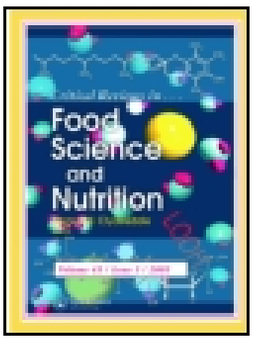

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# Functional peptides derived from rice bran proteins

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**ABSTRACT**

Rice bran has been predominantly used in the feed industry, and only recently it has attracted greater attention in terms of human nutrition with increasing knowledge of its bioactivity. A growing interest is the analysis of physiologically active peptides derived from rice bran proteins. In this paper, the bioactivities of rice bran proteins hydrolysates and peptides are reviewed based on recent studies. These enzymatic hydrolysates and peptides exert various biological activities including antioxidant, antidiabetic, anticancer and inhibitory activity for angiotensin converting enzyme (ACE), which may ultimately prevent certain chronic diseases. Nevertheless, these functionalities can be highly associated with their corresponding structural characteristics, in particular specific sequences and molecular weight distribution. This article may facilitate the expansion of the prospective applications of the bioactive peptides in a number of fields and provide some clues of the relationship between peptides structure and functionality for future research.

**KEYWORDS**

Rice bran; Peptide; Antioxidant; Antidiabetes; Anticancer; ACE-inhibitory activity

## Introduction

Rice bran is the major by-product of the rough rice milling process, most of which is utilized in the feed industry, in addition, a small amount is also used in other applications, such as nutritional supplements, constituents of microbiological media, and fuel in boilers (Luh, 1991; Inglett et al., 2004; Saman et al., 2011; Yadav et al., 2011). Rice bran has a high nutritional value with significant protein content (12-20%). The proteins in rice bran are of a complex nature mainly including albumin, globulin, glutelin, and prolamin with a high digestibility of up to 90% (Wang et al., 1999). Rice bran is considered a good source of hypoallergenic proteins and dietary fiber (Gnanasambandam and Hettiarachchy, 1995; Abdul-Hamid and Luan, 2000), and a previous study suggested that rice bran protein (RBP) can be a suitable ingredient in infant formula (Burks and Helm, 1994), which increases the diversity and improves dietary restriction among children with food allergies (Wang et al., 1999). The RBP has considerable potential to be an alternative protein resource due to its specific composition and functionality. However, RBP is under-utilized in the food production practice due to its poor food functionality caused by the extensive disulfide bond cross-linking (Hamada, 1995), contributing to low solubility and strong aggregation of RBP (Hamada, 1997). In addition, rice bran contains high level of phytate and fiber which are extensively bound to proteins, making the protein bodies more difficult to be separated from other components (Juliano, 1985; Adebiyi et al., 2009a).

 

Research has shown that the controlled hydrolysis of protein is an effective method for modifying functional properties and enhancing the extraction of RBP, and in a wide range of pH values and temperatures, the protein still demonstrates high solubility (David et al., 2009). Acid or alkaline chemical hydrolysis method can be applied to hydrolyze RBPs for producing potentially bioactive peptides. Though the use of acid or base is a simple and straightforward method, harsh conditions of strong acid or base hydrolysis conditions can modify and even destroy some essential amino acids, and create toxic chemical residues or undesirable side reactions, which ultimately decreases the quality and application value of the proteins (Humiski and Aluko, 2007).

Enzyme based hydrolysis shows increased specificity and the reaction process is milder, resulting in less destruction of amino acids and is also more environmentally benign. More importantly, enzymatic hydrolysis can improve the physicochemical, functional and immunological properties of native proteins with lower toxic side-reactions or products (Kristinsson and Rasco, 2000). For these reasons, enzymatic treatment with various proteases is the most commonly used method for hydrolyzing proteins to produce bioactive peptides. This article will review the potential physiological and functional activities of bioactive peptides derived from the hydrolysis of RBPs through enzymatic proteolysis. In this review, the use of different proteolysis enzymes (such as Papain, Flavorzyme, Protamex, and Trypsin), separation

and analytical methods for producing peptides from RBPs with various bioactivities are summarized.

