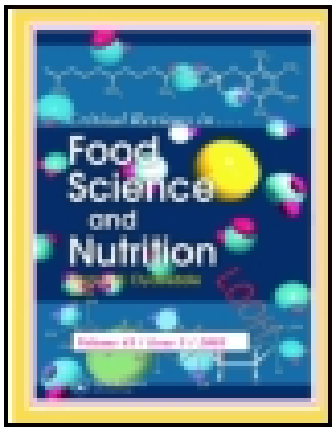

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Recent research in antihypertensive activity of food protein-derived hydrolyzates and peptides

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Recent research in antihypertensive activity of food protein-derived hydrolyzates and peptides

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Abstract

Year to year obesity prevalence, reduced physical activities, bad habits/or stressful lifestyle, and other environmental and physiological impacts leading to increase of diseases such as coronary heart disease, stroke, cancer, diabetes and hypertension worldwide. Hypertension is considered as one of the most common serious chronic diseases; however, discovery of medications with high efficacy and without side effects for treatment of patients remains a challenge to scientists. Recent trends in the functional foods have evidenced that food bioactive proteins play a major role in the concepts of illness and curing: therefore, nutritionists, biomedical scientists, and food scientists are working together to develop improved systems for discovery of peptides with increased potency and therapeutic benefits. This review presents the recent research carried out to date for purposes of isolation and identification of bioactive hydrolyzates and peptides with angiotensin I-converting enzyme (ACE) inhibitory activity and antihypertensive effect from animal, marine, microbial and plant food proteins. Effects of food processing and hydrolyzation conditions as well as some other impacts on formation, activity and stability of these hydrolyzates and peptides are also presented.

Keywords angiotensin-converting enzyme (ACE), antihypertensive activity, food peptides, hypertension

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INTRODUCTION

Hypertension is a major health problem of epidemic proportions, which affects about 30% of all adults worldwide. It is defined as a systolic blood pressure (SBP) above 140 mm Hg and a diastolic blood pressure (DBP) above 90 mm Hg. Hypertension plays a major etiologic role in the development of cardiovascular disease, ischemic heart disease, cardiac and renal failure **(Balti *et al.*, 2010; WHO, 2003).** It has been estimated that inadequate blood pressure control is responsible for 62% of cases of cardiovascular disease, 49% of cases of ischemic heart disease and 7.2 million deaths per year **(WHO, 2002).** However, treating hypertensions has been associated with about a 40 % reduction in the risk of stroke and about a 15% reduction in the risk of myocardial infraction **(Collins *et al.*, 1990; WHO, 2003).** Moreover, epidemiological studies have evidenced that a small reduction in blood pressure (about 10 mm Hg) can be associated with significant benefits in population **(Rousseau-Ralliard *et al.*, 2010)**.

Although the chemical synthetic drugs are the most common used in hypertension treatment, lifestyle modifications have also been found in many clinical trials to lower blood pressure and to reduce the incidence of hypertension. These modifications include weight loss in overweight, smoking reduce, physical activity, moderation of alcohol intake, a diet with increased fresh fruits and vegetables, reduced saturated fat content, reduction of dietary sodium intake and increased potassium intake. Therefore, regardless of the level of blood pressure, all individuals should adopt appropriate lifestyle modifications **(WHO, 2003).** On the other hand, **i**ncreasing consumer knowledge of the link between diet and health increased the awareness and demand for functional food ingredients to avoid undesirable side effects associated with consumption of organically synthesized chemical drugs and also to avoid the increasing cost of drug therapy **(Hong *et al.*, 2008).** However the challenge of improving the food supply is to realize that the success of food-based health care intervention is dependent on both efficacy and compliance. Efficacy relates to the ability of the food-based intervention to alter the biological pathways that improve health, whereas compliance relates to the propensity for an individual to actually consume the health promoting product (**Decker and Park, 2010)**.

The interest in functional foods reflected in a large number of studies and research carried out to produce and identify bioactive peptides from food proteins with different biological effects and therapeutic purposes. At present and based on published literature, there are several natural peptides that have been isolated and selected as antihypertensive peptides from various foods such as milk, fish, meat, cereal grains, legumes and seed. Food peptides are inactive when they are encrypted within the parent proteins and they only show biological activity when they are

released by some strategies such as enzymatic hydrolysis, gastrointestinal digestion, and food processing or by a combination of different strategies **(Hernández-Ledesma *et al.*, 2011b)**. Chemical synthesis of specific bioactive peptides having similar amino acid structures with naturally food derived bioactive peptides is also strategy used. However, in the *in vitro* hydrolysis approach, the challenges lie in choosing the protein and enzymes combination that gives rise to a high yield of the bioactive peptides **(Otte *et al.*, 2007).** Measurement of angiotensin I converting enzyme (ACE) inhibitory activity using a number of methods including spectrophotometric, fluorimetric, radiochemical, high performance liquid chromatography (HPLC) and capillary electrophoresis is the most common strategy followed in the selection of antihypertensive hydrolyzates and peptides derived from food sources **(Lopez-Fandino *et al.*, 2006).** The potency of an ACE-inhibitory peptide is usually expressed as an IC_{50} value, which is equivalent to the concentration of peptide mediating 50 % inhibition of ACE-activity. ACE-inhibitory activity may also be expressed as an ACE-inhibition index or the percentage inhibition achieved by a defined concentration of an inhibitor **(Murray and FitzGerald, 2007**). In addition, a number of studies in spontaneously hypertensive rats (SHR) and in hypertensive human volunteers have been performed to determine the antihypertensive effect of food derived peptides **(Erdmann *et al.*, 2008)**. However, production of sufficient and effective antihypertensive peptides from food protein hydrolyzates on a commercial scale facing several challenges such as quality of the raw materials and cost effectiveness of the final product **(Raghavan and Kristinsson, 2009).**

The main objective of this work is to present the recent research carried out to date for purposes of isolation and identification of bioactive hydrolyzates and peptides with ACE-

inhibitory activity and antihypertensive effect from animal, marine, and plant food proteins. Effects of food processing and peptides preparation conditions as well as some other impacts on formation, activity, and stability of these hydrolyzates and peptides are also discussed.

ACE AND BLOOD PRESSURE REGULATION

Blood pressure is controlled and impacted by different interacted biochemical pathways. One of the most known blood pressure regulators is the angiotensin I converting enzyme (ACE, a dipeptidyl carboxypeptidase) through the renin–angiotensin system (RAS). RAS is identified as a hormone based pathway that responds to low blood pressure by initiating the kidneys to secrete the enzyme renin. Renin (also referred to as angiotensinogenase) circulates in the blood where it hydrolyses angiotensinogen into angiotensin I (Ang I). ACE, which is associated with endothelial cells on blood vessel walls especially in the lungs, converts the inactive decapeptide Ang I by cleaving dipeptide from the C-terminus into the potent vasoconstricting octapeptide Angiotensin II (Ang II) (Fig. 1). Converting Ang I to Ang II in blood vessels induces the release of aldosterone, increases the sodium concentration and constriction of arteries and then a subsequent increase in blood pressure (**Decker and Park, 2010; Hernández-Ledesma *et al.*, 2011b).** However, it has been estimated that Ang II is not the only effector of the RAS and other peptides generated by the RAS influence renal function and structure as well. Moreover, the discoveries that Ang II can be generated by enzymes other than ACE and that Ang II and other RAS derived peptides bind to various receptors with different functional consequences have further added to the complexity of this system (**Wolf and Ritz, 2005).** ACE also cleaves the C-

terminal dipeptide from bradykinin (a potent vasodilator) and stimulates the release of aldosterone in the adrenal cortex (**Pihlanto-lepala, 2001; Lopez-Fandino et al., 2006; Ricci et al., 2010).**

Currently there is a number of ACE-inhibitory drugs mainly captopril and enalapril, marketed under various trade names and have an established role in the treatment of patients across the cardiovascular disease continum, from uncomplicated hypertension to established cardiovascular disease **(Ferrari, 2008: Ahhmed and Muguruma, 2010**). ACE-inhibitors alter the balance between the vasoconstrictive, salt-retentive, and hypertrophic properties of Ang II and the vasodilatory and natriuretic properties of bradykinin and alter the metabolism of a number of other vasoactive substances. They differ in the chemical structure of their active moieties, in potency, in bioavailability, in plasma half-life, in route of elimination, in their distribution and affinity for tissue-bound ACE, and in whether they are administered as prodrugs **(Brown and Vaughan, 1998).** It has been suggested that based on their effect mechanisms, ACE-inhibitors can be generally divided into two categories: (1) those that compete with available ACE substrate to react with ACE; and (2) those that combine with the ACE bioactive sites to inhibit its enzymatic activity **(Huang *et al.*, 2011)**.

