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Bioactive Peptides from Agriculture and Food Industry Co-Products: Peptide Structure and Health Benefits

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Abstract

Co-products from food processing are typically disposed or turned into low value animal feed. Proteinaceous co-products can be converted to bioactive peptides exerting health benefits, which can lead to development of nutraceuticals and functional foods. This is an effective means for valorization of these co-products. The release of encrypted peptides exhibits various bioactivities, including anti-hypertension, antioxidant, immunomodulatory activities among others, *in vitro*, and some activities have been demonstrated *in vivo*. Structure modification of bioactive peptides occurring under gastrointestinal digestion and cellular transport remains the important factor determining the health benefits of bioactive peptides. Understanding peptide transformation in gastrointestinal tract and in blood circulation before reaching the target organs would shed some lights on its bioavailability and subsequently ability to exert physiological impact. In this chapter, the potential health promoting properties of peptides encrypted in various sources of co-products will be reviewed based on evidence on *in vitro*, *in vivo* and clinical trial studies. Structural changes of bioactive peptides under physiological condition will also be discussed in relation to its bioactivities.

Keywords: bioactive peptide, transepithelial transport, bioavailability, peptide sequence

1. Introduction

A large number of food co-products is annually generated from various plant- and animal-based food processing plants. The Food and Agriculture Organization (FAO) recently reported that 14% of global food production is lost prior to getting the retails [1]. There is also an estimation of generating co-products and waste of 38 percent in food processing industries [2]. These co-products are usually disposed, landfilled, incinerated and/or processed into animal feed and other low value products such as compost and fertilizer [3], which would cause economic losses and environmental concerns.

Bioactive peptide production is a promising approach to fully utilize proteinaceous co-products. These peptides could also exert physiological functions, leading to health benefits, such as antioxidant, antihypertension, antidiabetic,

immunomodulatory activities among others. But these peptides are encrypted in the protein chain and needed to be released to exert bioactivities. Fermentation, enzymatic and chemical (by acid or alkali) hydrolysis could be applied to produce bioactive peptides as well as using solvents which is normally used to extract natural peptides. Among them, enzymatic hydrolysis known to be more effective as it is a mild, ecofriendly, and controllable process. Bioactivities of the released peptides are affected by their size, hydrophobicity, charge, amino acid composition and their sequence which are different based on various enzyme and substrate as well as the hydrolysis conditions. After production of peptides with the certain bioactivity, their structural modification upon gastrointestinal digestion and epithelial transportation and absorption must be taken into consideration to determine their potential bioavailability. Several bioactivities and physiological functions of peptides derived from both animal and plant-based agricultural co-products have been reported, which are mainly focused on their antioxidant, antihypertensive, antidiabetic and antibacterial properties. In this chapter, their main biological activities along with the associated structure will be considered. Besides, the structure-bioavailability relation of the peptides will be demonstrated and procedures to keep them intact upon gastrointestinal digestion and transepithelial transportation will also be proposed.

2. Agricultural co-products as a source of bioactive peptides

Agricultural co-products involve a vast variety of materials with great potential as a substrate for bioactive peptide production due to the low value and high protein content. It is estimated that about 9000 tons in dairy, 3 and 9 million tons in seafood and livestock industries, respectively, are annually discarded [4]. Skins, bones and heads are rich in collagen. Collagen is known as a promising source of peptides with several bioactivities which could exert physiological functions. Collagen comprises of hydrophobic amino acids, particularly proline and hydroxyproline, offering a higher chance of being absorbed through epithelial membrane. These amino acids also make them stable against proteases in gastrointestinal digestion tract and brush border, so that the intact peptides could be absorbed and reach the target organ. Blood is also high-protein co-product obtained in a large volume in a slaughterhouse. It has been reported that about 2.5 billion tons of blood are annually generated only in Europe [5]. Hemoglobin in red corpuscles and albumin, globulins, and fibrinogen in plasma are the major proteins [6]. Whey is a predominant co-product of dairy industry with an annual production of approximately 180–190 million tons [7]. The whey-derived peptides have also lower allergenicity beyond their bioactivities, because β -lactoglobulin (β -lg) as a main whey protein contributing to allergenicity would degrade within hydrolysis process [8].

