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Biological activity and health benefits of food-derived bioactive peptides

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ABSTRACT: Bioactive peptides (BPs) generated by enzymatic hydrolysis possess diverse biological properties. In recent years, reports on novel BPs derived from both food and non-food protein sources, such as animals, plants, fermented food products, and microorganisms, have drastically increased. The biological activities of BPs include antioxidant, antihypertensive, antimicrobial, antidiabetic, immomodulatory, opioid, and anticancer properties, among others. Of these, BPs with antioxidative and antihypertensive properties have been studied the most, but their tests were commonly conducted *in vitro*. Since the mechanism of their biological functions and safety in real situations might be required to support health claims, future research on BPs with the aforementioned activities and/or alternative functions should employ *in vivo* models and human subjects. Furthermore, the cost of production and formulation for stability should be evaluated prior to commercialization.

KEYWORDS: antihypertension, antimicrobials, antioxidant, bioactive peptides, opioid activity

INTRODUCTION

Bioactive peptides (BPs) are food-derived, short protein fragments between 2 and 20 amino acid residues that exert physiological effects on the body in addition to providing nutritional values [1]. They are encrypted as inactive peptides inside long protein chains and can be released upon digestion of proteins from animals [2], plants [3] or even from microorganisms such as yeast [4] or lactic acid bacteria [5]. Some BPs are generated by food fermentation processes [6].

The diverse activities of BPs include antioxidant, antihypertensive, antimicrobial, anticancer, antidiabetic, opioid, immunomodulatory, and many other bioactivities [7, 8]. In addition, certain BPs impart more than one of these effects simultaneously — for example, BPs from ginger show both antioxidant and antihypertensive properties [9], while four peptides from egg yolk display antidiabetic, antioxidant and antihypertensive activities [10]. The ability of BPs to confer these benefits is consistent with the numerous reports that naturally occurring peptides possess health-promoting biological activities. For instance, a tripeptide-glutathione found in various tissues has antioxidant activity, whereas defensins, a 2–5 kDa peptide which is a cysteine rich cationic peptide has a strong antimicrobial activity [11].

Non-communicable diseases (NCDs) such as cancer, hypertension, diabetes and heart diseases are the major health problem worldwide. Many social behaviors such as consuming unhealthy diet and less physical activity contribute significantly to trigger NCDs [12]. A strategy that increases the availability of healthy diet is one of the factors that can help to prevent and control of NCDs. Nowadays, there are many food supplements and healthy drinks which contains a mixture of BPs available in the market. However, searching for novel BPs and development of functional foods and drinks are still increased due to their impact on health benefit. Over the past decade, a large number of BPs from both food-and non-food proteins have been isolated and identified.

Upon food protein intake, food proteins are digested by the combined action of several gastrointestinal enzymes such as pepsin, trypsin, chymotrypsin, pancreatin and many peptidases. The inactive BPs within the protein chains are released and these free BPs can then exhibit biological activity. During or after cellular uptake, a portion of BPs may remain intact while some may be further hydrolyzed by cellular peptidases. The functional BPs are then circulated to their target and exert biological activity (Fig. 1). In this review, bioactive peptides derived from different food sources will be discussed, with emphasis on their isolation, identification and functions, including antihypertensive, antioxidant, antimicrobial, anticancer, antidiabetic and opioid activities

ISOLATION AND IDENTIFICATION OF BPS

BPs are encrypted in protein chains as inactive sequences and can be activated only after being released by protease digestion. Therefore, as the first step in the characterization of BPs, food proteins must be extracted from the source of interest and hydrolyzed by proteolytic enzymes, which can be microbial enzymes [13, 14], mammalian digestive enzymes, plant enzymes [15], or combinations thereof. Alternatively, BPs can be released from precursor proteins via mi-