MILK AND MILK PRODUCTS PROTEIN-DERIVED HYDROLYZATES AND PEPTIDES

Several bioactive hydrolyzates and peptides have been isolated and identified from many dairy products including milk, cheese and yoghurt and they have been found to exhibit various physiological activities such as antihypertensive, opioid, immunomodulatory antimicrobial,

antioxidative, antithrombotic, and cytomodulatory **(Haque *et al.*, 2009)**. However, behave like ACE-inhibitors and at the end of the 20th century; attempts were already being made to market several casein hydrolyzates with antihypertensive effect **(Miguel *et al*, 2009)**. Bioactive peptides derived from milk proteins have been reviewed recently by **Haque and Chand, 2008; Saito (2008); Jäkälä and Vapaatalo (2010)**; and **Ricci *et al*. (2010).**

Raw milk proteins (Casein and Whey)

Milk is known to be a rich source for the supply of bioactive peptides compared to other food protein sources. Casein is the major protein which occupies about 80% of the total protein in bovine milk and exists mainly in macromolecular complexes as casein micelles consisting of more than 1,000 casein submicelles. It is a group of phosphoproteins and consists mainly of αs1-, αs2-, β-, and k-casein and they are known to be precursors of a number of different bioactive peptides **(Saito, 2008).** Isolation and identification of bioactive peptides with different biological effects from casein have been studied extensively.

Based on literature data summarised in table 1, there are several hydrolyzates and peptides with ACE-inhibitory activity and antihypertensive effect have been identified from bovine and other species milk proteins. Measurement of ACE-inhibitory activity is the most common strategy followed in the selection of antihypertensive hydrolyzates and identified peptides from milk. However, structure activity relationship of ACE-inhibitory peptides from milk proteins is not well clarified. Structure activity correlations between different peptide inhibitors of ACE

indicate that binding to ACE is strongly influenced by C-terminal tri-peptide sequence of substrate. In addition, although the precise substrate specificity is not fully understood ACE prefers substrates or competitive inhibitors containing hydrophobic (aromatic or branched side chains) amino acid residues at each of the three C-terminal positions **(Haque and Chand, 2008).** On the other hand, the antihypertensive effect of peptides derived from milk is usually tested in SHR, which is considered as a good experimental model to test antihypertensive peptides because the hypertensive process in these animals present many similarities with essential hypertension in humans. However, the antihypertensive effect of the *in vitro* ACE-inhibitory peptides has not always been evaluated. Moreover, it has been estimated that although the activity of such peptides is easy to establish in assay *in vitro*, measuring their efficiency *in vivo* is more difficult, because no clear link between *in vitro* and *in vivo* activities has been established (**Rousseau-Ralliard *et al.*, 2010).** This mainly because of some hydrolyzates and peptides were found to have potent ACE-inhibitory activity, but did not exert antihypertensive effect or showed weak effect **(Maruyama *et al.*, 1987; Walsh *et al.*, 2004; Rousseau-Ralliard *et al.*, 2010)**. In addition, the antihypertensive potential of milk protein derived peptides is dependent on their ability to reach their target site without being degraded and as a consequence inactivated by the action of intestinal or plasma peptidases **(Haque *et al.*, 2009; Miguel *et al.*, 2010)**. Therefore the weak antihypertensive effect can be attributed both to the rapid degradation of the active components absorbed from the homogenates and to their weak inhibitory capacity compared with pharmaceutical drugs **(Rousseau-Ralliard *et al.*, 2010)**. However, peptides without ACE-inhibitory activity and antihypertensive properties, can release after oral ingestion other minor peptides which present blood pressure lowering effects (**Miguel *et al.* 2010)**. It has also been

estimated that milk peptides and other milk components may lower blood pressure through mechanisms other than ACE-inhibition mechanism such as opioid-like activities, mineral-binding, and antithrombotic properties **(Pihlanto-lepala, 2001; Jauhiainen and Korpela, 2007).**

From table1, it can also be seen that there are several researchers have prepared hydrolyzates with antihypertensive properties from whey proteins by enzymatic hydrolysis using various proteases or fermentation by microorganisms or a combination of both. Whey is the second major protein in bovine milk after casein and account about 20% of the total milk protein. The major proteins found in whey are immunoglobulins, α-lactalbumin, ß-lactoglobulin, bovine serum albumin, immunoglobulin, lactoferrin as well as proteosepeptone fractions and transferring. Whey proteins stand out for their high nutritional value in terms of biological value and composition in essential amino acids. Different bioactivities have been associated to these proteins, among them antihypertensive, antimicrobial, opioid, antioxidant and immunomodulant activity being the most studied **(Séverin and Wenshui, 2005; Hernández-Ledesma *et al.*, 2011a)**. In human study, whey protein beverages reduced blood pressure in young men and women in a six week controlled intervention. There were no differences in systolic blood pressure (SBP), diastolic blood pressure (DBP), or mean arterial pressure (MAP) observed between groups consuming 28 g per day of either hydrolyzed or non-hydrolyzed whey protein in a beverage. However, in young adults with elevated DBP and SBP, whey beverage consumption significantly decreased SBP, DBP, and MAP by 8.0, 8.6, and 6.4 mm Hg; respectively, for all comparisons (**Fluegel *et al.*, 2010)**. In contrast, it has been found that daily consumption of a milk drink supplemented with whey peptides presumed to contain ACE-inhibitory peptides did

not significantly affect blood pressure and/or inflammation markers in mildly hypertensive subjects **(Lee *et al.*, 2007).** Whey bioactive components and peptides have been reviewed by **Pihlanto-lepala (2001); and Hernández-Ledesma *et al.* (2011a)**.

Manufactured Milk Products

Fermented milk products, in addition to providing both energy and nutrients, are an excellent source of bioactive peptides. Fermented food products prepared using various microorganisms have a long history in their use because of their many beneficial effects. Many industrially utilised dairy starter cultures are highly proteolytic. Bioactive peptides can, thus, be generated by the starter and non-starter bacteria used in the manufacture of fermented dairy products **(Donkora *et al*., 2007; Haque and Chand, 2008)**. Several hydrolyzates and peptides isolated from manufactured milk products such as fermented milk, yogurt and cheese were found to have ACE-inhibitory activity and antihypertensive effect. In human study done by **Seppo *et al.* (2003),** *Lactobacillus helveticus* LBK-16H fermented milk containing bioactive peptides in normal daily use resulted in a blood pressure– lowering effect in hypertensive subjects with mean difference of - 6.7 mm Hg in SBP and -3.6 mm Hg in DBP between test product and control group. Further, demographic factors had no significant effect on the responses. Age, sex, and antihypertensive medication had no significant interaction effects. Another study conducted by **Aihara *et al.* (2005)** reported that administration of supplementary tablets containing powdered *Lactobacillus helveticus*-fermented milk with two tripeptides (VPP and IPP), which have ACE-inhibitory activity, to subjects with high-normal blood pressure or mild hypertension

resulted in a significant decrease in blood pressure without any adverse effects. In hypertensive rats, **Kim *et al.* (2010)** found that SBP and ACE-activity of SHR were significantly ameliorated in fermented milk peptides (10 mg/kg of body weight (BW)/day) group compared to those of the skimmed milk. Furthermore, **Liu *et al.* (2011)** found that milk fermented with *Lactobacillus paracasei* subsp. *Paracasei* (101FM) or *Lactobacillus plantarum* (102FM) significantly decreased SBP and DBP in the SHR after eight hours of a single oral administration or after 8 weeks of weekly (chronic) administration.