Production of bioactive peptide from some plant-based materials are restricted due to difficulties in protein recovery from their unique rigid structure of polysaccharides. To meet the challenge, pretreatment of these substrates would improve protein recovery and provide other benefits such as reduction of time and energy consumption, leading to a higher efficacy in bioactive peptide production [9]. Corn gluten meal was immersed in alkali solution, treated with α -amylase and cooked prior to proteolysis [10]. Ultrasound exposure of watermelon seed caused structural changes, leading to production of peptides with higher antioxidant activity than those without any treatment. Ultrasound could degrade interactions in matrix and unfold proteins, so that more hydrophobic residues and reactive sites are exposed, resulting in a higher efficacy in protein hydrolysis [9]. Oat bran polysaccharide was digested by cellulase and viscozyme to ease protein recovery prior to proteolysis and production of antioxidant peptides [11].

Bran from rice and wheat, is a main protein-rich co-product in cereal industry with production of about 50–60 and 90 million tons every year, respectively. Albumin, globulin, prolamin and glutelin are the major proteins in bran with a total protein content of 12–20% [12]. Seeds are also another source of bioactive peptides, among them those harvested for oil production generate a large mass of co-product. Soybean, rapeseed and canola meals with the crude protein contents of about 48, 36 and 38%, respectively, are important cases of protein-rich co-products obtained from oil industries [13]. Corn gluten meal is also a major proteinaceous co-product generated in a large quantity from corn wet milling process in which, zein and glutelin are the main proteins (68 and 28%, respectively). Zein composes of mainly hydrophobic amino acids by which, more hydrophobic peptides are likely produced [14]. Hydrophobicity is an important feature of peptides to exert some bioactivities such as antioxidant and antihypertensive properties.

Thus far, peptides with various bioactivities *in vitro* and *in vivo* levels have been recognized from these low value co-products. Conversion of these co-products to more value-added hydrolysates and/or peptides would ultimately lead to development of health promoting food products.

3. Biological activities

Several bioactivities and physiological functions of peptides derived from both animal- and plant- based agricultural co-products have been reported. These activities include antioxidant, antihypertensive, antidiabetic and antibacterial properties.

3.1 Antioxidant activity

Reactive oxygen species (ROS) are formed during normal cellular metabolism of providing energy, respiration and also when cells are exposed to exogenous oxidative stress [15, 16]. Normally, these products are neutralized by endogenous antioxidant defense systems, such as antioxidant enzymes (superoxide dismutase: SOD, glutathione peroxidase: GPx and catalase), glutathione (GSH) and others. But, the excessive level of ROS could result in many health disorders, such as cancer, cardiovascular, respiratory, neurodegenerative and other diseases [16, 17]. Therefore, antioxidant-containing diet could help to overcome ROS and subsequently their corresponding disorders. Nowadays, attempts have been made to find new antioxidant compounds from natural resources due to their benefits over synthetic compounds. To that direction, antioxidant peptides from agricultural co-products are gaining more attractions, because of their nontoxicity and safety besides their nutritional properties. Antioxidant hydrolysates/peptides production from agricultural co-products including skins, bones, viscera, whey as animal-based and brans, seeds, leaves, gluten as plant-based co-products have been extensively reviewed [17–21].

Lower molecular weight (MW) peptides with hydrophobic and aromatic amino acids (HAAs and AAAs, respectively) have been generally reported to exert good antioxidant activities [22]. The HAAs could improve peptides accessibility toward ROS through binding with lipid and reaching to free radicals, so that the peptides could quench them effectively. The AAAs including Trp, Phe and Tyr are also correlated with the strong antioxidant activity via their high electron transferring capacities of their aromatic rings. Besides HAAs and AAAs, some of hydrophilic amino acids, such as His could improve the activity through its imidazole ring which has been indicated as strong electron donator [23]. Presence of charged amino acids in peptide structure could also improve the activity. A higher negatively charged amino acids (NCAA) have been observed in plasma hydrolysates prepared from

chicken blood that showed higher antioxidant activity than those prepared from blood corpuscles with lower NCAs [6]. Presence of NCAs are reported to correlate with the strong antioxidant activity, because they can neutralize free radicals by giving their excess electrons.