**Peptides and functionalities from RBP hydrolysis**

The RBPs are highly nutritious plant proteins, which are particularly attractive as potential food ingredients because they are efficiently digested, rich in essential amino acids, colorless, hypoallergenic and hypocholesterolemic (Kahlon and Smith, 2004). Moreover, RBP was found to have the ability to regulate human physiological rhythm, enhance immunity and resist disease. The enzymatic hydrolysis of protein to prepare bioactive peptides has always been the focus of this area (Liu et al., 2014). Soluble oligopeptides can be obtained by enzymolysis technology to achieve specific physiological functions. For example, they can act as nutritional supplements and functional factors for improving lipid and glucose metabolism and even have regulatory effects on the nervous system (Yu et al., 2009). The content of albumin in rice bran is about 6-7 times higher than that in rice, and the hydrolysis of albumin produced eight peptides with enhanced immune function (Kunwadee et al., 2009), indicating that the development of peptides from rice bran is becoming more interesting than ever.

The functional properties of these active peptides are closely related to their degree of hydrolysis and the structure, which could be characterized with the number of ionizable groups ($-NH^{+}_{4}$, $-COO^{-}$) on peptide molecules. With an increased hydrolysis degree, the hydrophilicity

and net charge were correspondingly promoted. As the hydrolysis proceeds, the number of polypeptide chains decreases gradually, which enhances the absorbability, hypoallergenicity and hypoantigenicity of the protein hydrolysates (Kim et al., 2001). The most reported properties of peptides derived from RBP include antioxidant, antihypertensive, anti-diabetic, and immunomodulatory.

***Antioxidant activities of rice bran peptides***

Studies have found that some protein hydrolysates and peptides demonstrate high level of antioxidant capacity, and may act as potentially natural antioxidants (Aida and Xiong, 2003; Elias et al., 2008). The antioxidant peptides, ranging in size from 2 to 10 amino acid residues inhibit the oxidation of biological macromolecules by scavenging free radicals *in vivo* (Nam et al., 2006). Free radicals and singlet oxygen are associated with the occurrence of various diseases including cancer, cardiovascular disorders, diabetes, inflammatory disease and aging (Lin and Chang, 2004). Furthermore, oxidative processes caused by reactive oxygen species (ROS) play a major role in spoilage of food products, leading to a loss of quality and reduced shelf life (Adebiyi et al., 2009b). The use of synthetic antioxidants such as butylated hydroxyanisole (BHA) in food products is common, and it is strictly regulated due to their safety concerns (Eresha et al., 2005). The RBP hydrolysates are rich in hydrogen donors, which can scavenge free radicals and terminate free radical reaction chains, thus achieving anti-aging function (Fan et al., 2008). The antioxidant capacity of rice bran peptides has been evaluated by free radical scavenging activity,

such as superoxide anion and hydroxyl radical scavenging and due to different preparation and analytical methods, the antioxidant ability of protein hydrolysates is also significantly different (Zhang et al., 2005).

Various proteases (Papain, Flavorzyme, Neutrase, Protamex, and Trypsin) have been applied to hydrolyze the RBP to obtain antioxidant peptides (Mei et al., 2013). Consecutive chromatographic approaches including SP-SephadexC-25 cation-exchange chromatography, SephadexG-25 gel filtration chromatography and reversed phase high-performance liquid chromatography (RP-HPLC) have also been used to isolate antioxidant peptides. The antioxidant activity of the peptides was determined using the methods of DPPH radical-scavenging capacity, hydroxyl radical scavenging ability and reducing power activity. Results have shown that the peptides with a MW < 3kDa had the highest DPPH and hydroxyl radical scavenging abilities (6.56±0.28, 5.43±0.22 mg/mL, respectively, both expressed by IC50 value), and the greatest reducing power activity (1.02±0.18, absorbance at 700nm at sample concentration of 4 mg/mL). In addition, the purified peptide F2-5 (fractioned from MW < 3kDa peptides) possessed the strongest DPPH radical scavenging activity (58.2±1.63%). Purity test showed that the component of F2-5 consisted of only one peptide. Furthermore, the purified peptide also showed significant inhibition on the cell proliferation rate of SGC-7901 (a human gastric cancer cell line). The determination of amino acid composition showed that the purified peptides contained seven essential amino acids, Thr, Val, Met, Ile, Leu, Phe, and Lys, which were inferred to play an

