotometers and fluorescence spectrophotometers (Lahogue et al., 2010), to study the impact of bioactive peptides on a target enzyme. During the testing of antihypertensive bioactive peptides, the standard substrate N-α-hippuryl-L-histidyl-L-leucine is commonly used to form the hydrolysis products (hippuric acid and histidyl-leucine) which are monitored at 228 nm. Similarly, the screening process for hypoglycemic peptides involves the observation of changes in the fluorescence signal generated by the

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tested bioactive peptides resulting from the cleavage of the fluorescent substrate Gly-Pro-AMC hydrobromide. Zhou *et al*. (Zhou et al., 2021) used this method to discover a DPP-IV inhibitory peptide VPLVM derived from broccoli. Nevertheless, these methods can ascertain only the relative potency of the test peptides when compared to a standard and thus are not suitable for high-throughput screening of bioactive peptides. Using an *in-silico* approach for screening provides an efficient solution to this issue.

The use of computer-aided approaches has hastened the discovery of FBPs. Such approaches use diverse databases and associated algorithms to model the pharmacological and structural characteristics of these peptides. These *in silico* methods comprise mainly bioinformatics and biomolecular simulations. Bioinformatic methods use extensive databases to provide robust statistics and classification based on factors including bioactivity, absorption, distribution, metabolism, excretion, and toxicity of FBPs (Agyei et al., 2018; Shakya et al., 2020). Online *in silico* tools such as BIOPEP, PreAIP, iDPPIV-SCM, and AHTPIN facilitate the rapid screening of FBPs (Minkiewicz et al., 2019), including anti-inflammatory, hypoglycemic, and antihypertensive peptides (Kumar et al., 2015; Khatun et al., 2019; Charoenkwan et al., 2020). Different machine-learning models are essential for successful bioactivity prediction. However, two limiting steps hinder model performance: model selection and peptide representation (Du et al., 2023b). Recently, a screening model for antihypertensive peptide discovery has been released that uses a protein language model with evolutionary scale modeling embeddings that are refined through training on experimental datasets (Du et al., 2024). Using confident learning theory for bioactive peptide dataset cleaning and a pretrained language model for peptide embeddings, this model exhibited superior performance in precise bioactivity prediction than the twelve traditional embedding methods. Understanding the protein-peptide interactions is as vital as the bioactivity prediction. Computational methods commonly used for analyzing the binding sites and affinity effects of FBPs include qualitative structure-activity and property-relationship analyses (QSAR/QSPR), molecular docking, and molecular dynamics simulations (MDS). Pei *et al*. (Pei et al., 2022b) validated the robust binding affinity between the DPP-IV enzyme and VPLVM through molecular dynamics simulations (MDS). Meanwhile, it is necessary to obtain the 3D structure of the targeted protein which is used for binding analysis (Du et al., 2023a). In the last five years, Alpha-Fold2 and cryo-electron microscopy have been useful for circumventing the limitations of traditional X-ray crystallography and nuclear magnetic resonance spectroscopy (NMR) (Callaway, 2020; Jumper et al., 2021).

Although molecular docking and MDS have enhanced the understanding of the SARs of FBPs, their potential limitations often translate to discrepancies with experimental data. Molecular docking approximations of real molecular interactions suffer from fluctuations in the body's environment and sampling method limitations (e.g., solvent, pH), which restrict prediction accuracy (Trott and Olson, 2010). Predicting the time evolution of a system that comprises particles (i.e., atoms, molecules) and external factors (i.e., temperature, electric field) is a complex challenge for biomolecular simulations. To handle such a complex system, researchers often rely on MDS simulations to solve the equations of the motion of particles interacting within the system. However, the computational demands and extended processing times limit the practicality of these simulations by using force field equations in modeling systems (Vidal-Limon et al., 2022). Unfortunately, most FBPs are characterized by unknown pharmacokinetics, a short half-life ($t_{1/2}$), and rapid elimination by peptidases in the body (e.g., pepsin, trypsin), thereby limiting their potential development as oral supplements or drugs (Pei et al., 2022a). For example, casein-derived peptides (VPYPQ) and their fragments VP, VPYP, YPQ, and VPY, exhibited DPP-IV inhibitory bioactivity *in vitro*, with VPYPQ administration lowering blood glucose levels *in vivo*. However, their $t_{1/2}$ was less than 20 min (Zheng et al., 2019), highlighting the insufficiencies of current research methodologies.