Although antioxidant activity of agricultural co-product has been widely evaluated, few studies were conducted to assess the activity *in vivo* and there is still a gap for clinical trial. The chemical *in vitro* studies are not able to reflect the activity in biological systems due to their complicated physiological conditions. In addition, cellular evaluation might be able to provide a comparable environment to biological systems. Antioxidant properties of peptides from fish sauce increased with the increasing of peptides concentration based on chemical assays, while these peptides could act as pro-oxidants in higher concentration ($>50 \mu\text{g L-leucine equivalent/ml}$) in cellular experiments [24]. Hydrolysates prepared from corn gluten meal and ham seed meal as well as ACFL, a peptide from horse mackerel viscera, increased the level of antioxidant enzymes based on *in vivo* models upon exposure to oxidative stress [10, 25, 26]. In a human trial study, a reduction in plasma malondialdehyde and an increase in SOD level was observed after daily ingestion of 4.5 g black soy derived peptides for 8 weeks [27].

3.2 Antihypertensive activity

Hypertension is a major risk for many disorders including coronary heart disease, stroke, heart failure, vision loss, chronic kidney disease, and dementia [28, 29]. Renin-angiotensin system (RAS) is responsible in blood pressure regulation. The liver-released angiotensin is converted to angiotensin-I (Ang-I) by renin, then the Ang-I is easily degraded to Ang-II, a potent vasoconstrictor, by angiotensin converting enzyme (ACE), leading to vasoconstriction and hypertension. Thus, hypertension can be controlled using renin and ACE inhibitors, in which the latter is more important due to its dual functions, catalyzing Ang-II (a potent vasoconstrictor) and inactivation of bradykinin (a vasodilator) [30]. However, controlling blood pressure by peptides from agricultural co-products also reported to be achieved through other mechanisms, such as nitric oxide production and blocking calcium channel and Ang-II receptor. The receptor blockers are able to hinder vasoconstriction and other functions mediated by Ang-II. Calcium channel blocker can avoid calcium availability in blood vessel cell wall and heart which make them to have a lower extent contraction, leading to relaxation and lower blood pressure [27, 31]. Arg-containing peptides have also been reported to suppress hypertension as Arg is a precursor in nitric oxide production. Nitric oxide (NO) is synthesized by the reaction of Arg and oxygen in the presence of nitric oxide synthase as a catalyst. NO is a vasodilator which act against Ang-II and a balanced level of these two compounds could lead to normal blood pressure [32]. Peptides namely, LIWKL, RPYL, RRWQWR, blocking the Ang-II receptor and HRW, a calcium channel blocker, have been reported to exert antihypertensive effects [33, 34].

The antihypertensive activity of peptides depends on several factors including amino acid composition and their position, molecular weight and charge. Normally, peptides with lower molecular weight exert higher activity due to their higher affinity to bind with ACE, in which the most potent reported peptides are di- and tri-peptide. Moreover, small peptides can stay intact through gastrointestinal digestion and epithelial transportation, reaching to blood circulation system and the target organ [31]. The structure–activity relationship of 168 dipeptides and 140 tripeptides with ACE inhibitory activity was assessed [35]. The authors reported that presence of bulky side chain and hydrophobic amino acids in dipeptides could result in higher activity, while presence of aromatic amino acids at C-terminus

and positively charged amino acids in the middle and hydrophobic residues at N-terminus brought them higher ACE inhibitory activity. The interaction of peptides with zinc ion in active site of ACE could effectively deactivate the enzymes. It has been reported that Leu could bind with Zn^{2+} by its carboxyl group and inhibit the enzyme activity [36].

Antihypertensive activity of hydrolysates/peptides from agricultural co-products was extensively explored by *in vitro* and *in vivo* studied, mostly based on their inhibition capacity against ACE and rat with hypertension (either SHR or hypertensive-induced rat), respectively (**Table 1**). However, clinical studies are still needed to confirm their health benefits. Clinical studies on the antihypertensive effect of IPP and VPP showed a controversial result as it reduced systolic and diastolic blood pressure in Asian case studies to a higher extent than Caucasians, while no effect was observed in Dutch and Danish cases [46, 47]. They reported that the difference could be associated to variations in genetics and dietary habit which should be taken into consideration. Kwak et al. [27] reported antihypertensive effect of black soy peptide in human trial, in which systolic blood pressure decreased in hypertensive subjects likely through reduction in ACE activity and increase in nitric oxide production. The authors concluded that the activity of black soy peptides might also be associated with higher arginine content which is a substrate for nitric oxide formation, known as a strong vasodilator.