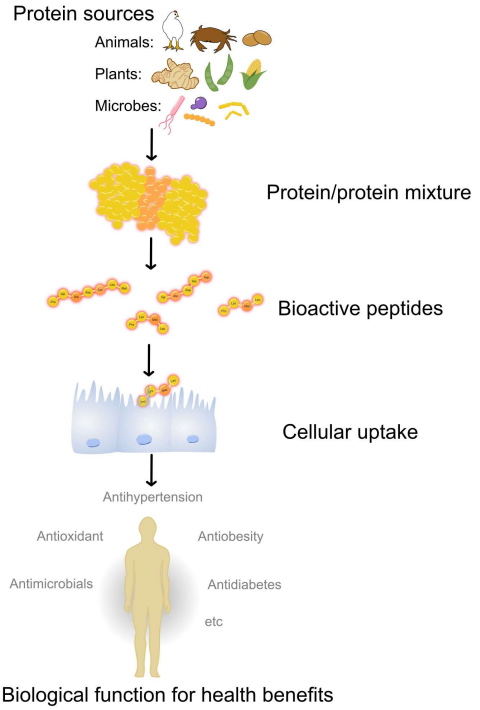

Fig. 1 Biological functions of food-derived bioactive peptides.

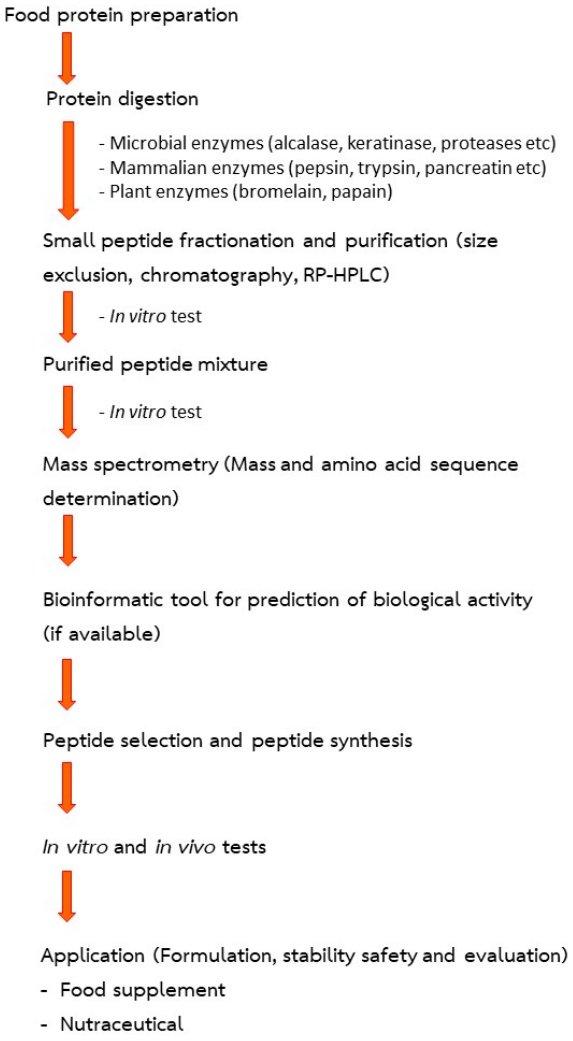

Fig. 2 Common protocol to isolate bioactive peptides from food proteins.

crobial fermentation. To this end, microbes (bacteria or yeast) are cultured on the protein substrates of interest, whereupon they release enzymes to breakdown proteins during growth. (The interested readers are referred to a recent review on BPs from fermented foods [16].) After confirming their efficient digestion by Tricine-SDS gel electrophoresis [17], the hydrolysates subjected to sequential fractionation, in which they are filtered through a series of ultrafiltration membranes with decreasing molecular weight cutoffs (MWCO). For example, peptides smaller than 1 kDa are collected using the MWCOs of 30, 10, 5, 3 and 1 kDa, respectively. This could be done sequentially using larger pore size membrane till a small molecular mass peptide fraction is obtained. The collected fraction is lyophilized and subjected to gel filtration (such as Sephadex G-25 column) and then to reverse phase-high performance liquid chromatography (RP-HPLC). The RP-HPLC peaks showing bioactivity are collected, and the peptide masses and sequences are analyzed by mass spectrometry — e.g., liquid chromatography-mass spectrometry (LC-MS/MS) or matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF-MS). However, as a very sharp RP-HPLC peak may still consist of several peptides with various masses and lengths, the peptides of interest should be individually prepared via chemical synthesis for their bioactivity tests.