Antihypertensive peptides have been produced from milk yoghurt proteins through fermentation by bacteria starters and during manufacture processing. In one study, ACE-inhibitory activity of peptide fractions from different yoghurt batches indicated that all probiotic yoghurts showed appreciable activity during initial stages of storage compared with the control yoghurt; with a significant ($p < 0.05$) decrease afterwards **(Donkora *et al.*, 2007).** It has also been found that peptide content and the ACE-inhibitory activity of the water-soluble extracts of sheep milk yoghurt increased throughout storage **(Papadimitriou *et al.*, 2007).** In a recent study, at the end of the feeding period (8 week) the reduction in SBP of rats fed skim milk diet supplemented with freeze dried low fat yogurt was 3.7% (- 9.5 mm Hg) and 2.7% (- 6.4 mm Hg) in those fed freeze dried low-fat probiotic yogurt while reduction in DBP was 30% (–9.4 mm Hg) and 44% (–13.8 mm Hg), respectively **(Ramchandran and Shah, 2011)**.

Bioactive peptides were also found to be produced during cheese manufacturing. In Spain, different Spanish cheeses, made with diverse technologies and milk from different species were

studied for their ACE-inhibitory activity. Among the water soluble extract <1000-Da of the different samples, Cabrales cheese was the most active with an inhibitory activity of 76.1%. However, Mahon cheese showed the lowest ACE-inhibitory activity (56.6%) **(Gomez-Ruiz *et al.*, 2006).** In Italy, the ACE-inhibitory activity of water soluble extracts from Asiago cheeses was assayed in two cheese production systems and with different ripening times. The cheese production systems had no significant effect on the ACE-inhibitory activity, whereas 6-month-old cheeses had higher inhibitory potency than the more ripened ones. Moreover, the fraction containing peptides smaller than 3 kDa made a more considerable contribution to ACE-inhibitory activity than the fraction smaller than 10 kDa, suggesting an inhibitory effect due to short peptides **(Lignitto *et al.*, 2010).** In Australia, extracts of three commercial Australian Cheddar cheeses were also found to exhibit antimicrobial, antihypertensive and antioxidant properties (**Pritchard *et al.*, 2010)**.

Effect of processing and preparation conditions on milk and milk products protein-derived hydrolyzates and peptides

Several studies were performed to investigate effect of food processing methods such as fermentation, atomization, homogenization and pasteurization and the processing conditions on the activity and stability of the antihypertensive peptides derived from milk proteins. The effects of protein hydrolyzation or digestion with different enzymes under different conditions were also investigated. It has been reported that a peptide with ACE-inhibitory activity (IC_{50} = 19.9 µM) was purified from skim milk after fermentation by *Lactobacillus helveticus* 130B4 showed a

stability to digestive protease and heat treatment at 100 ◦C for 20 min **(Shuangquan *et al.*, 2008).** A recent study has been established that although the amount of active peptides increased with the hydrolysis time, the IC_{50} values did not vary significantly. In addition, the ACE-inhibitory activity and the antihypertensive properties of the casein hydrolyzed products were maintained after drying. The active peptides were also resistant to atomization, homogenization and pasteurization. Moreover, the hydrolyzates were incorporated into liquid yoghurt and no significant reduction of either peptide was detected during the shelf-life of the product (**Contreras *et al.*, 2011)**. It has also been found that sonication enhanced the production of ACE-inhibitory peptides from casein and therefore shortened the time for bioprocessing (**Madadlou *et al.*, 2011)**.

For studying the influence of fermentation conditions, milk was fermented to defined pH values with 13 strains of lactic acid bacteria. The highest ACE-inhibitory activity was obtained with two highly proteolytic strains of *Lactobacillus helveticus* and with the *Lactococcus* strains. Fermentation from pH 4.6 to 4.3 with these strains slightly increased the ACE-inhibitory activity, whilst fermentation to pH 3.5 with *Lactobacillus helveticus* reduced the ACE-inhibitory activity. Further, cold storage dramatically increased the ACE-inhibitory activity of some products (**Nielsen *et al.*, 2009)**. Moreover, **Pan and Guo (2010)** studied the effect of fermentation conditions on the production of ACE-inhibitory peptide from sour milk fermented by *Lactobacillus helveticus* LB10 using response-surface methodology. Optimal conditions to produce the maximum production of ACE-inhibitory peptides were found to be 4% (v/w) inoculum, 7.5 initial pH of medium and 39.0 °C. The fermented milk resulted in 75.46%

inhibition in ACE activity. Further evidences , **Otte *et al*. (2011**) documented that fermentation temperature of milk significantly influenced the bacterial growth, extent of lysis, and ACE-inhibitory activity. Also they suggested that the cell wall proteinase was the primary catalyst in release of ACE-inhibitory peptides. In contrast, **Pihlanto *et al*. (2010)** reported that modification of fermentation conditions or pH control did not affect the ACE-inhibitory activity. Further, the development of ACE-inhibitory activity during fermentation was found to be correlated with degree of hydrolysis. **Ong and Shah (2008)** found that increase in ripening temperature of cheese from 4 ◦C to 8 and 12 ◦C increased the percentage of ACE-inhibition. Hence, probiotic *Lactobacillus acidophilus* L10 can be added into cheddar cheeses to improve proteolysis and ACE-inhibitory activity**.**

For studying the influence of the microbe type, among nine *Lactobacillus* studied, *L. brevis, L. helveticus* and *L. paracasei* were found to be the most effective species inducing ACE-inhibitory peptides with inhibition rate ranging from 93.3 to 100% at a concentration of 200 mg/ml From 2% (w/w) whey powder in growth media **(Ahn *et al.,* 2009)**. Furthermore, fresh low-fat milk was fermented for up to 30 h at 42°C with five mixed lactic acid bacteria. The whey was separated from the fermented milk and freeze-dried. As the fermentation time extended to 30 h, the ACE-inhibitory activity increased as shown by a decrease of IC_{50} from 1.18 to 0.24 mg/ml **(Chen *et al.,* 2009).** It has also been estimated that when Raftiline HP® (a commercial inulin product, use commonly as a fat replacer in low-fat fermented milks such as yogurt) was added at the rate of 1, 2 and 3 g/100 ml to reconstituted skim milk, the generation of ACE-

inhibitory peptides by *L. casei*, *L. delbrueckii ssp*. *bulgaricus* and *B. longum* was improved **(Ramchandran *and* Shah, 2010)**.