Source	Enzyme	Hydrolysate/ Peptide	Activity		Ref.
			<i>In vivo</i>	<i>In vitro</i>	
Animal based co-products					
Bovine whey lactoferrin	Pepsin	Partially purified hydrolysate (<3 kDa) LIWKI, RPYL and RRWQWR		ACE inhibitory activity, Blocking Ang-II receptor	[33]
Fish (Cobia) skin	Protamex	Hydrolysate WAA, AWW, IWW, WL	Systolic and diastolic blood pressure reduction at 600 mg/kg: −21.9 and − 15.5 mmHg, respectively, after 4 h in SHR	ACE inhibitory activity ACE inhibitory activity IC ₅₀ ² : 41, 4.3, 0.2 and 8.5, respectively	[37]
Chicken bone	Pepsin	YYRA	Systolic blood pressure reduction at 10 mg/kg in SHR: −2 mmHg after 6 h	ACE inhibitory activity IC ₅₀ : 33.9	[38]
Bovine bone gelatin	Alcalase	RGL-(Hyp)-GL and RGM-(Hyp)-GF	Systolic blood pressure reduction at 30 mg/kg: −31.3 and −38.6 mmHg for RGL and RGM after 4 and 6 h, respectively, in SHR	ACE inhibitory activity IC ₅₀ : 0.9 and 6.9, respectively	[39]
Bovine blood plasma	Flavourzyme	HPY		ACE inhibitory activity IC ₅₀ : 0.7	[40]

Source	Enzyme	Hydrolysate/ Peptide	Activity		Ref.
			<i>In vivo</i>	<i>In vitro</i>	
Poultry viscera	Autolysis	ARIYH, LRKGNLE and RVWCP		ACE inhibitory activity IC ₅₀ : 8.9, 8.9 and 4.9, respectively	[29]
Whey		IW	ACE activity reduction in human plasma by 32% at 50 mg administration		[41]
Plant based co-products					
Wheat bran	Alcalase	Hydrolysate, peptides <1 kDa including NL, QL, FL, HAL, AAVL, AKTVF, TPLTR	Systolic blood pressure reduction at 100 mg/kg in SHR ¹ : –20 and – 35 mmHg after 6 h for hydrolysates and the peptides <1 kDa, respectively	Renin and ACE inhibition activity	[42]
Corn gluten meal	Trypsin	AY	Systolic blood pressure reduction at 50 mg/kg in SHR: –9.5 mmHg after 2 h	ACE inhibitory activity IC ₅₀ : 3.6	[28]
Cottonseed	Papain	FPAIGMK		ACE inhibitory activity IC ₅₀ : 46.7	[43]
Flaxseed protein isolate	Thermoase	Partially purified hydrolysate (3–5 kDa)	Systolic blood pressure reduction at 200 mg/kg in SHR: –37 mmHg after 8 h	Renin and ACE inhibitory activity	[44]
Red seaweed (<i>Porphyra columbina</i>) by-product	Trypsin and Alcalase	Hydrolysate		ACE inhibitory activity	[45]

¹Spontaneously hypertensive rat.
²IC₅₀ based on µg/ml.

Table 1.
Peptides/hydrolysates derived from agricultural co-products involved in antihypertension activity.

3.3 Antidiabetic activity

The diabetes is a chronic health problem which involved 463 million adults in 2019 and it is estimated to reach 700 million by 2045. In the health disorder, the elevated blood glucose cannot be treated properly due to either pancreas failure in insulin production (type-I) or insulin resistance in the body (type-II), in which the latter comprised the majority of about 90–95% [48].

The diabetes type-I treatment is associated to insulin injection, while the diabetes type-II can be prevented by controlling the pathways, by which the blood glucose elevates. The enzymes, α -amylase and α -glucosidase, play roles in carbohydrate digestion through breaking them down to oligosaccharides and subsequently

to glucose, which is easily absorbed from intestine to blood, leading to hyperglycemia. In addition, dipeptidyl peptidase IV (DPP-IV), a protease located on endothelial, epithelial and some other cells, could easily degrade hormones stimulating insulin secretion during food ingestion, including glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic peptide (GIP), and cause dysregulation of blood glucose. Therefore, many studies have attempted to find bioactive peptides to inhibit these enzymes, so that the diabetes type-II would be cured or prevented. Bioactive peptide with *in vitro* enzyme inhibitory and *in vivo* antidiabetic activities derived from agricultural co-products are summarized in **Table 2**.