To minimize the number of peptides that must be synthesized, bioinformatics tools for predicting biological activity of peptides can be leveraged. For instance, *in silico* analysis of the angiotensin I-converting enzyme (ACE) inhibitory peptides can be achieved by analyzing their ability to bind to the active site of human ACE, which can then be used to inform the selection of BP candidates with antihypertensive properties [18]. On the other hand, antioxidant peptides are selected based mainly on their amino acid composition and sequences: peptides with a large number of hydrophobic amino acid residues have been found to show high antioxidant activity [19]. Similarly, opioid peptides can be selected *in silico* based on the presence of Tyr and Pro amino acid within their sequences. This strategy was successfully employed to identify three

opioid peptides in wheat storage proteins [20]. The summary of entire workflow to generate BPs from their sources is shown in Fig. 2.

After peptides are selected and synthesized, their bioactivites are tested using *in vitro* and *in vivo* assays. However, as the process described thus far usually produces low peptide yields and are therefore not cost-effective, economically viable processes for commercial production of functional BPs remain to be developed. Alternatively, crude hydrolysates with confirmed bioactivity can be commercialized in lieu of purified peptides. Importantly, prior to commercialization, the formulation, stability, safety, and health benefits of BP-based products should be thoroughly evaluated using *in vivo* tests and human subjects.

BIOLOGICAL ACTIVITIES OF BPS

BPs possess diverse bioactivities with possible application as functional foods, nutraceuticals and many others. Only BPs with antihypertensive, antioxidant, opioid, antimicrobial, anticancer and antidiabetic activities will be focused. The assay methods for each activity are also discussed. Most BPs have a single bioactivity but some BPs may exert multifunctional bioactivities.

BPs with antihypertensive activity

Hypertension is prevalent in the global population. One of the proposed strategies for alleviating hypertension is to inhibit the ACE, which is a peptidyl dipeptide hydrolase (EC 3.4.15.1) associated with the renin-angiotensin system and playing a key role in regulating peripheral blood pressure. The enzyme converts angiotensin I to angiotensin II, which mediates vasoconstriction and consequently elevates blood pressure. Moreover, ACE inactivates bradykinin, a vasodilator peptide [21]. Therefore, lower ACE activity should result in reduced blood pressure and decreased hypertension.

Numerous BPs with antihypertensive activity have been identified in various food protein sources. The bioactivity of BPs as ACE inhibitors is mostly determined using ACE from rabbit lung with hippuryl-L-histidyl-L-leucine (HHL) as the substrate. When active, ACE cleaves HHL into hippuric acid (HA), which can be extracted by ethyl acetate, dried and redissolved in water, and quantified by measuring the absorbance at 228 nm [22]. The change in absorbance can therefore indicate the degree of ACE inhibition. Alternatively, the ACE kit WST can be employed. The assay detects 3-hydroxybutyric acid [3HB], which is produced when ACE cleaves 3-hydroxybutyryl-Gly-Gly-Gly (3HB-GGG). The color generated by the coupling reaction is measured at 450 nm [18]. In our hand, the latter method is more convenient and quite reproducible, albeit rather expensive.

BPs with antioxidant activity

In biological systems, free radicals and other reactive oxygen species are commonly generated during metabolic reactions. When these free radicals are not properly eliminated, they may cause damage to DNA, proteins, and other cellular molecules. This oxidative stress plays an important role in promoting and initiating chronic diseases such as aging and cancer [23]. Many BPs derived from plant, animal proteins and their by-products have been reported to exhibit antioxidant activity. The relationship between peptide structure and antioxidant activity depends on their molecular masses, amino acid sequences, and composition, in addition to hydrophobicity. Among these factors, composition and sequence of amino acid have the greatest impact on the antioxidant activity. Peptides with a large number of hydrophobic amino acid residues have been found to show high antioxidant activity, as hydrophobic interactions between antioxidant peptides and lipid bilayer proteins of cells may facilitate radical scavenging. Hydrophobic amino acids with strong radical-scavenging properties include Gly, His, Ile, Lys, Phe, Pro, Trp and Val [23, 24]. In addition to hydrophobicity of each amino acid, the sequence of each peptide also contributes to its antioxidant property [24].