## 5 Oral delivery of FBPs

Oral administration of FBPs is the most favorable and expedient route. While small molecular weight

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FBPs exhibit tolerance to hydrolases in the GIT (Udenigwe and Aluko, 2012), recent studies demonstrate oral administration of FBPs may lead to their degradation, thereby reducing their bioactivity or causing deactivation. Deactivation of orally administered FBPs is influenced by proteases in the GIT, peptidases in the epithelial cells, and transepithelial peptide transport. Brush-border peptidases regulate the degradation and bioavailability of orally administered FBPs by breaking them down into smaller peptides (Wang and Li, 2017b). Peptide transportation across the intestine is facilitated by three vital factors: peptide transporter 1, responsible for the transfer of di- and tripeptides; a paracellular transporter, facilitating the movement of hydrophilic, low molecular weight peptides; and transcytosis, involving endocytic uptake and subsequent basolateral release (Shimizu et al., 1997; Daniel, 2004; Regazzo et al., 2010). Regrettably, the oral bioavailability of most FBPs is typically less than 1%, significantly limiting their development as promising oral drugs.

### 5.1 Barriers to oral delivery

Absorption of FBPs through the GIT faces three major obstacles, namely the biochemical environment, mucus, and epithelial lining (Brown et al., 2020). The bioavailability of FBPs may be affected by active biochemical barriers, including pH and enzymes (e.g., brush-border and cytosol peptidases) (Allen and Carroll, 1985). The pH gradient from the stomach (pH 1.0–2.0) to the intestine (pH 4.0-7.5), along with gastrointestinal (GI) motility and abundant digestive enzymes, heavily impact the absorption of oral FBPs (Brown, et al., 2020). The coating of the GIT, comprising a firm mucus layer bound to the epithelial lining and a loose mucus layer adherent to the lumen, acts as an efficient physical barrier that frequently limits the diffusion of oral FBPs (Brown, et al., 2020; Drucker, 2020). The epithelial lining that includes enterocytes heavily influences the absorption process by inhibiting their transport from the GIT to the bloodstream. Furthermore, tight junctions between the epithelium, enterocytes, and active efflux pumps (e.g., P-glycoprotein) directly impact the bioavailability of oral FBPs by regulating the paracellular and transcellular pathways (Brayden et al., 2020; Brown, et al., 2020). Once absorbed, FBPs undergo hepatic first-pass metabolism, which significantly shortens their elimination $t_{1/2}$.

### 5.2 Approaches to overcome oral barriers

Despite being a relatively new field (under 40 years old), there have been significant efforts to develop strategies that enable the clinical translation of orally delivered medical FBPs. Common strategies that have been used to enhance BP absorption entail promoting penetration, impeding enzyme hydrolysis, and mucus adhesion, as well as physical insertion. Several strategies for enhancing the stability and absorption of oral bioactive peptides in the GIT have been outlined (Fig. 3). These include encapsulation methods to protect FBPs from hydrolysis and promote their penetration and absorption, permeation enhancers (PEs) that stabilize peptides by neutralizing pH, and device interventions such as bioadhesive patches and biodegradable microneedles, which facilitate direct transportation of FBPs to target sites, bypassing biochemical barriers, mucus, and the epithelial lining (Brayden, et al., 2020; Brown, et al., 2020; Drucker, 2020). For instance, semaglutide, a glucagon-like peptide-1 receptor agonist that is used in T2DM treatment (Mosenzon et al., 2020), is successfully delivered orally when co-formulated with the absorption enhancer sodium N-[8-(2-hydroxybenzoyl) aminocaprylate] (SNAC) (Buckley, et al., 2018). SNAC inhibits enzyme hydrolysis, neutralizes acidic pH, and enhances transcellular transport, thereby improving the oral bioavailability of BPs (Buckley, et al., 2018; Brayden, et al., 2020). Besides, Chiasma Pharmaceuticals, MA, USA, developed Transient Permeation Enhancement (TPE) technology, an oily suspension technique, used to increase the transcellular transport of octreotide, a cyclic octapeptide (Brayden, et al., 2020). Using TPE technology, the oral formulation of octreotide involves sodium caprylate, medium-chain fatty acid (as a permeation enhancer), and oil-based excipients, which generate a lipophilic suspension of hydrophilic particles in a hydrophobic environment (Tuvia et al., 2014). Other strategies used to facilitate oral absorption include microneedle-pill and enhancing paracellular transport (Vecchio and Stroud, 2019; Brayden, et al., 2020). For metabolically fragile peptides, the absorption risk could be overcome by exploring lymphatic transport as an alternative route. This

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route includes chylomicron and microfold cell pathways, which bypass hepatic first-pass metabolism (Zhang et al., 2021b).