Source	Enzyme	Hydrolysate/ Peptide	Activity		Ref.
			<i>In vivo</i>	<i>In vitro</i>	
Animal based co-products					
Chicken feet	Neutrase	Hydrolysate	Reduction of glycemia in glucose-intolerant rats at 300 mg/kg	DPP-IV inhibitory activity	[49]
Collagen from pig and cattle skin, fish scale and chicken feet	Collagenase	GA-Hyp GPA GP-Hyp		DPP-IV inhibitory activity IC ₅₀ : >20, 5.03 and 2.51 mM, respectively	[50]
Mare whey	Papain	NLEILR TQMVDEEIM-EKFR		DPP-IV inhibitory activity IC ₅₀ : 86.3 and 69.84 μM, respectively	[51]
β-Lactoglobulin from bovine whey	Trypsin	VAGTWY	Reduction of glucose level in mice at 300 mg/kg	DPP-IV inhibitory activity IC ₅₀ : 210 μM	[52]
	Trypsin	IPAVF		DPP-IV inhibitory activity IC ₅₀ : 44.7 μM	[53]
Atlantic salmon skin	Flavourzyme	GPAAE, GPAA		DPP-IV inhibitory activity IC ₅₀ : 49.6 and 41.9 μM, respectively	[54]
Cuttlefish viscera	Crude protease extracts from smooth hound and cuttlefish hepatopancreas	Hydrolysate		Stimulation cholecystokinin (CCK) and GLP-1 release in enteroendocrine STC-1 cells, DPP-IV inhibitory activity	[55]
Bovine haemoglobin	<i>in vitro</i> GI digestion by pepsin and pancreatin	VAAA KAAVT, YGAE, ANVST and TKAVEH		DPP-IV inhibitory activity, IC ₅₀ : 141 μM Stimulation of GLP-1 secretion in STC-1	[56]

Source	Enzyme	Hydrolysate/ Peptide	Activity		Ref.
			<i>In vivo</i>	<i>In vitro</i>	
Plant based co-products					
Wheat gluten	Debitrase HYW20	Pro-containing peptides		DPP-IV inhibitory activity	[57]
	Protease from ginger	QPQ, QPG, QPF, LPQ, SPQ		DPP-IV inhibitory activity IC ₅₀ : 79.8, 70.9, 71.7, 56.7, and 78.9 μM, respectively	[58]
Oat globulin	Trypsin	LQAFEPLR		DPP-IV inhibitory activity IC ₅₀ : 103.5 μM	[59]
<i>Luffa cylindrica</i> seed	Pepsin Trypsin Alcalsae	Hydrolysate		α-amylase and α-glucosidase inhibitory activity	[60]
Rice bran	UmamizymeG Alcalase	IP 13 peptides containing 6–32 amino acids		DPP-IV inhibitory activity IC ₅₀ : 0.41 mM α-amylase and β-glucosidase inhibitory activity	[61] [62]
Hemp seed meal	Alcalase	LR, PLMLP		α-glucosidase inhibitory activity	[63]

Table 2.
Examples of agricultural by-product peptides and hydrolysates exhibiting antidiabetic activity.

In a clinical study, Goudarzi and Madadlou [64] indicated that hydrolysate prepared from whey proteins stimulated insulin production, so that plasma glucose got back to normal level in postprandial hyperglycaemia cases, while the hydrolysate had no effect in prehypertensive cases. Although studies indicated that hydrolysates/peptides might stimulate secretions of hormones involving in insulin production [55, 56, 64], most studies have focused on major enzymes involving in carbohydrate digestion and DPP-IV. The structure–activity relation of peptides possessing the diabetes-involving-enzyme inhibition has not been completely understood yet. Nongonierma et al. [57] identified di- and tri-peptides inhibiting DPP-IV in wheat gluten hydrolysate. These peptides had some main characteristics including the presence of Pro at carboxyl terminus or penultimate position and Phe or Leu at amino terminus. Li-Chan et al. [54] described that peptides with DPP-IV inhibitory activity required presence of hydrophobic amino acids, particularly Pro, as Pro placed at 1–4 (preferably at second) positions from N-terminal end and bounded with Leu, Val, Phe, Ala and Gly. Dipeptides as X-Pro with X as a small size hydrophobic amino acid would likely be an effective inhibitor. Presence of hydrophobic and aromatic amino acids at N-terminal end of peptides with DPP-IV inhibitory activity was also reported by Lima et al. [65]. Ren et al. [63] evaluated the α-glucosidase inhibitory capacity of peptides from hemp seed and indicated that hydrophobicity of peptides was a prime factor affecting inhibitory activity and molecular weight as a second priority. The authors have also reported that larger molecular weight peptides could also enhance α-glucosidase activity. α-Amylase is another enzyme involving in carbohydrate digestion and it has been reported that presence of branched and aromatic amino acids such as Lys, Phe, Tyr and Trp and positively charged amino acid could help to inhibit the enzyme [60].