The radical scavenging activity of BPs can be assessed using several types of methods. In chemical assays, BPs are assessed for their ability to scavenge synthetic free radicals, such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) and 2,2-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) [18]. In addition, the ferric reducing ability of plasma (FRAP) assay can also be used to measure antioxidant activity [25]. In biological assays, the ability of BPs to protect bacteria lacking scavenging enzymes, such as catalase (Cat) and/or superoxide dismutase (Sod), from oxidative stress is evaluated. For example, in the presence of oxidative stress from menadione treatment, a triple *sod* mutant of *Agrobacterium tumefaciens* (*sodBI sodBII sodBIII*) was used to evaluate antioxidant peptides from ginger, turmeric and white turmeric [9].

BPs with opioid activity

Opioid refers to all substances that, similar to morphine, relieve pain by binding to opioid receptors on nerve cells, thereby blocking the transmission of pain signals from the body to the brain via the spinal cord [26, 27]. Opioid receptors (OR) are classified into 5 types; Mu (MOR), Kappa (KOR), Delta (DOR), Nociceptin (NOR) and Zeta (ZOR) [28]. All of these receptors are capable of mediating analgesia. Morphine is an important opioid alkaloid substance that binds to the Mu opioid receptor and is used efficiently

to relieve pain, but it also generates many undesirable side effects such as headache, nervousness, dry mouth, drowsiness, and euphoria [29]. It is known that the endogenous ligands for opioid receptors are peptides which share the common N-terminal sequence of Tyr-Gly-Gly-Phe-(Met or Leu). This opioid motif is followed by a C-terminal end of various lengths, yielding peptides between 5 and 31 residues-long [30]. A Tyr residue at the N-terminus and an aromatic amino acid at the third or fourth position provide an essential structural motif for interaction with opioid receptors. Functionally, peptides that bind to opioid motif, can act as agonists, which trigger pain-relieving signals and cause analgesia, or antagonists which produce no functional response. In addition, it can block agonists from binding to the same receptor and consequently display antiopioid activity. Hence, opioid peptides can be opioid receptor ligand with either agonistic or antagonistic activities. Opioid peptides can be classified into two types; endogenous opioid peptides produced by the body, and exogenous peptides derived from food proteins [31]. Enkephalins and endorphins are examples of endogenous opioid peptides. Recently, it has been reported that Interleukin-4 (IL-4) induces macrophages to release an endogenous opioid peptide (Met-enkephalin), which then activates peripheral opioid receptors in male mice with an injured sciatic nerve [33]. The most studied exogenous opioid peptides are derived from casein proteins from human, cow, buffalo, goat and camel milk [31, 32]. β-casomorphin, for example, is a group of opioid peptides derived from the proteolytic cleavage of β-casein in animal and human milk [33]. Soy proteins and gluten from wheat also generate exogenous opioid peptides upon protease hydrolysis [20, 34]. Most food-derived opioid peptides have a conserved Tyr at their N-termini, although some opioid peptides from cow milk and wheat high molecular-weight glutenin do not [31].

The opioid activity can be determined using the radioreceptor assay [35], in which potential peptides are tested for their ability to electrically evoke the contraction of the myenteric plexus of the guinea pig's ileum longitudinal muscle. However, this assay is rather complicated and uses animals. Recently, an alternative cAMP assay for opioid activity has been described [20]. In this assay, Chinese hamster ovary (CHO) cells are incubated with different concentrations of tested peptides, and the change in cAMP concentration in each cell lysate is then measured using the Lance cAMP detection kit. In addition to biological assays, it is also possible to predict opioid peptide sequences *in silico* using the BIOPEP database, and rank their activity via the Peptide Ranker. Using this strategy, Garg and colleagues were able to identify three potential opioid peptides from wheat storage proteins [20].

BPs with antimicrobials

Antimicrobial peptides (AMPs) are a diverse group of molecules found in all living organisms. They usually contain 10–55 amino acids and weigh less than 10 kDa [36]. The amphipathic (i.e., having both hydrophilic and lipophilic portions) and cationic nature of AMPs allows them to interact with and integrate into membrane bilayer, and consequently kill several types of microorganisms (bacteria, fungi, virus and protozoa) [36]. Similar to other types of BPs, AMPs have also been detected in food protein hydrolysates, where they are encrypted within the native protein precursors and unleash the antimicrobial activity after enzymatic hydrolysis [37]. Food-derived AMPs present a great advantage in terms of safety as they originate from safe protein sources. However, high doses may be required to kill the target microbes. For example, up to 8 mg/ml of pea protein hydrolysate was required to completely inhibit *Escherichia coli* O157:H7 [38]. Antimicrobial activity of BPs can be evaluated using any standard microbiological methods that report minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), or IC_{50} (i.e., the inhibitory concentration that inhibits the growth of half of the inoculated bacterium) [39].