For purpose of studying the effect of enzyme type and hydrolysis conditions the effect of alcalase, neutrase, trypsin and their combined system, i.e. alcalase-neutrase and trypsin-neutrase, under two different hydrolysis conditions, i.e. pH-controlled and pH spontaneous drop, on the formation of ACE-inhibitory peptides and the characteristics of whey protein hydrolyzate was investigated. The hydrolyzate of whey protein isolate obtained after 3 h incubation with alcalase plus 2 h with neutrase under pH-spontaneous drop condition possessed the highest ACE-inhibitory activity of 54.30% **(Wang *et al.*, 2010).** The impact of a simulated gastrointestinal digestion on the stability of eight peptides previously identified in fermented milk with antihypertensive activity was investigated. Two of these identified peptides with sequences LHLPLP and LVYPFPGPIPNSLPQNIPP, possess ACE-inhibitory activity and antihypertensive effect. The results showed that LHLPLP was resistant to digestive enzymes. In contrast, LVYPFPGPIPNSLPQNIPP was totally hydrolyzed and its activity decreased after incubation with pepsin and a pancreatic extract **(Quiros *et al.*, 2009)**. It was also shown that enzyme/substrate (E/S) ratio of 0.60, pH of 9.18 and temperature of 38.9 °C were found to be the optimal conditions to obtain high ACE-inhibitory activity close to 92.2% and degree of hydrolysis of the whey protein was 18.8% **(Guo *et al.*, 2011).** Furthermore, the hydrolysis of bovine whey protein concentrate (WPC), α-lactalbumin (α-La), by aqueous extracts of Cynara cardunculus, was optimized using response surface methodology. Degree of hydrolysis (DH), ACE-inhibitory activity and antioxidant activity were used as objective functions, and hydrolysis

time and E/S ratio as manipulated parameters. Maximum DH was 18% and 9%, for WPC and α-La, respectively. 50% ACE-inhibition (IC_{50}) was produced by 105.4 (total fraction) and 25.6 μg/ml (<3 kDa fraction) for WPC, and 47.6 (total fraction) and 22.5 μg/ml (<3 kDa fraction) for α-La **(Tavares *et al.*, 2011a).** However none of whey ACE-inhibitory peptides remained stable in the presence of gastrointestinal enzymes. They were partially or even totally hydrolyzed to smaller peptides, but observed ACE-inhibitory effects were not severely affected for two of them **(Tavares *et al*., 2011b).**

The ACE-inhibitory activity of hydrolyzates and peptides was also found to be varied with the hydrolysis time under the same conditions. **Ferreira *et al.*, (2007)** reported that extensive hydrolysis was required to obtain peptides suitable for functional ingredients, namely ACE-inhibitory effect, more effective gastrointestinal absorption and reduced food allergies. In contrast, **Guo *et al.*, (2009)** reported that high DH can not lead to high ACE- inhibitory activity of the hydrolyzate and a relatively 20% DH was required to produce potent ACE- inhibitory activity**.** Furthermore, **Contreras *et al.* (2009)** found that the ACE-inhibitory activity of casein soluble fraction increased during the first 3 h of hydrolysis (1-24 h) and the highest activity with IC_{50} value of 22.19 mg/ml was observed after 3 h hydrolysis. However, **Jiang *et al.* (2010)** reported that casein hydrolyzate obtained at 12 h hydrolysis showed the highest ACE-inhibitory activity.

Based on literature data, it can be concluded that milk is known as one of the most important sources of nutrients and compounds with various biological activities. In addition, milk and its manufactured products are available to large number of people worldwide with low cost

compared with other animal food sources. Therefore, a large number of studies were performed in order to investigate milk components in terms of their health benefits. On the other hand, because milk is a protein-rich food, biological activities of hydrolyzates and peptides derived from milk proteins such as casein and whey were extensively studied, especially their ACE-inhibitory activity and antihypertensive effect. However, most of studies that have been done to test the antihypertensive activity of milk peptides were carried out *in vitro* and/or in animal models and few studies were performed in human. Thus, there is essential need to more clinical trials to verify the efficiency of these peptides in human body. Moreover, bioactive stability of these peptides should be studied in long terms. Much more research to develop their availability in easy oral administration forms such as encapsulation and to investigate their safety to human are also needed. Mechanisms other than ACE-inhibitory activity and potential role of minerals such as calcium in milk and other protein-combined components should be clarified.

MARINE FOOD PROTEIN-DERIVED HYDROLYZATES AND PEPTIDES

Marine foods are well known as rich source of different bioactive components. Based on emerging evidence of potential health benefits, these components show significant promise as functional food ingredients. Activities including antihypertensive, antioxidant, anti-microbial, anti-coagulant, anti-diabetic, anti-cancer, immunostimulatory, calcium-binding, hypocholesteremic and appetite suppression have been reported **(Harnedy *and* FitzGerald, 2011).** Among the bioactive compounds, marine food-derived bioactive peptides are well known to have a potential in use as functional ingredients in nutraceuticals and pharmaceuticals due to

their effectiveness in both prevention and treatment of hypertension in addition to their nutritive value (**Wijesekara and Kim, 2010).** Moreover, the health benefits of seafood consumption have primarily been associated with protective effects against some chronic diseases such as cardiovascular diseases. Further, intake of seafood has also been associated with improved fetal and infant development as well as several other diseases and medical conditions **(Larsen *et al.*, 2011).** Therefore, a great interest has been developed nowadays to isolate bioactive hydrolyzates and peptides with antihypertensive activity from different marine organisms such as fish, shellfish, seaweeds and algae.

Fish and shellfish

Researchers have shown an increased interest in finding antihypertensive peptides in fish and shellfish proteins. Currently, there are several hydrolyzates and peptides with ACE-inhibitory activity were isolated and identified. In addition, some *in vivo* studies were performed to test the antihypertensive effect of these peptides in hypertensive rats **(Table 2).** However, studies conducted to determine their effect in human are limited. The hydrolysis by various selected proteases is the most common strategy followed to release bioactive peptides from fish and shellfish proteins. *In vitro* digestion model to stimulate the human gastrointestinal digestion is also used.

From data summarised in table 2, it can be seen that the ACE-inhibitory activity of fish hydrolyzates and the IC_{50} values are different according to the fish species. The IC_{50} values are also varied for hydrolyzates and peptides derived from the same fish species **(Wang, Hu, Cui *et***

***al.*, 2008; Shiozaki *et al.*, 2010).** These variations can be attributed to the difference between the preparation methods used, purity of the derived peptides, and the amino acid composition of the derived peptides. On the other hand, the antihypertensive effects in the hypertensive rats are also varied and this variation may be dependent on doses volume and the administration method as well as these affects. It has been estimated that an active inhibitory peptide must contain an aromatic amino acid at the C-terminal and Trp was the most effective among C-terminal amino acids of investigated dipeptides **(Cheung *et al.*, 1980)**. In the same line, **Ono *et al.* (2003)** suggested that amino acid sequence, as well as the kind of amino acids, is a crucial factor in the ACE-inhibitory activity of the antihypertensive peptides. They also suggested that peptides composed of Trp at the C-terminal inhibited ACE much more effectively than did those composed of Trp at the N-terminal.

A study was carried out for purpose of investigation the antihypertensive effect of a peptide with IC_{50} value of 1.2 μM from salmon on SHR and the dose dependency for the antihypertensive effect in mild hypertensive subjects**.** The results indicated that a single intravenous administration of the salmon peptide showed a significant reduce in SBP of SHR at a dose of 30 mg/kg BW. In addition, although no significant difference was observed, the oral administration at a dose of 300 mg/kg BW also tended to reduce the SBP of SHR. On the other hand, the oral administration of the salmon peptide in mild hypertensive subjects showed the dose dependency of the antihypertensive effect. The SBP in 1.0 g of salmon peptide intake group was significantly reduced whereas no changes were observed in the 0.3 g of salmon peptide intake and placebo groups during the experimental period. As a result, the effective dose of the salmon peptide was considered to be a 1.0 g intake once a day **(Enari *et al.*, 2008).**

Some studies were also performed to investigate the effect of some treatments and preparation conditions on stability and ACE-inhibitory activity of peptides derived from fish. It has been reported that the type of enzymes did not significantly (p <0.05) affect ACE-inhibitory activity of hydrolystes from tilapia protein. In addition, hydrolyzates resulted from 25% DH showed higher ACE-inhibitory activity compared to 7.5% DH hydrolyzates, indicating that low MW peptides are better ACE-inhibitors than high MW ones. Further, ultrafiltration significantly decreased (p <0.05) the ACE-inhibitory activity of fractionates compared to the whole hydrolyzates (**Raghavan and Kristinsson, 2009)**. In one study, a purified ACE-inhibitory peptide with amino acid sequence VVYPWTQRF and IC_{50} value of 66 µM isolated from oyster protein hydrolyzate (Table 2) showed good heat and pH stability and strong enzyme resistant properties against gastrointestinal proteases **(Wang, Hu, Cui *et al.,* 2008).** In another study, to investigate heat-stability of peptides purified from cuttlefish muscle protein hydrolyzates, each peptide was subjected to incubation for 2 h at temperatures ranging from 4 to 100 $^{\circ}$C, and then ACE-inhibitory activities were determined. The results showed that preincubation did not change the inhibitory activity of the purified peptides and IC_{50} values of peptides before and after preincubation were the same **(Balti *et al.,* 2010)**. Furthermore, **Lahogue *et al.,* (2010)** reported that non-purified fish ACE-inhibitory hydrolyzate with IC_{50} value of 43 µg/ml showed a relatively good stability even after several months of storage at -20°C.