3.4 Antibacterial activity

The antibacterial activity of hydrolysates/peptides has been studied to a lesser extent when compared to other aforementioned properties. Hydrolysates/peptides with antibacterial activity have been obtained from co-products of milk, seafood, meat and others which are summarized in **Table 3**. Conventional antibiotics and preservatives are extensively applied to control pathogens, which lead to antibiotic-resistant strains. Therefore, an alternative antimicrobial agent has been sought. Peptides with antibacterial properties could be one of alternative agents as they are non-toxic and could act against both Gram-negative and Gram-positive as well as antibiotic-resistant bacteria [76]. Typically, chemical antibiotics have specific targets and bacteria can develop various defense strategies towards antibiotics. In contrast, antimicrobial peptides target cell membrane and can cause serious damage which make it difficult to develop resistance [77].

The antibacterial activity of these peptides is associated to their molecular weight, charge and hydrophobicity [67]. Peptides are attached by negatively-charged residues of cell membrane, like lipopolysaccharides and lipoteichoic acid on Gram-negative and Gram-positive bacteria, respectively, through electrostatic

Source	Peptide/Hydrolysate	Test bacteria	Activity		Ref.
Animal based by-products					
Rainbow trout Viscera	Hydrolysate prepared by pepsin	Flavobacterium psychrophilum, Renibacterium salmoninarum	MIC ¹ (mg/ml)	2 5	[66]
Yellowfin tuna viscera	Partially purified (<3 kDa) hydrolysate prepared by Protamex	Listeria. monocytogenes, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa	MIC (mg/ml)	0.5 0.5 0.5 0.5	[67]
Bovine hemoglobin	TSKYR obtained by pepsin-hydrolyzed hemoglobin	Total viable colonies Coliform bacteria Yeasts Molds	Reduce microbial counts in ground beef stored in refrigerator		[68]
Porcine blood proteins: albumin globulin	Hydrolysate prepared by Alcalase, Flavourzyme, Protamex, trypsin and papain	Bacillus cereus	IZ ² (mm): 2.30–2.55		[69]
Camel whey	Crude hydrolysate prepared by trypsin/ partially purified (<3 kDa)	E. coli S. aureus Salmonella typhimurium Streptococcus mutans	MIC (mg/ml)	130/65 260/130 NI ³ /260 NI/260	[70]
Cow whey	Crude hydrolysate prepared by trypsin/ partially purified (<3 kDa)	E. coli S. aureus S. typhimurium S. mutans	MIC (mg/ml)	260/130 260/130 NI/260 NI/260	
Snow crab co-products	Hydrolysate prepared by Protamex	E. coli L. innocua	IZ (mm)	9 7.5	[71]

Source	Peptide/Hydrolysate	Test bacteria	Activity		Ref.
Plant-based co-products					
Palm kernel cake	Hydrolysate prepared by Alcalase	<i>Lisinibacillus sphaericus</i>	MIC (µg/ml)	150	[72]
		<i>Bacillus thuringiensis</i>		150	
		<i>B. cereus</i>		200	
		<i>Clostridium perfringens</i>		250	
		<i>B. subtilis</i>		450	
<i>Jatropha curcas</i> meal	CAILTHKR obtained by Protamex-hydrolyzed meal	<i>E. coli</i>	MIC (µg/ml)	29	[73]
		<i>Shigella dysenteriae</i>		46	
		<i>P. aeruginosa</i>		58	
		<i>S. aureus</i>		45	
		<i>B. subtilis</i>		34	
		<i>S. pneumoniae</i>		68	
Rice bran	KVDHFPL obtained by bromelain-hydrolyzed bran	<i>Listeria monocytogenes</i>	MIC (µg/ml)	0.25	[74]
		<i>L. monocytogenes</i> biofilm		8	
	LRRHASEGGHGPHW EKLLGKQDKGVIIRA SSFSKGVQRAAF obtained by pepsin-hydrolyzed bran	<i>Porphyromonas gingivalis</i> (PG), <i>Candida albicans</i> (CA)	IC ₅₀ : (PG, CA; µM)	289, ND ⁴ ND, 75.6 ND, 78.5	[75]

¹Minimum inhibitory concentration.