BPs with anticancer activity

Cancer is a deadly disease characterized by abnormal cell growth and uncontrollable cell division, resulting in cancer cells that can infiltrate and destroy normal tissues throughout the body. Anticancer peptides (ACPs) have increasingly garnered attention as cancer therapeutics owing to their high specificity, low toxicity and minimal side effects. Eleven ACPs have been approved by the US Food and Drug Administration (USFDA) and European Medicine Agency (EMA) for treatment of multiple myeloma, prostate cancer, T-cell lymphoma, hepatocellular carcinoma, hormone responsive cancers and osteosarcoma [40]. Similar to AMPs, ACPs possess cationic, hydrophobic and amphipathic properties, which enhance their affinity to cell membrane and enable them to kill the target cancer cells through membrane rupture [37]. In addition, ACPs can be designed to target a well-known immune checkpoint PD1/PD-L1, in which cells containing PD1 protein can use it to bind PD-L1 on T cells, thereby evading the T cell-mediated killing. As PD-L1 is present in excess in some types of cancer cells, targeting the PD1/PD-L1 checkpoint with anticancer drugs, ACP peptides or monoclonal antibodies presents a potential cancer treatment strategy. For instance, PD-L1 peptide mimic, PL120131, interferes with the PD1/PD-L1 interaction by competitively binding to PD1 [41]. Another engineered ACP, FITC-YT-16, targets PD1, efficiently blocking the PD-1 signaling pathway and increasing the antitumor activity of T-cells [42]. To identify food-derived ACPs that potentially target this checkpoint, *in*

silico analysis and an inhibitory activity assay against cancer cell proliferation can be employed in tandem [41, 42].

BPs with anti-Type II Diabetes Mellitus (TIIDM) activity

Diabetes is a metabolic disease caused by insulin deficiency, insulin resistance, or both, resulting in elevated blood glucose levels. The disease is classified into two types. Type I Diabetes Mellitus (TIDM) is insulin dependent and caused by the lack of or inadequate insulin production by the pancreas. In contrast, Type II Diabetes Mellitus (TIIDM) is insulin independent and caused by an imbalance between insulin secretion and blood sugar absorption [43]. Many enzymes, including α-glucosidase, α-amylase and dipeptidyl peptidase IV (DPP-IV), are implicated in hyperglycemia, and are therefore recognized as therapeutic targets for TIIDM, which accounts for approximately 90 percent of DM patients. Thus far, BPs with anti-TIIDM activity have been identified in egg [43], salmon milt [44], soy protein [45], pumpkin [46] and pork loin [47]. The anti-TIIDM activity of BPs can be evaluated by determining their IC_{50} values, which is the concentration of BPs required to inhibit 50 percent of the activity of the aforementioned enzymes [43–47].

ABSORPTION OF BPS INTO THE BLOOD CIRCULATION SYSTEM

For BPs to exert their intended biological effects and impart health benefits, they must be able to pass through the membranes of internal organs and enter the blood circulation system. Many studies have shown that di- and tri-peptides are significantly absorbed in the small intestinal epithelial cells through a proton-coupled symporter called PepT1, while oligopeptides (i.e., larger than tetrapeptides) are transported less efficiently [48]. However, a study in male Wistar rats fed orally with collagen hydrolysates demonstrated that several large peptides (up to 15 amino acid residues) could in fact be absorbed and enter blood circulation. Additionally, large peptides were detected in portal vein blood and liver, suggesting that peptides larger than di-/tripeptides could pass through the liver and reach the blood circulation system [49]. The intestinal absorption of larger peptides in this study was assumed to be passive, though the exact molecular mechanism of peptide uptake should be further investigated. Nonetheless, these findings support the feasibility of using BPs larger than di- and tripeptides as functional foods.