Fish products and byproducts

A number of peptides were isolated from fish products and many of them have been shown to have ACE-inhibitory activities and antihypertensive effect. **Je *et al*. (2005)** documented that rat blood pressure significantly decreased after injection by purified ACE-inhibitor isolated from fermented oyster sauce**. Itou *et al*. (2007)** found that a single oral administration of the hot water extract from *Narezushi* (a fermented mackerel product) at a dose of 10 mg peptide/kg significantly and maximally decreased the SBP by 27 mm Hg at 4 h after administration, although the SBP recovered to its initial level after 8 h. The *Narezushi* extract decreased the SBP more than raw mackerel extract, the ACE-inhibitory peptide produced during processing was considered to be involved in this difference.

Tuna cooking juice, a protein-rich by product containing 4% water- soluble protein, is discarded as drainage in commercial canned tuna factories **(Ko and Jao, 2000).** It has been established that Orientase (a commercial protease from *Bacillus subtillus*) effectively released peptides from tuna cooking juice protein with ACE-inhibitory activity and antihypertensive effect in SHR **(Hwang and Ko, 2004).** Further evidences, **KO *et al*. (2006)** found that the oligopeptides OA3, derived from tuna cooking juice, significantly reduced SBP in SHR at doses of 0.5, 0.75, and 1.0 g/kg BW and their efficacies were exhibited in a dose-dependent manner. In addition, they suggested that emulsification, microencapsulation, and lipophilization could further enhance and extend the antihypertensive effect of OA3 oligopeptides. In a recent study, **Hwang (2010)** examined peptides obtained from tuna cooking juice for the stability of their inhibitory properties and composition changes during processing and in the presence of gastrointestinal proteases. The results showed that the ACE-inhibitory peptides retained 95–99%

activity after simulated digestion and the peptides reserved almost the same composition before and after various temperatures (20–100 °C), levels of pressure (50–300 MPa) and pH (2–10) treatments**. Bougatef *et al.,* (2008**) isolated hydrolyzates with ACE-inhibitory activity by various proteases from heads and viscera byproducts protein of sardinelle. The degrees of hydrolysis and the ACE-inhibitory activities increased with increasing proteolysis time. In addition, the protein hydrolyzate generated with alkaline proteases from the viscera of sardine was then fractionated by size exclusion chromatography on a Sephadex G-25 into eight major fractions (P1–P8). The IC_{50} values for ACE inhibitory activities of sardinelle by-products protein hydrolyzates and fraction P4 were 1.2 ± 0.1 and 0.81 ± 0.01 mg/ml, respectively. Further, fraction P4 showed resistance to *in vitro* digestion by gastrointestinal proteases**.**

Fish industry wastes

Like many other food processing industries, the seafood processing sector gives rise to a significant volume of organic waste. Environmental issues, economic concerns and legal restrictions regarding the disposal of processing wastes have led to increased research in the discovery of alternative value added products, such as bioactive peptides from these waste streams **(Wilson *et al.,* 2011).** Several marine processing waste derived protein hydrolyzates and constituent peptides have been shown to display potent ACE-inhibitory activity and also antihypertensive effect in SHR. Bioactive peptides from marine processing waste and shellfish have been reviewed by **Harnedy and FitzGerald (2011).**

Tuna frame proteins are usually discarded as processing waste or used for animal feed because of its poor functional properties. However, frame proteins can be converted into value-added products by enzymatic hydrolysis, which may be widely applied to improve functional and nutritional properties of proteins. Tuna frame protein was hydrolyzed using alcalase, neutrase, pepsin, papain, a chymotrypsin and trypsin. Peptic hydrolyzate exhibited the highest ACE-inhibitory activity among them. During consecutive purification, a potent ACE-inhibitory peptide from tuna frame protein, which was composed of 21 amino acids, GDLGKTTTVSNWSPPKYKDTP (MW: 2,482 Da, IC_{50}: 11.28 μM), was isolated. In addition, antihypertensive effect in SHR revealed that a single oral administration (10 mg/kg BW) of that peptide can decrease SBP significantly **(Lee, Qian, Kim, 2010)**.

ACE-inhibitory peptide (YFP) was also purified using consecutive chromatographic techniques from yellowfin sole (*Limanda aspera*) frame protein, which is normally discarded as industrial waste in the process of fish manufacture. The YFP has a molecular mass of 1.3 kDa and consisted of 11 amino acids, MIFPGAGGPEL, and IC_{50} value of 28.7 μg/ml. In addition, antihypertensive effects of YFP in SHR following oral administration were determined as the blood pressure significantly decreased after peptide ingestion (**Jung, Mendis, Je *et al*., 2006)**. However, in the process of clam tsukudani production, a large volume of solid waste is discarded from its manufacture. Solid wastes of freshwater clam, including mainly mantle, were hydrolyzed by different enzymes to produce peptides with ACE-inhibitory activity. The results showed that a primary hydrolyzate, produced during hydrolysis by 2 crude peptidases, exhibited a strong ACE-inhibitory activity with IC_{50} value of 0.23 mg/ml. In addition, the ACE-inhibitory

activity of all secondary hydrolyzates digested by pepsin and trypsin was significantly increased as compared to primary hydrolyzates and the peptides produced during pepsin digestion had a greater ACE-inhibitory activity than those produced during trypsin digestion. Further, peptides with molecular weight of less than 1 kDa possessed the stronger ACE-inhibitory activity and their inhibitory pattern was found to be competitive (**Sun *et al*., 2011)**.

Other marine sources

Marine plants food such as seaweeds, algae and other small marine organisms were also found to contain bioactive peptides with *in vitro* and *in vivo* activity. In one study, protease hydrolyzate of Acetes chinensis (an under utilised shrimp species thriving in Bo Hai Gulf of China) has been shown to have high ACE-inhibitory activity with IC_{50} value of 0.97 mg/ml. Further, five peptides with high ACE-inhibitory activity were purified from the hydrolyzates and their sequences were identified by amino acid composition and molecular weight analysis. Three of them, FCVLRP, IFVPAF and KPPETV, with IC_{50} values of 12.3 µM, 3.4 µM and 24.1 µM, respectively were novel ACE-inhibitory peptides **(Hai-Lun *et al*., 2006)**. In another study, Acetes chinensis were fermented by *Lactobacillus fermentum* SM 605 and the fermented sauce presented high ACE-inhibitory activity. The minimum IC_{50} value of 3.37 ± 0.04 mg/ml was achieved by response surface methodology with optimized process parameters such as fermentation time of 24.19 h, incubation temperature at 38.10°C, and pH 6.12. In addition, three novel ACE-inhibitory peptides were purified by ultrafiltration, gel filtration, and reverse-phase high performance liquid chromatography. Their amino acid sequences identified by mass

spectrometry to be DP, GTG, and ST with IC_{50} values of 2.15 ± 0.02, 5.54 ± 0.09, and 4.03 ± 0.10 μM, respectively **(Wang, He, Chen *et al.*, 2008).**