²Inhibition zone.

³No inhibition.

⁴Not detected.

Table 3.
Antibacterial peptide/hydrolysate prepared from agricultural co-products.

interactions, by which the structure of cell surface was disrupted. Subsequently, peptides could permeate to the cell and reach to cytoplasmic membrane, causing leakage of cytoplasmic fluid [78].

Antibacterial activity of hydrolysates and/or peptides has mostly evaluated by their directly exposure to pathogens and less studies have conducted to assess their application in food. A peptide, TSKYR, obtained from bovine hemoglobin [68] and hydrolysate prepared from yellowfin tuna waste [67] were added to ground beef and minced fish, respectively. The peptide, TSKYR, could reduce total viable colonies, yeasts, molds and particularly coliform bacteria within 14 days storage in a refrigerator. Moreover, the peptide (0.5% w/w) in ground beef was able to diminish lipid oxidation by 60% which was reported to be comparable to BHT. Pezeshk et al. [67] reported that hydrolysates prepared from yellowfin tuna were able to increase fish (silver carp) mince shelf-life in a refrigerator through inhibition of psychrophilic and total count bacteria as well as prevention of oxidative degradation.

4. Bioavailability

Studies revealed that peptides prepared from agricultural co-products have great potential of health promoting properties. However, bioavailability of these peptides is a challenge, in which they need to stay intact within gastrointestinal tract (GIT) and epithelial transportation to reach their target organs and exert physiological

functions. Proteases and peptidases in GIT, brush border and cytoplasm are able to break down the peptide bond to a higher extent, leading to changes in structure and subsequently the activity. However, some peptides have been reported to be stable within the digestion and transportation. There are several factors affecting the peptides stability, which are associated to proteases specificities in GIT. Lower molecular weight, negatively charged, hydrophilic and acidic amino acid containing peptides are reported to be more stable against GI digestion. Negatively charged peptides from milk are more stable against GI digestion followed by positively charged and neutral peptides [79, 80]. Hydrophobic peptides reported to have less stability, which might be due to pepsin specificity towards hydrophobic amino acids [81]. Peptides containing more acidic amino acids, and also those with lower molecular weight showed more stability against GI digestion [6, 79]. Peptides with the molecular weight of larger than 3 kDa were easily digested by GI proteases, while peptides with <1 kDa mostly survived and no change in their antioxidant activity upon GI digestion [82]. Savoie et al. [83] reported that peptides from animal- (casein and cod fish) and plant- (soy and gluten) based substrates with Pro and Glu showed higher stability. Pro has a rigid ring structure bonded to β -carbon which makes it resistant against proteolytic degradation [80]. Thus, Pro containing peptides, IAGRP and PTPVP, have been reported to stay intact after in vitro GI digestion [84].

Epithelial permeation of bioactive peptides into blood circulation system is another challenge that affects physiological activities. Peptides may undergo some structural modification induced by brush border proteases (**Table 4**). For instance, a peptide with ACE inhibitory activity, KPLL, can be degraded to KP and LL within epithelial permeation, resulting in lower activity than the intact form [96]. The permeation could occur through four pathways, including peptide transporter 1 (PepT1), passive paracellular transportation through tight junctions, transcytosis and simple passive transcellular diffusion. Peptide properties such as size, hydrophobicity, charge and amino acid sequence are important factors affecting their absorption. Briefly, small (di- and tri-) peptides can be transported via PepT1 route, however peptide properties have effects on its efficacy. Non charged and hydrophobic peptides have higher affinity towards PepT1. Hydrophilic and negatively charged low molecular weight peptides can pass through energy-independence paracellular route. Transcytosis is an energy-dependent route, by which long chain peptides, particularly hydrophobic, can be transported. A highly hydrophobic peptide is likely transported through simple passive transcellular diffusion. To evaluate the effect of molecular weight on the permeation, Wang and Li [97] reported that hydrolysates with the molecular weight lower than 500 Da (mostly di- and tri-peptides) showed higher bioavailability and were able to pass through Caco2 cell via PepT1 route, while those with the molecular weight ranging 500–1000 and 1300–1600 Da permeated through paracellular route. Besides molecular weight, peptide sequence also affects its bioavailability. A Pro-containing peptide has more stability towards brush border proteases and peptides with Leu at N-terminus have been reported to be highly susceptible to hydrolysis [86, 90, 91].