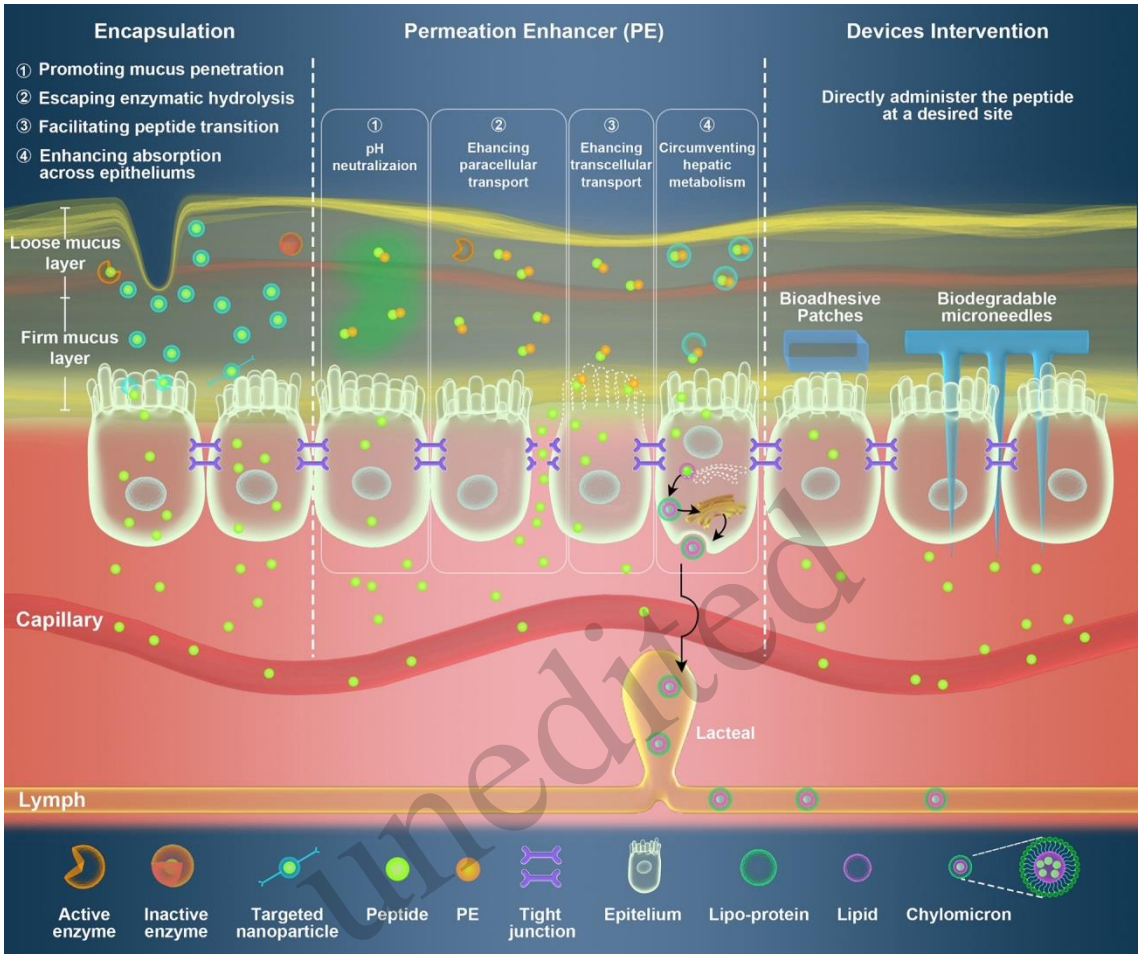


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**Fig. 3 Schematic representation of strategies to enhance the bioavailability of oral food-derived bioactive peptides (FBPs) in the gastrointestinal tract, including encapsulation, permeation enhancers, and device interventions.**

Encapsulation is a technology used to safeguard bioactive compounds from unfavorable conditions in the GIT environment. Currently, nanoencapsulation (ranging from 10 to 1000 nm) and microencapsulation (ranging from 1 to 1000 μm) are at the forefront of enhancing the stability and bioavailability of orally administered FBPs (Witika et al., 2020; Cian et al., 2022). Whereas VLPVP is vulnerable to degradation in the GIT, it remains active in simulated GI fluids due to the deployment of Shirasu Porous Glass membrane emulsification technology (Huang et al., 2017). However, poor absorption and low tolerance in the GIT still constrain the development of oral delivery systems for bioactive compounds (Nur and Vasiljevic, 2017). Core-shell nanoparticles with a thiolated hyaluronic acid coating for encapsulation of insulin have achieved high mucus-penetration ability and high oral bioavailability (11.3%) in type 1 diabetic rats (Tian et al., 2018). Electrospinning, an adaptable encapsulation method that uses micro- and nanosized fibers to protect bioactive compounds from deactivation and increase bioavailability prospects, has shown great potential in improving the bioavailability of FBPs (Wen et al., 2017).