important role in antioxidant activities. Wattanasiritham et al. (2016) hydrolyzed Khao Dawk Mali 105 RBP with Papain and Trypsin and evaluated the antioxidant activity of RBP fractions and hydrolysates using the Oxygen Radical Absorbance Capacity (ORAC) assay. The results indicated that denatured albumin hydrolyzed by trypsin exhibited the highest antioxidant activity with an ORAC value of 4.07 μmol of Trolox equivalent (TE) $mg^{-1}$ protein. The MW of peptides arising from fractions with high antioxidant activity was 800–2100 Da and the peptides were composed of 6-21 amino acid residues. More importantly, this is the first report that the antioxidant activities of denatured RBP hydrolysates are remarkably higher than native RBP hydrolysates (Wattanasiritham et al., 2016). Similarly, the hydrolyzed RBP using Alcalase, Flavourzyme and Neutrase, also showed that Alcalase generated the most effective hydrolysates with the highest antioxidant activity while Neutrase provided hydrolysates with the lowest activity, which suggested in addition to time, enzyme type had a significant effect on the antioxidant efficacy of protein hydrolysates. Moreover, this research also indicated that the hydrolysis time had a lower influence on antioxidant activity (Thamnarathip et al., 2016).

Specifically, rice bran albumin has been hydrolyzed using commercial proteases (Alcalase, Neutrase, Flavourzyme, and Protamax) and the antioxidant activities of hydrolysates were determined by DPPH and ABTS radical scavenging assays. These results also indicated that Alcalase hydrolysate had the highest DPPH and ABTS radical scavenging activities, which

suggested that rice bran hydrolysates have the potential to be used as dietary or nutraceutical agents beneficial for human health (Uraipong and Zhao, 2016).

Recently, a novel antioxidant peptide F2-a was isolated from the hydrolysis of RBP using trypsin, followed by purification using a membrane bioreactor, gel filtration and RP-HPLC. This peptide exhibited strong DPPH free radicals scavenging activity (IC50 = 0.15 mg/mL) and reducing power (0.125±0.013, absorbance at 700nm at sample concentration of 0.05 mg/mL). The amino acid sequence, Tyr-Ser-Lys (MW: 395.0 Da), was determined by Q-TOF ESI mass spectroscopy. This research also provided the evidence that rice bran is a potential source of bioactive peptides with strong antioxidant capacity (Wang et al., 2017).

***ACE-inhibitory activity***

Recently, a large number of studies have found that many peptides derived from plant and animal proteins exert antihypertensive properties. Hypertension is a worldwide chronic disease with a high risk factor for stroke, arteriosclerosis, myocardial infarction and end-stage renal disease (Jung et al., 2006). The ability to inhibit the angiotensin I converting enzyme (ACE) reflects the potential anti-hypertensive activities of peptides. ACE is a multifunctional Zn-metallopeptidase and plays an important physiological role in regulating blood pressure acting mainly on the rennin-angiotensin system (Fujita et al., 2000). The ACE catalyzes the conversion of angiotensin from an inactive decapeptide (angiotensin I) form to a potent vasoconstrictor octapeptide (angiotensin II) and also inactivates antihypertensive vasodilator bradykinin (Wang

et al., 2008). Blocking the production of angiotensin II with ACE inhibitors can relax constricted blood vessels, lower blood pressure, and reduce energy consumed by the heart during pulsation (as shown in Figure 1) (Kostis and DeFelice, 1987). The potent synthesized ACE inhibitors such as captopril, enalapril, alacepril and lisinopril, are commercialized for the regulation of blood pressure. However, these synthetic drugs have side effects such as coughing, taste disturbances, and skin rashes. Therefore, food derived ACE inhibitor based peptides would be more appealing in terms of safety and minimal side effects (García et al., 2013). The study from Wang et al. (2017) also demonstrated that hydrolyzed RBP using Trypsin obtained a hydrolysate with MW < 4 kDa which exhibited the strongest ACE inhibitory activity with a relative low IC50 value of 300 mg/mL. In addition, antihypertensive effects in spontaneously hypertensive rats (SHRs) showed that oral administration of hydrolysates of < 4 kDa could decrease systolic and diastolic blood pressure significantly. A molecular docking study further revealed that the ACE inhibitory effect was associated with a specifically isolated peptide Tyr-Ser-Lys (MW: 395.0 Da), which was shown to be related to the formation of very strong hydrogen bonds within the active pockets of human ACE. These results indicate that rice bran and its hydrolysates may have potential application in the prevention of hypertension. RBPs have been fractioned into albumin, globulin, prolamin and glutelin followed by using the four proteases: Alcalase, Neutrase, Flavourzyme and Protamax. RBP hydrolysates showed significant ACE-inhibitory activities. In particular, Protamax-produced albumin hydrolysate showed the highest ACE-inhibitory activity with an $IC_{50}$