Peptides structural changes usually occur in GI tract and transepithelial transportation that would likely have effects on their physiological functions. To meet the challenge, some approaches have been applied to improve the stability of these peptides such as using permeation enhancer, enzyme inhibitor and encapsulation. Sodium caprate has been used to improve the permeability of two antihypertensive peptides, IPP and LKP, through paracellular route in Caco2 cell [98]. The authors reported that sodium caprate could intensify the peptides absorption via paracellular mechanism and inhibited PepT1 route, leading to antihypertensive effect in SHR model. An antihypertensive peptide, RLSFNP, would degrade to RLSF, SFNP, FNP and F during the epithelial transportation. Permeation enhancers including

Peptide	Bioactivity	Permeated peptides	Route of transport	Ref.
VLPVPQK	ACE inhibitor Antioxidant	VLPVPQK, VLPVPQ	PepT1/ SOPT2	[85]
LHLPLP	ACE inhibitor Antihypertensive	LHLPLP, HLPLP	Paracellular	[86]
IPP, LKP	ACE inhibitor	IPP, LKP	PepT1 and paracellular	[87]
IWHHT	ACE inhibitor Antihypertensive Antioxidant Anti-inflammatory	IWHHT, IWH, IW	Paracellular, PepT1 and paracellular, PepT1	[88]
YQEPVLGPVRGPFPIIV	Immunomodulatory	YQEPVLGPVRGPFPIIV, QEPVLGPVRGPFPIIV, YQEPVLGPVR-GPFPII	Transcytosis	[89]
LSW	ACE inhibitor Anti-inflammatory	LSW, SW	PepT1 and paracellular	[90]
RRWQWR	ACE inhibitory Antihypertensive	RWQ, WQ	Paracellular	[91]
WDHHAPQLR	Antioxidant	WDHHAPQLR, DHHAPQLR, WDHHAP, QLR	Paracellular PepT1	[92]
YWDHNNPQIR	Antioxidant	YWDHNNPQIR, WDHNNPQIR, DHHNNPQIR and YWDHNNPQ	Transcytosis	[93]
YFCLT, GLLLPH	Antioxidant	YFCLT, GLLLPH	Paracellular and transcytosis	[94]
WGAPSL	Cholesterol- Lower	WGAPSL, WGAPS, WGAP, GAPSL, GAP, SL	Paracellular	[95]

Table 4.
Peptide modification within epithelial permeation and their transportation route across Caco-2 cell.

sodium glycocholate hydrate, sodium deoxycholate and Na₂EDTA as well as enzyme inhibitors, bacitracin and leupeptin, have been applied to improve the intact peptide bioavailability [99]. Na₂EDTA was the most effective to enhance RLSFNP absorption through enlarging intracellular junctions. They also reported that bacitracin could exert permeation enhancer activity beyond its protease inhibitory effect. Permeation enhancer is believed to cause damages in cell membrane in case of long-term usage, leading to inflammation. However, major destructive effects were not observed by using bacitracin in rat intestine [100]. Besides, encapsulation of RLSFNP by liposome could also facilitate the intact peptide transportation through transcytosis in Caco2 cell [101]. In addition, Li et al. [102] used nano-encapsulation of antidiabetic peptides made by chitosan coated liposome to maintain the stability of peptides.

5. Conclusions

Co-products are inevitably generated in food production, distribution, processing and consumption. These protein rich and low value materials could provide a

great source of bioactive peptides production. Many peptides with various activities have been purified and identified, however, their activities need to be confirmed via *in vivo* studies and human trials, so that they could be further develop to functional food products. The main obstacle of developing these peptides in functional foods is their structural modification after ingestion via proteolytic degradation in gastro-intestinal tract and epithelial absorption, which would likely lead to a reduction in bioactivity. Although some peptides, particularly those containing Pro, could stay intact within digestion and absorption, structural modification usually happen in the route. Encapsulation of susceptible peptides or applying protease inhibitor as well as permeation enhancer in epithelial cells could facilitate the intact peptides absorption. Although these strategies might allow peptides to reach the target organ and exert certain physiological effect, their safety, particularly the use of protease inhibitors, needs further investigation regarding their side effects under physiological condition.

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Conflict of interest

The authors declare no conflict of interest.

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