SOURCES OF BPS AND THEIR FUNCTIONS

Numerous BPs have been isolated from animals (i.e., casein in milk and muscle protein in meat) and plants, (i.e., soybean and cereals). Microorganisms and their food fermentation products also serve as rich sources of BPs. As BPs are small peptides, it is possible to obtain BPs with the same sequence and biological activity from different sources. Examples of BPs from various protein sources and their activities as well as protease enzymes used in protein digestion are shown in Table 1. Several types of enzymes have been used to hydrolyze proteins such as digestive tract enzymes (pepsin, trypsin, chymotrypsin, pancreatin), microbial enzymes (alcalase from *B. licheniformis*, umamizyme from *Aspergillus oryzae*), plant enzyme (papain, bromelain), and protease enzymes from *B. subtilis* or lactic acid bacteria used in food fermentation. Only one or two types of protease is generally used in most cases of protein digestion. Many BPs with either antihypertensive and/or antioxidant effects are reported from goat casein [50], beef loin [51], porcine blood [52], hen egg white lysozyme [53] and mung bean proteins [54]. Insect larva of Asian weaver ant [18] and black soldier fly [55], which are edible and rich in proteins, also release various BPs with either of the aforementioned activities (Table 1). BPs with both anti-TIIDM and anti-obesity activities, and BPs with only anti-TIIDM activity are found in camel milk protein digested with alcalase [56]. In addition, the digestion of camel milk protein with bromelain generates a BP capable of inhibiting pancreatic lipase, which could potentially be used as an anti-obesity agent [56].

Several large peptides confer antimicrobial properties. For example, a 16-amino acid peptide from turbot viscera hydrolysate was found to be effective against both Gram positive (*Staphylococcus aureus*, *Listeria monocytogenes*) and Gram negative (*E. coli*, *Salmonella* Typhimurium) bacteria [57]. Another antimicrobial peptide from brown kidney bean contains 25 amino acid residues and has antifungal activity [58]. However, a small antimicrobial BP (pentapeptide) has also been isolated [59]. For anticancer peptides, several small BPs (6–8 amino acids long) obtained from hydrolyzed quinoa seed proteins can inhibit the proliferation of Caco-2 cells [60]. Apart from the sources already described, a large number of novel BPs have also been isolated from microalgae [61], food fermentation using either lactic acid bacteria [62] or *B. subtilis* [63], and low-value byproducts such as wine lees [64] and defatted rice bean [65]. Hence, by varying the types of food proteins and proteases used for hydrolysis, it is possible to obtain BPs with diverse biological functions, as shown in Table 1.

CONCLUSION AND FUTURE PERSPECTIVES

BPs are gaining popularity and are increasingly used as functional foods due to their plethora of biological activities and potential health benefits. This review provides the examples of BPs derived from common food proteins as well as new protein sources such as insects, edible rhizomes, wine lees and animal coagu-

Table 1 Protein sources and bioactivities of bioactive peptides.