value of 5.2 mg protein/mL. The ACE-inhibitory activities of Protamax- and Alcalase-produced glutelin hydrolysates and Alcalase-catalysed albumin hydrolysates were also relatively high with IC50 values of 6.2, 8.4 and 9.2 mg protein/mL, respectively. Among the four proteins fractions, the hydrolysates of albumin and glutelin exhibited higher inhibitory activities than that of globulin and prolamin whereas the latter exhibited the lowest activities (Uraipong and Zhao, 2015). As mentioned above, Uraipong and Zhao (2016) hydrolysed rice bran albumin using the proteases (Alcalase, Neutrase, Flavourzyme, and Protamax) under their respectively optimal conditions and found that Protamax hydrolysates demonstrated the highest ACE-inhibition activity. The bioactivities of Neutrase hydrolysate followed those of Alcalase and Protamax hydrolysates whereas the bioactivity of Flavourzyme hydrolysate was the lowest. The active peptides derived from the Protamax-catalysed albumin (AP) hydrolysates were purified by ultrafiltration and ion-exchange liquid chromatography and the fractions APU-I (MW < 3 kDa) was found to have the smallest molecular masses but with the highest ACE-inhibitory activity, whilst the fraction APU-III (MW > 10 kDa) showed the lowest activity. In addition, the ACE-inhibitory activity in crude hydrolysate of AP was slightly higher than that of APU-I, which may be due to a synergistic effect among the peptides in the three fractions (Uraipong and Zhao, 2016). In a separate study, another commercial enzyme, protease G6 was also used to obtain rice bran hydrolysates and peptides, and its function was analyzed using a rat model of two kidney-one clip (2K-1C) renovascular hypertension. The results showed that the plasma ACE activity was higher in the

2K-1C animals, and the ACE levels were significantly reduced after the treatment with RBPs, leading to a reduced blood pressure and peripheral vascular resistance compared to the controls. These results also suggest that bioactive peptides-derived from RBPs have the potential to be developed as alternative therapeutics for prevention and treatment of hypertension in humans (Boonla et al., 2015).

***Antidiabetic effects of RBP***

Some peptides from food based proteins have been identified to possess antidiabetic activity, and one of research activities focused on the finding of new, natural and safe dipeptidyl peptidase (DPP) inhibitors from food proteins or their hydrolysates. The insulinotropic hormone glucagon-like peptide-1 (GLP-1) is metabolized rapidly by the enzyme dipeptidyl peptidase IV (DPP-IV) and DPP-IV inhibitors have been shown to be effective in controlling postprandial glycemia in type 2 diabetes through a proposed mechanism as shown in Figure 2 (Richard, 2008; Richter et al., 2008). Hatanaka et al. (2012) used two commercially available proteases (Umamizyme G and Bioprase SP) to hydrolyze defatted RBPs and a DPP-IV inhibitory protein hydrolysate was successfully achieved. The rice bran peptides obtained from Umamizyme G hydrolysis showed an enhanced DPP-IV inhibition activity which was 10 times greater that those produced from Bioprase SP treatment. The IC50 value of the rice bran peptides produced by Umamizyme G was 2.3 ± 0.1 mg/mL and the specific inhibitory peptides, Leu-Pro and Ile-Pro were identified from the rice bran peptides. Further analysis demonstrated that Ile-Pro had the

strongest inhibiting DPP-IV activity among the tested 15 Xaa-Pro dipeptides and Pro-Ile. In addition, Ile-Pro was found to competitively inhibit DPP-IV ($K$i = 0.11 mM). The LC–MS-SIM analysis indicated that the content of Leu-Pro and Ile-Pro in the peptides derived from RBPs obtained by Umamizyme G enzymolysis was 2.91±0.52 μg $mg^{-1}$, suggesting that the functional peptides derived from RBPs can be purified to a reasonable level and may have the potential to prevent or treat type 2 diabetes.