### 5.3 Challenges and solutions for taste and odor

The taste and odor of FBPs are critical determinants of their palatability, particularly in forms like suspensions, powders, and granules. Most bioactive compounds, FBPs included, have a bitter or astringent taste

that negatively impacts patient adherence (Perry and Mcclements, 2020). Therefore, it is crucial to mask these unpleasant flavors to enhance patient acceptability. Taste masking is achieved through diverse methods, including the addition of sweeteners and encapsulation (Nasr et al., 2022). For example, Zhao *et al*. (Zhao et al., 2020) used oxidized starch hydrogel microencapsulation to mask the astringency of proanthocyanidins in beverages. Encapsulating gluten-derived peptides using water-in-oil high internal phase emulsions has been effective in improving gastrointestinal stability and reducing bitterness (Gao et al., 2022). While direct applications of taste masking for FBPs are infrequent, they offer a spectrum of possibilities for the development of FBP-based products.

## 6 Conclusions and perspectives

Food protein serves not only as a source of nutrients but also as a reservoir of FBPs with multifunctional activities, offering potential therapeutic benefits. Despite the growing body of evidence indicating their health-promoting bioactivities, few FBP-related products have been approved for clinical applications. The following research avenues could facilitate the application of FBPs as functional foods or potential medications:

(1) Therapeutic targets and mechanistic studies: The activities of FBPs have been extensively studied in multiple diseases, such as AD, hypertension, T2DM, liver diseases, and IBD. However, the therapeutic targets of most BPs are not clear, and their mechanisms of action remain to be elucidated. There are known targets in each disease, such as Aβ and tau for Alzheimer's disease, ACE and angiotensin receptor for hypertension, α-amylase and DPP-IV for T2DM, ECM and TIMPs for liver diseases, NF-κB and COX-2 for IBD, which can be used as a screening tool to identify novel BPs for metabolic diseases and in turn reveal the underlying mechanisms of action. Furthermore, the use of biotin-labeled BPs may open up a new path for specific receptor identification and is also conducive to elucidating pharmacological mechanisms, especially direct evidence for target binding by cryo-electron microscopy, x-ray crystallography, or nuclear magnetic resonance techniques.

(2) Pharmacological potential of FBPs: Given the pharmacological functions and SARs of FBPs, it is vital to develop them into potential active ingredients for disease prevention and treatment. Recently, studies of FBPs have focused on the relationship between their regular pattern of structure and activities, which provide a structural basis for the design of new FBPs. Modification of the peptide backbone, such as replacing L-amino acids with D-amino acids or substituting amino acid residues, will improve the bioavailability and potency of FBPs for potential pharmacological applications.

(3) Stability and pharmacokinetics of FBPs: *In silico* analysis has expanded the scope of methods available for screening bioactivity and exploring the SARs of FBPs. This approach assumes that the FBPs are stable *in vivo*. However, most FBPs have a short $t_{1/2}$, which requires confirmation of their stability and pharmacokinetics before proceeding with *in silico* analysis. Combining plasma stability assays with ultra-high-performance liquid chromatography−tandem mass spectrometry (UHPLC-MS/MS) could be instrumental in discovering new FBPs.

(4) Bioavailability improvement: FBPs usually exhibit low water solubility, poor stability in the GIT, and unacceptable taste, which can potentially limit their bioavailability. The drug delivery system can help to overcome these barriers. For example, the side chain of BPs carries a variety of active functional groups, such as carboxyl, hydroxyl, amino, and thiol groups, which can be modified to overcome the physiological barriers of oral delivery, such as water solubility, hydrolysis through the digestive tract, permeability through the gastrointestinal mucus and epithelial lining and first-pass metabolism. Moreover, the introduction of functional peptides (i.e., targeting, cell-penetrating, responsive, and self-assembling peptides) provides a new strategy to enhance the targeting, stability, taste, and bioactivity properties, which may provide a new insight for the precise treatment of diseases.

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## Author contributions

Hongda CHEN: conceptualization, writing original draft, and visualization. Jiabei SUN: writing original draft. Haolie FANG: conceptualization, and visualization. Yuanyuan LIN, Han WU, Dongqiang LIN, Zhijian YANG, Bingxiang ZHAO, Tianhua ZHOU, Jianping WU, and Shanshan LI: writing – review & editing. Quan ZHOU and Xiangrui LIU: funding acquisition and writing – review & editing. All authors have read and approved the final version..

## Compliance with ethics guidelines

Hongda CHEN, Jiabei SUN, Haolie FANG, Yuanyuan LIN, Han WU, Dongqiang LIN, Zhijian YANG, Quan ZHOU, Bingxiang ZHAO, Tianhua ZHOU, Jianping WU, Shanshan LI, and Xiangrui LIU declare that they have no conflict of interest.

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