The sea cucumber is an important cultured aquatic species and is also enjoyed by people in China and Japan. Gelatin from the sea cucumber (Acaudina molpadioidea) was hydrolyzed sequentially with bromelain and alcalase. The hydrolyzate was fractionated into three ranges of molecular weight (GH-I, <10 kDa; GH-II, <5 kDa; GH-III, <1 kDa) using an ultrafiltration membrane bioreactor system. The GH-III hydrolyzate showed a high ACE-inhibitory activity with IC_{50} value of 0.35 mg/ml. The GH-III hydrolyzate was also used as drinks administered to renal hypertensive rats (RHR) for 1 month and led to a significant reduce in SBP and DBP of the RHR. Further, an ACE-inhibitory peptide was isolated from the GH-III with a molecular weight of 840 Da and amino acids sequence identified as GAPGA and its IC_{50} value was 0.0142 mg/ml **(Zhao *et al.*, 2007).** Furthermore**,** a novel ACE-inhibitory peptide identified as MEGAQEAQGD was isolated from body wall protein of the sea cucumber (Acaudina molpadioidea). The inhibitory activity of the peptide was intensified by 3.5 times from IC_{50} 15.9 to IC_{50} 4.5 μM after incubation with gastrointestinal proteases. However, after 3 h of administrating the ACE-inhibitory peptide at dose of 3 μM/kg BW, SBP of SHR decreased by -19 mm Hg and the activity was maintained for 5 h **(Zhao *et al.*, 2009)**.

Wakame, *Undaria pinnatifida* containing almost 15% protein has been a very popular food in the oriental countries **(Suetsuna and Nakano, 2000)**. The ACE-inhibitory activity and antihypertensive effect of the hot water extract of wakame, *Undaria pinnatifida* were examined

by **Suetsuna *et al.* (2004)**. Ten dipeptides were isolated from the extract by several steps of chromatography. In addition, both single and repeated oral administration of synthetic YH, KY, FY, and IY significantly decreased blood pressure in SHR**.**

Among marine resources, marine algae are valuable sources of structurally diverse bioactive compounds **(Wijesekara *et al.*, 2011).** In South Korea, Korean seaweeds were screened by ACE-inhibitory and peroxynitrite assays. It has been suggested that the brown alga *E. stolonifera* and its components, most notably the phlorotannins, appear to harbor potential as a natural source of ACE-inhibitors (**Jung, Hyun, Kim, and Choi, 2006).** In addition, **Sheih *et al*. (2009)** purified an ACE-inhibitory peptide with amino acid sequence identified as VECYGPNRPQF and IC_{50} value of 29.6 μM from pepsin hydrolyzate of algae protein waste (a mass-produced industrial by-product of an algae essence from microalgae *Chlorella vulgaris*). The peptides also showed good pH and heat-stability (40–100 $^{\circ}$C for 1 h, pH 2–10), low gastrointestinal enzyme susceptibility, and established safety. Furthermore, **Qu *et al.* (2010)** produced several hydrolyzates from *Porphyra yezoensis* (an important alga mainly cultivated in China, Japan, and Korea) using various proteases. Among them three with IC_{50} values of 7.0 for albumin, 8.1 for gliadin, and 1.5 mg/ml for glutelin hydrolyzates. Further, the antihypertensive hydrolyzates produced remained at high stability at temperatures of 4, 25, and 37 °C, pH values of 2.0 and 8.0, and after pepsin and trypsin treatments, but was sensitive to high storage temperature (90°C). In addition, the acidic and slightly alkaline conditions were more favourable for preserving the antihypertensive activity of hydrolyzates than strong alkaline environment. In

a recent study, **Ko *et al.* (2011)** isolated an ACE- inhibitory peptide with IC_{50} value of the 24.7 μM from Styela plicata after hydrolysis with various proteases.

In one study, the optimal hydrolyzing conditions of the collagen extracted from jellyfish (Rhopilema esculentum) with alcalase to prepare the ACE-inhibitory peptide, using response surface methodology were determined. The optimal conditions were found to be at temperature of 52.7 °C, pH of 8.63 and E/S ratio of 3.46%. The ACE-inhibitory activity of the obtained hydrolyzates could reach 81.7% (**Zhuang *et al*., 2010)**. In another study, enzymatic conditions for producing ACE-inhibitory peptides from collagen were optimized**.** The results showed that through a single-enzyme hydrolysis, the ACE-inhibitory activity could reach an average of 78.06%**.** However, when a combination of pepsin and trypsin was used for a multiple-proteases hydrolysis, the ACE-inhibitory activity could be significantly improved to an average of 88.25% **(Kong *et al*., 2011)**. Furthermore, **Alemán *et al.* (2011)** hydrolyzed gelatin obtained from giant squid (Dosidicus gigas) inner and outer tunics by seven commercial proteases to produce bioactive hydrolyzates. The alcalase hydrolyzate was the most potent ACE-inhibitor (IC_{50}=0.34 mg/ml) **(**for further review about collagen and gelatin bioactive peptides see **Gómez-Guillén *et al*., 2011).**

In conclusion, no doubt that fish and shellfish are important sources of protein and play a major role in diet of many people worldwide. In the recent years and based on published literature, it can be observed that much attention has been paid to investigate bioactive hydrolyzates and peptides derived from fish and shellfish proteins as well as from other marine food sources in terms of their health benefits. Thus, there are several hydrolyzates and peptides

with ACE-inhibitory activity and antihypertensive effect were isolated and identified from marine food proteins. In addition, fish and shellfish by- products and industry wastes were found to possess bioactive peptides that may provide a source for these peptides with higher cost-effectiveness and contribute in solving of the environment problems. However, studies performed to investigate effect of marine food derived peptides in human are scarce; therefore, further research is needed to clarify their functions in human body. Developed systems and techniques are also needed for purification and isolation of bioactive peptides from fish and shellfish and to increase their acceptability to human, specially reducing of their fish-odor. Also studies for purpose of testing their safety and bioactive stability should be performed.

MEAT AND POULTRY PROTEIN-DERIVED HYDROLYZATES AND PEPTIDES

In the food guide pyramid, meat is categorized as a protein food group along with poultry, fish and eggs. It is well known that meat is a major source of food proteins with high biological value in many countries **(Arihara, 2006).** In the last 10 years, many researchers worldwide have paid considerable attention to the use of certain food constituents including meats to prevent the action of ACE in elevating blood pressure **(Muguruma *et al.*, 2009).**

Sarcoplasmic protein extracts from beef rump (biceps femoris) were found to possess hydrolyzates and fractions with ACE-inhibitory activities after hydrolysis using three enzymes or their paired combinations for different times. In addition, peptide with amino acid sequence VLAQYK from the hydrolyzates with ACE-inhibition rate of 30.1% was identified **(Jang and**

Lee, 2005). Once again, four peptides with high ACE-inhibitory activity were separated from sarcoplasmic protein hydrolyzates using commercial enzymes. They were identified as GFHI, DFHING, FHG, and GLSDGEWQ with IC_{50} values of 117, 64.3, 52.9, and 50.5 μg/ml, respectively **(Jang *et al.*, 2008)**. In a current study, **Bernardini *et al.* (2012)** reported that bovine brisket sarcoplasmic protein extracts and their 3 kDa filtrates displayed ACE-inhibitory activity. For commercial meat products, **Vaštag *et al.* (2010)** evaluated the ACE-inhibitory activities of the water-soluble protein extracts throughout the petrovac sausage ripening (Petrovská Kolbása, commercial sausage in Serbia). The results showed that the examined protein extracts exhibited ACE-inhibitory activity, which also enhanced; from 27.11 (day 0) to 73.74 % (day 90).