Insulin resistance is a status where peripheral tissues have a decreased response to normal circulating levels of insulin and is largely associated with an increased risk of type II diabetes (Lionetti et al., 2009). It has been reported that the pro-inflammatory cytokine IL-6 has the strongest correlation with insulin resistance and type 2 diabetes (Pickup et al., 2000; Kern et al., 2001). Boonloh et al. (2015) prepared peptides derived from RBP using Protease G6 for evaluating the effect of RBPs on insulin signaling in HepG2 cells treated with IL-6. Results clearly demonstrated that the peptides derived from RBPs possessed an insulin sensitizing effect tested by the cell models of insulin resistance induced by IL-6 and high glucose-exposure.

Amylases such as α-amylase and α-glucosidase are the key enzymes involved in the digestion of dietary starch. Thus, the inhibition of α-amylase and α-glucosidase benefits the control of glucose release and has been used for managing diabetes (Lin et al., 2012). Similarly, Uraipong and Zhao (2015) hydrolysed 4 proteins of albumin, globulin, prolamin and glutelin using four proteases (Alcalase, Neutrase, Flavourzyme and Protamax) for investigating the inhibitory

activities of the hydrolysates against α-amylase and α-glucosidase. They found that glutelin hydrolysate in the MW<3 kDa fraction obtained by Alcalase-catalysed hydrolysis exhibited the highest inhibitory activity. Furthermore, purification by ion-exchange chromatography revealed that the highest α-glucosidase inhibitory activity fraction was GAIE1 (glutelin Alcalase ion exchange fraction 1), which was identified to contain 13 unique peptide sequences containing 6 to 32 amino acid residues. These RBP hydrolysates with strong α-amylase and α-glucosidase inhibitory activities have the potential to be developed as an alternative to currently available α-glucosidase inhibitor drug, acarbose, in the form of dietary or nutraceutical supplements for the management of diabetes.

***Anticancer***

Cancer is the second leading cause of death after heart disease worldwide (Kannan et al., 2009). Colorectal and breast cancers are the most common causes of cancer related illnesses and deaths in the United States (Kannan et al., 2009). Kannan's study has shown that gastrointestinal-resistant rice bran peptide fractions have promised the activities in vitro against the viability and proliferation of colon and liver cancer cells (Kannan et al., 2008). Hydrolysates and peptides were prepared from heat-stabilized defatted rice bran using a food grade Alcalase, followed by simulated gastric and intestinal juices treatment to fractionate the GI-resistant peptides into >50, 10-50, 5-10, and <5 kDa sizes. Then inhibitory and cytotoxicity activities in human colon (HCT-116) and breast (HTB-26) cancer cell lines were investigated and the results showed that <5

kDa fraction of rice-bran protein hydrolysate had a potent anti-tumor activity particularly when cells were treated with 500 μg $mL^{-1}$. The specific fraction of peptide also showed cytotoxic effects on the two cancer cells which was more pronounced with an IC50 of approximately 750 μg $mL^{-1}$. Furthermore, a clonogenic assay indicated that the <5kDa fraction had significant growth inhibitory and cytotoxic effects on colon cancer cells at a higher dosage and over prolonged treatment period. The results showed that the <5kDa fraction of RBP hydrolysates demonstrated the potential anti-cancer activities as confirmed by an *in vitro* study (Kannan et al., 2009).

In a subsequent study, Kannan et al. (2010) also purified and characterized peptides from RBPs and found the MW <5 kDa fraction of the peptide may be associated with anti-cancer properties. In particular, the pure peptide at 600–700μg $mL^{-1}$ showed 84% inhibition on colon cancer cells (Caco-2, HCT-116) growth, 80% inhibition on breast cancer cells (MCF-7, MDA-MB-231) growth and 84% inhibition on liver cancer cells (HepG-2) growth. Mass spectrometry analysis and de novo peptide sequencing revealed an amino acid sequence of Glu-Gln-Arg-Pro-Arg. Furthermore, the peptide was characterized by matrix-assisted laser desorption ionization (MALDI) and confirmed its homogeneity with a molecular mass of 685.4 Da.