Source	Protease	Peptide sequence[a]	Function[b]	Ref.
Animals				
goat casein	pepsin/pancreatin	SWMHQPP QSLVYPFTGPIPNSL YPYQGPIVL	1, 2 2 2	[50]
beef loin	thermolysin	LSW, FGY, YRQ	1	[51]
larvae and pupae of Asian weaver ant (*Oecophylla smaragdina*)	pepsin/trypsin	FFGT, LSRVP CTKKHKPNC	1 2	[18]
larvae of black soldier fly (*Hermetia illucens*)	alkaline protease	GYGFGGGAGCLSMDTGAHLNR VVPSANRAMVGIVAGGGRIDKPILK AGLQFPVGR, GFKDQIQDVFK GFKDQIQDVFK	2 2 2 2	[55]
porcine blood (red blood cells) porcine plasma	pepsin/trypsin	TPYPCV, VVYPWR, FLTC YTFPFH, WGHGNPHV, VPLW	1 1	[52]
bovine blood (α-haemoglobin)	pepsin	TSLYR	5	[59]
camel milk	alcalase alcalase bromelain	KDLWDDFKGL DNLMPQFM, WNWGWLLWQL MPSKPPLL	3, 4 3 4	[56]
hen egg white lysozyme	pepsin/trypsin/chymo trypsin	KVF, MKR, AMK, AKF, GIL RGY, WIR, VAW	1 1, 2	[53]
pork loin	pepsin/pancreatin	APPPPAEV, APPPPAEVH, KLPPLPL, RPLLP, VATPPPPPK VPTPVPLGM, VPLPVPVPI	3 3 3	[47]
Plants				
ginger rhizome (*Zingiber officinale*)	pepsin/trypsin	VTYM, RGPFH, AEPPR GSGLVP, KMSPV	1, 2	[9]
turmeric rhizome (*Curcuma longa*)	pepsin/trypsin	WTLTPLTPA CACGGV, DVDP, CGVGAA	2 1	[9]
white turmeric rhizome (*Curcuma manga*)	pepsin/trypsin	HVVV RSC	2 1	[9]
mungbean protein	neutral protease	WGN, AW, RGWYE, GVPFW	2	[54]
soy bean protein	alkaline protease	LLPLPVLK, SWIRL, WLRL	3	[45]
quinoa seeds	pepsin/pancreatin	FHPFPR, NWFPLPR, PNFHPFPR, HYNPYFPG	6	[60]
brown kidney bean (*Phasiolus vulgaris*)	– (water extraction)	KTCENLADTYKGPCFTTGSCDDHCK	5	[58]
wheat storage protein	– (*in silico* search)	YPG, YYPG, YIPP	7	[20]
Microorganism				
single-cell microalgae (*Chlorella sorokiniana*)	protease N	WV, VW, IW, LW	1	[61]
Food fermentation				
pork muscle	enzymes from *Lactobacillus plantarum* and *Staphylococcus simulans*	KPVSPL, KPVSPLL, KPVSPLLL, THLDT, VKVG, VLLFH	2	[62]
rice beans	enzymes from *Bacillus subtilis*	PFPIPFPIPIPLP, IPFPPIPELPPI	1	[63]
Byproducts				
wine lees hydrolysate	Flavourzyme	FKTTDQQTRTTVA NPKLVTIV, TVTNPARIA LDSPSEGRAPG, LDSPSEGRAPGAD	1 1 1	[64]
defatted rice bran	Umamizyme G	IP, MP, VP, RP, TP, LP, KP, HP, YP, FP, WP, PP, SP, AP	1	[65]
turbot viscera hydrolysate	pepsin/trypsin	GITDLRGMLKRLKKMK	5	[57]

[a] A = alanine; R = arginine; N = asparagine; D = aspartic acid; C = cysteine; E = glutamic acid; Q = glutamine; G = glycine; H = histidine; I = isoleucine; L = leucine; K = lysine; M = methionine; F = phenylalanine; P = proline; S= serine; T = threonine; W = tryptophan; Y = tyrosine; V = valine.

[b] 1 = antihypertension; 2 = antioxidant; 3 = anti-diabetes (TIIDM); 4 = anti-obesity; 5 = antimicrobials; 6 = anti-cancer; 7 = opioid activity.

lated bloods.

Searching for novel BPs can be a tedious, costly, and time-consuming task. To meet the soaring demand for BPs, high throughput strategies for identifying new sources of food proteins and isolating functional BPs from them are necessary. Systematic approaches, including bioinformatics, may also be used to predict new BPs based on the structure-activity relationship of peptides and their interaction with the target receptors, thereby reducing trials and errors. It is worth noting, however, that bioinformatics tools are not foolproof, and it is common that only few or none of the predicted peptides will truly have bioactivities. Therefore, the results obtained *in silico* should be confirmed experimentally by *in vitro* and *in vivo* assays. For industrial applications, a cheaper and versatile protocol for large scale production should be developed. Producing BPs with value added from low-value byproducts could be a promising cost reduction-strategy. Alternatively, food protein hydrolysates with bioactivities may be directly manufactured as food supplements, whereas pure BPs are reserved for nutraceuticals. Regardless of the formulation, the products must pass the regulatory requirements and have their bioavailability and safety validated. Finally, since potent bioactivity *in vitro* does not necessarily translate to high efficacy *in vivo*, studies using human subjects are essential for the approval of BPs as health foods and nutraceuticals.

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