Porcine muscle was also found to release bioactive peptides with antihypertensive properties. **Sentandreu and Toldra (2007)** evaluated the ACE-inhibitory activity of dipeptides generated by the action of porcine muscle dipeptidyl peptidases. Among the assayed dipeptides, a peptide identified as RP showed the strongest activity, being able to suppress more than 60% of initial enzyme activity at a concentration of 25 μM. Further evidences, **Muguruma *et al.* (2009)** suggested that peptides identified as M6 (KRVIQY) and A5 (VKAGF) from porcine skeletal myosin B with IC_{50} value of 6.1 and 20.3 μM, respectively, are peptides that may serve several purposes. In addition, when both synthesized peptides were administered orally to SHR at doses of 10 mg/kg BW, antihypertensive effect was observed after 6 h (-23 mm Hg for M6 and -17 mm Hg for A5).

A thermolysin digest of chicken muscle was found to have ACE-inhibitory activity with IC_{50} value of 45.0 μg/ml. Further, seven peptides were identified from the digest and showed ACE-inhibitory activities; however, some of them failed to show antihypertensive effect in SHR after intravenous or oral administration **(Fujita *et al.*, 2000).** Enzymatic hydrolyzates with antihypertensive activity were also obtained from the leftover residues of a chicken essence factory. Among many proteases tested, alcalase was found to be the best to produce high ACE-inhibitory activity (IC_{50} of 273 μg/ml). In addition , the *in vivo* experiment revealed that 1% and 3% supplementation of the alcalase- treated hydrolyzate of chicken essence residues added to the control diet fed to SHR lowered the SBP (-18 and -26 mm Hg) of the rats after 16 weeks of treatment **(Chen *et al.*, 2002).** Furthermore, untreated chicken breast muscle extract showed ACE-inhibitory activity with IC_{50} value of 10.60 mg/ml, whereas the activity of the extract became stronger after treatment with an Aspergillus protease and gastric proteases (trypsin, chymotrypsin, and intestinal juice), reaching IC_{50} value of 11 μg/ml. On the other hand, the SHR fed the untreated chicken extract exhibited the greatest decrease in blood pressure by 50 mm Hg at 3 h after administration. The administration of the chicken extract treated with the *Aspergillus* protease also caused a great decrease by 45 mm Hg at 2 h after administration **(Saiga *et al.*, 2003).** In a recent study, **Terashima *et al.*, (2010)** isolated and identified four peptides with ACE-inhibitory activity from the hydorlysate of boneless chicken leg meat digested with artificial gastric juice (pepsin). Among them, two peptides P1 (MNVKHWPWMK) and P4 (VTVNPYKWLP) were identified as the peptides encrypted in myosin heavy chain. The IC_{50} values of P1 and P4 were determined as 228 and 5.5 μM, respectively, and they were suggested as novel peptides from chicken.

Collagen and gelatin extracted from animal sources have also been shown to be a good source of antihypertensive peptides by enzymatic digestion. The ACE-inhibitory activity described for collagen and gelatin hydrolyzates and peptides may be related to the high concentration of hydrophobic amino acids, as well as to high proline levels **(FitzGerald, Murray, Walsh, 2004).** Collagen extracted from chicken legs (which are the yellow keratin parts containing a nail) was hydrolyzed with various enzymes, and the ACE-inhibitory activity of each hydrolyzate was determined. The filtrate of this hydrolyzate obtained by ultrafiltration with a molecular-weight cutoff of 3 kDa showed a stronger activity (IC_{50} of 130 μg/ml) than the fractionated one. Further, when the filtrate was administered to SHR, a decrease in their blood pressure was observed after 2 h of administration, and a significant decrease in blood pressure (-50 mm Hg) was observed after 6 h **(Saiga *et al.*, 2008).** In a bovine gelatin hydrolyzate, seven small peptides with ACE- inhibitory activity were identified with IC_{50} range from 6.4 to 2500 μM and resistance toward gastrointestinal and mucosal enzymes *in vitro*. Further, the hydrolyzate of bovine gelatin thermolysin showed a marked blood pressure lowering effect in SHR with a maximum decrease of 17 mm Hg in SBP, observed 6 h after administration of 300 mg/kg BW; however, the unhydrolyzed gelatin showed no effect in SHR **(Herregods *et al.*, 2011).**

It can be concluded that meat and poultry and their products are a valuable source of several peptides with various biological activities. However, although the consumption of meat products is varies and affected by many factors such as social, economic and political influences, religious beliefs and geographical differences worldwide, but they are important ingredient in

human diet. Therefore, the bioactive efficiency of peptides with antihypertensive activity derived from meat and poultry should be investigated within further research in human trials. Moreover, development of techniques for isolation of bioactive peptides from the animal tissue with high purity is also needed. Further research for purposes of investigating the stability and safety of these peptides in long terms should also be performed.

EGG PROTEIN-DERIVED HYDROLYZATES AND PEPTIDES

Egg is a rich source of protein and other nutrients and an important ingredient of human diet. A number of bioactive peptides with antihypertensive activity and other biological effects have been isolated and identified from egg proteins. Ovalbumin was hydrolyzed by pepsin, trypsin, chymotrypsin, and thermolysin. Among the digests obtained, the peptic digest exerted the most potent ACE-inhibitory activity with IC_{50} value of 45.3 μg/ml. In addition, six peptides were identified from the digest and showed activity *in vitro*; however, some of them showed no effect in SHR (**Fujita *et al.,* 2000).** Oligopeptides obtained by hydrolyzing chicken egg yolks showed ACE-inhibitory activity. They also were orally administered at 20, 100 and 500 mg/kg BW to SHR for 12 weeks and the results revealed that the administered oligopeptides suppressed the development of hypertension at all dosages. In addition, after 12 weeks at 500 mg/kg BW, the values for systolic, mean, and diastolic blood pressure were approximately 10% less in SHR administered than control (**Yoshiia *et al.,* 2001)**. Furthermore, the hydrolysis of crude egg white with pepsin, trypsin, and chymotrypsin produced peptides with ACE-inhibitory activity were mainly derived from the proteolysis of ovalbumin. Among the identified peptides, two novel sequences with IC_{50} values of 6.2 and 4.7 μM were identified (**Miguel *et al.,* 2004).** Once

again , **Miguel *et al*. (2005)** isolated and identified several active peptide sequences from ovalbumin using tandem mass spectrometry, peptide synthesis, and confirmation of the ACE-inhibitory. In addition, the evaluation of the antihypertensive effect of the hydrolyzate of egg white proteins and of some synthetic peptide sequences, with strong ACE-inhibitory activity, revealed that they efficiently reduced blood pressure in SHR**.**

The impact of a simulated gastrointestinal digestion on the stability and activity of two bioactive peptides that derived from ovalbumin by enzymatic hydrolysis and identified as YAEERYPIL (IC_{50} of 5.4 µg/ml) and RADHPFL (IC_{50} 5.3 µg/ml) was evaluated. The results showed that both peptides were digested in the gastrointestinal tract and their ACE-inhibitory activity considerably decreased upon digestion (IC_{50} of 446 and 521µg/ml after digestion, respectively). The antihypertensive activity of the end products of the gastrointestinal hydrolysis, YAEER, YPI, and RADHP, was evaluated in SHR. The fragments YPI and RADHP significantly decreased blood pressure, 2 h after administration, at doses of 2 mg/kg BW. It has also been suggested that because of their high effect *in vivo* and low ACE-inhibitory activity (IC_{50}> 1000 µg/ml), therefore, they probably exert their antihypertensive effect through mechanisms other than ACE-inhibitory mechanism (**Miguel *et al*. (2006).** In a recent study, **Garcia-Redondo *et al*. (2010)** investigated vascular effects of egg white-derived peptides in resistance arteries from rats. They have concluded that these peptides could reduce the vascular resistance and be used as functional ingredients in the prevention and/or treatment of hypertension and other associated disorders. They also suggested that presence of Arg or Tyr at the N-terminal position may be important to produce the vasodilator effect in vascular bed.