Though the novel pentapeptide ( Glu-Gln-Arg-Pro-Arg) has been isolated, purified and characterized for showing strong inhibitions on several cancer cell lines including breast cancer cells (MCF-7, MDA-MB-231) and liver cancer cells (HepG-2) (Kannan et al., 2010). The mechanism of the *in vivo* inhibitory effect of the pentapeptide on breast cancer cells and whether it

involves the activation of apoptotic pathways needs further investigation. Thus, MCF-7 and MDA-MB-231 breast cancer cell lines were selected to investigate the induction of apoptotic pathways using pentapeptide in breast cancer cell models. The results determined by MTS assay and trypan blue assay showed that the rice bran pentapeptide significantly inhibited the growth of MCF-7 and MDA-MB-231 cells in a time- and dose-dependent manner and the apoptosis was induced through Caspase-dependent pathways. In particular, the maximum growth inhibitions were achieved after the treatment with pentapeptide (1000 μg $mL^{-1}$) on MCF-7 (90.9%) and MDA-MB-231 (87.0%) (Li et al., 2014a). Whether the cell death induced by the pentapeptide is as a direct lytic effect on cell membranes due to a unique amino acid composition or as an indirect result of apoptosis caused by membrane rupture, the evidence of no cytotoxic effects on normal human breast cells may support it as a novel anti-breast cancer agent (Li et al., 2014b).

***Other bioactive peptides***

In addition to above-mentioned common functional peptides, other active peptides with promising effects have also been reported. For example, tyrosinase inhibitory peptides were made from RBP, where chymotrypsin and trypsin were simultaneously used during the preparation (Ochiai et al., 2016). The peptides were further isolated from the hydrolysate fractions and found that the peptide CT-2 (Leu−Gln−Pro−Ser−His−Tyr) potently inhibited melanogenesis in mouse B16 melanoma cells without cytotoxicity and could be further developed as an agent to treat melanin-related skin disorders (Ochiai et al., 2016).

Moreover, cationic peptides with antimicrobial activity have also been prepared from rice bran hydrolysis (Taniguchi et al., 2017). In particular, pepsin was used to hydrolyze RBP to prepare peptide fractions with different pI values by isoelectrc autofocusing. The identified cationic peptides were chemically synthesized with corresponding pI values and were evaluated for their antimicrobial, lipopolysaccharide-neutralizing and angiogenic activities. Results demonstrated that the purified cationic peptides exhibited antimicrobial activity with little or no haemolytic activity, and were suggested to be used as safe functional ingredients with good antimicrobial activity in food products (Taniguchi et al., 2017).

In addition, peptides with significant opioid antagonistic activity were also noticed from trypsin hydrolysis of RBPs (Chen et al., 2005). Traditional opioids are commonly used in the clinical management of severe trauma or burns, but numerous side-effects are known with traditional opioids, including physical dependence, addiction, tolerance and respiratory depression (Buenaventura et al., 2008; Liu and Wang, 2012). Peptides derived from food with opioid activity provide significant advantages as safe and natural alternative as well as relatively less side-effects over traditional opioids. According to the previous research (Chen et al., 2005), the opioid antagonist activity of the peptides reached a maximum value at the degree of hydrolysis (DH) of 11.9% and the molecular weight distribution for these peptides mainly ranged from 125-5838Da. However, the high temperature treatment led to the absence of opioid antagonist

(Chen et al., 2005). The recent publications regarding the preparation, structure and bioactivity of the RBP peptides are summarized in Table 1.

**The application prospects of peptides derived from RBPs**

Bioactive peptides with special properties and functions can be obtained through specific enzymatic hydrolysis of RBPs, and these peptides have been shown to have a range of functions related to health benefit which could be incorporated into nutraceutical supplementation in a number of foods, such as beverages, meat products, medical care food and even the application for high performance sports nutrition, etc.

**Conclusion and outlook**

A series of recent studies have explored the potential of using RBPs to produce various bioactive peptides with health-promoting and nutritional functions. This paper reviewed the health-promoting functions of RBPs hydrolysates and peptides which exert various activities including antioxidant, anti-diabetic, anticancer, and ACE-inhibitory activity. However, the relationships between structural characteristics and the corresponding functional activities of the peptides require further study. In particular, clinical studies on the application of RBP peptides in human metabolism are still rare.

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**Table 1 Functional peptides derived from the hydrolysis of rice bran proteins.**

| | Enzymatic treatment | Fraction number | Sequence/Composition | MW | Protein type and class | Separation/Purification/ Analytical method | Reference |
|---|---|---|---|---|---|---|---|
| Antioxidant activity | Papain and flavorzyme | F2-5 | Thr, Val, Ile, Leu, Phe, Lys | <3 kD | | SP-SephadexC-25, SephadexG-25, HPLC | Mei et al., 2013 |
| | Trypsin | F14-16 | Rich in Gly, Ala | 726-1578 Da | Denatured albumin | RP-HPLC, LC-MS/MS | Wattanasiritham et al., 2016 |
| | Alcalase | F16, 121, 143 | | 6 kD | | Anion exchange chromatography | Parichart et al., 2016 |
| | Trypsin | F2-a | Tyr-Ser-Lys | 395.0 Da | | SephadexG-25, RP-HPLC | Wang et al., 2017 |
| | Alcalase | AAU-I | | <3 kDa | Albumin | Ion-exchange chromatography | Uraipong and Zhao, 2016 |
| | Trypsin | | Tyr-Ser-Lys | 395.0 Da | | SephadexG-25, RP-HPLC | Wang et al., 2017 |
| ACE-inhibitory activity | Protamax | | | | Albumin | | Uraipong and Zhao, 2015 |
| | Alcalase | AAU-I | | <3 kDa | Albumin | Ion-exchange chromatography | Uraipong and Zhao, 2016 |
| Anti-diabetic activity | Umamizyme G | | Ile-Pro | 250.0 Da | | HPLC, LC-MS-SIM | Hatanaka et al., 2012 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | Alcalase | GAIE1 | 13 unique peptides sequences with six to 32 amino acid residues | <3 kDa | Glutelin | Ultra-filtration, ion-exchange chromatography | Uraipong & Zhao, 2015 |
| Anti-cancer | Alcalase | | | <5 kDa | | | Kannan et al., 2009 |
| | Alcalase | <5k Da fraction | Glu-Gln-Arg-Pro-Arg | 685.4 Da | | RP-HPLC | Kannan et al., 2010 |
| Tyrosinase Inhibition Activity | Trypsin, chymotrypsin | CT-2 | Leu−Gln−Pro−Ser−His−Tyr | | | P-6 gel column chromatography, Superdex Peptide 10/300 GL column chromatography | Ochiai et al., 2016 |
| Antimicrobial Activity | Pepsin | F18-20 | | | | RP-HPLC, matrix-assised Laser/desorption ionization-time-of-flight mass spectroscopy | Taniguchi et al., 2017 |
| Opioid antagonistic activity | Trypsin | | | 125-5838 Da | | SE-HPLC | Chen et al., 2014 |

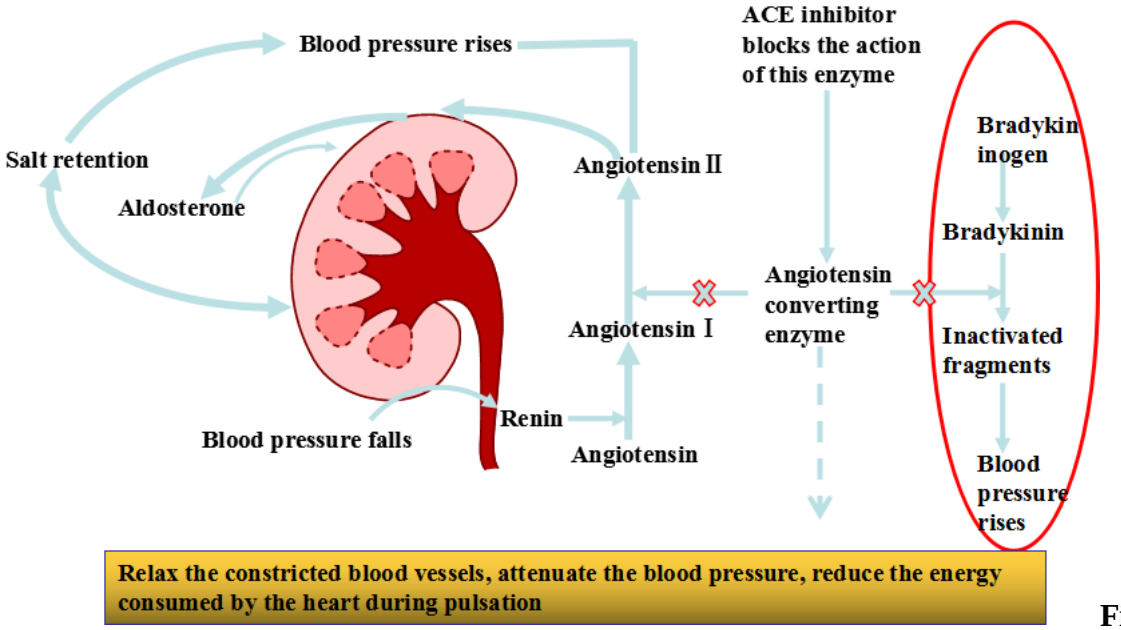


**Figure 1**

**Proposed anti-hypertensive properties of peptides derived from RBP (Erdös et al., 1999; García et al., 2013).**

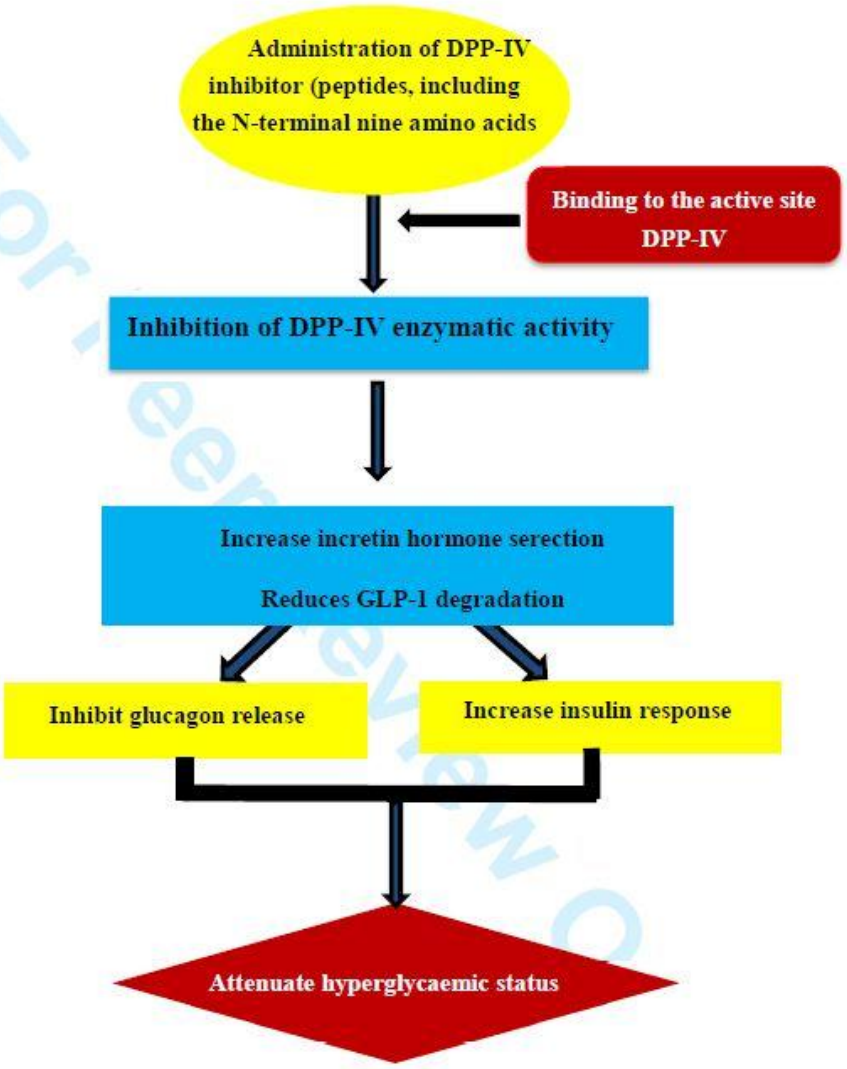


**Figure 2 A proposed mechanism of insulinotropic hormone glucagon-like peptide-1 involved in the regulation of hyperglycemia (Adapted from Drucker, 2006).**