The effect of cooking methods on the production of ACE-inhibitory peptides from egg was also studied. Boiled or fried eggs (in the forms of whites, yolks, and whole eggs) were digested by gastrointestinal tract proteases at simulated gut conditions. The results revealed that fried egg digests showed more potent activity than those of boil\ed egg digests and the fried whole egg protein digest showed activity with IC_{50} value of 0.009 mg/ml (**Majumder and Wu, 2009).** A recent study was aimed to explore the rationale behind the selection of conditions for the production of potent ACE-inhibitory peptides from egg proteins. Based on *in silico* digestion and quantitative structure and activity relationship model prediction, thermolysin-pepsin digestion of ovotransferrin was chosen as the best condition due to the presence of three potent peptides were identified as IWR, LKP and IQW. Further study showed that sonication or reducing agent pre-treatments could improve the activity of hydrolyzates over 20 times and the predicted peptides were successfully released from sonication-treated ovotransferrin hydrolyzate **(Majumder and Wu, 2010).** In another study, ACE-inhibitory peptides with sequences identified as DHPFLF, HAEIN and QIGLF were isolated from egg white protein and investigated further for their stability in gastrointestinal solution and for changes in their secondary structure in solution mixtures. QIGLF exhibited the highest activity (IC_{50} of 75 µM) and was resistant to digestion by proteases of the gastrointestinal tract **(Yu *et al.*, 2011).** Furthermore, egg protein hydrolyzates produced with non-gastrointestinal enzymes (thermolysin and alcalase) showed significantly higher ACE-inhibitory activity than that of hydrolyzates produced with gastrointestinal enzymes (pepsin and pancreatin). ACE-inhibitory activity significantly correlated with the amino acid composition, especially the proportion of positively charged amino acid. It has also been suggested that understanding the relationship between the

bioactivities and physicochemical properties of the hydrolyzates/fractions is important to facilitate the development technologies for preparing fractions with improved bioactivities **(You and Wu, 2011).**

Low molecular weight fraction sourced from egg white protein hydrolyzate by gel filtration showed ACE-inhibitory activity. Among the fractions, a novel peptide (RVPSL) with IC_{50} value of 20μM was identified **(Liu *et al.*, 2010).** Furthermore, hydrolyzate of hen egg white lysozyme protein was found to exhibit a potent ACE- inhibitory activity with an IC_{50} value of 15.6±1.4 μg/ml. In addition, three novel ACE- inhibitory peptides identified as MKR, RGY and VAW with IC_{50} values of 25.7±0.2, 61.9±0.1 and 2.86±0.08 μM, respectively, were isolated from the hydrolyzate **(Rao *et al.*, 2011).** In addition, peptides with ACE-inhibitory activity were isolated from hen egg white lysozyme and characterized. The most effective peptide was identified as FESNFNTQATNR (MW: 1428.6 Da) with IC_{50} value of 0.03 mg/ml and it was evaluated as an uncompetitive inhibitor against ACE **(Asoodeh *et al.*, 2012)**. From duck egg white protein, **Naknukool *et al.* (2011)** isolated potent ACE-inhibitory hydrolyzates identified as duck basic protein small 1 (dBPS1) and 2 (dBPS2) with IC_{50} values of 22.5 and 49.6 μg/ml, respectively. Further, the most potent ACE-inhibitory peptide was a nanopeptide (EKKGFCAGY) from dBPS1 and an octapeptide (KYCPKVGY) from dBPS2.

Egg is considered as one of foods that are available to large population with a moderate cost worldwide. It is also one of the best sources of protein with high biological value. Many hydrolyzates and peptides with antihypertensive activity could be released from egg proteins. However, their activity in human body as well as their safety and bioactive stability should be clarified in further research.

ROYAL JELLY PROTEIN-DERIVED HYDROLYZATES AND PEPTIDES

Royal jelly is composed mainly of proteins, sugars, lipids, free amino acids, vitamins, and minerals. It has been demonstrated to possess a number of pharmacological activities and is widely used in commercial medical products, health foods, and cosmetics in many countries **(Guo *et al.*, 2009).** ACE-inhibitory activity of royal jelly protein was newly observed for pepsin hydrolyzate with IC_{50} value of 0.358 mg /ml, and the subsequent trypsin and chymotrypsin hydrolyzate with IC_{50} value of 0.099 mg/ml. In addition, single oral administration of gastrointestinal royal jelly hydrolyzate at dose of 1000 mg/kg BW in 10-week SHR resulted in a significant reduction in SBP (-22.7 ± 3.6 mm Hg) at 2 h **(Matsui *et al.*, 2002).** It has also been estimated that Protease N treated Royal Jelly and its derived peptides identified as IY, VY, IVY showed ACE-inhibitory activity and were also found to have an antihypertensive effect in SHR after repeated oral administration for 28 day **(Tokunaga *et al.*, 2004)**. A recent study has shown that 7 peptide fractions from royal jelly protein hydrolyzate caused antihypertensive effect in SHR after oral and intravenous injection administration at different doses. Further, the antihypertensive effect of royal jelly protein hydrolyzate was dependent on the Molecular weights of its ACE-inhibitory peptides and the time required to digest them. However the unhydrolyzed royal jelly protein showed no effect in rats **(Takaki-Doi *et al.*, 2009).**

From literature data, it can be concluded that although royal jelly proteins were found to contain hydrolyzates and peptides with ACE-inhibitory activity and antihypertensive effect as well as it is well known to posses several biological activities, but there is a fact that royal jelly is

produced in a small amounts and that may makes them more expensive and not available to large number of people compared with other animal food sources. In addition, the bioavailability and effectiveness of these identified peptides should be investigated in human. Moreover, other honeybee products such as honey and pollen grains can be studied in terms of potential antihypertensive activity and other health benefits.

MICROBIAL PROTEIN-DERIVED HYDROLYZATES AND PEPTIDES

The yeast *Saccharomyces cerevisiae* has been extensively studied and is considered as one of the most important and useful yeasts in the food industry. In one study, **Kim, Lee *et al.* (2004)** measured the ACE-inhibition rate of cell-free extracts from number of yeasts. The inhibitory activity of the extracts was found in rang of 11.6-42.1% and the highest activity of 42.1 % was exerted by *Saccharomyces cerevisiae yeast extract.* Therefore, *S. cerevisiae* was selected for further studies and its cultural conditions for maximum ACE-inhibitor production were investigated. The results showed that maximal cell growth was reached at 48 h of cultivation, whereas maximal production of the ACE-inhibitor was obtained at 24 h of cultivation at 30C and the inhibitory activity was increased by about 1.5 times after treatment of the cell-free extract with pepsin. After the purification with ultrafiltration, Sephadex G-25 column chromatography, and reverse-phase HPLC, an active fraction with an IC_{50} of 0.07 mg/ml was obtained. The purified peptide was a novel decapeptide with amino acid sequence identified as YDGGVFRVYT and competitively inhibited ACE. Further, SBP of SHR was decreased from 192 to 161 mm Hg after 2h of the identified peptide administration at a dose of 1 mg/kg BW. In

another recent study done by **Jang *et al.* (2011b)**, a cell-free extract of *Saccharomyces cerevisiae* containing ACE-inhibitory peptide was treated in a successive simulated gastric-intestinal bioreactor (step 1: amylase digestion, step 2: gastric fluid digestion, step 3: intestinal fluid digestion) to illustrate the absorption pattern of antihypertensive ACE-inhibitory peptide, and the ACE-inhibitory activities of each step were determined. The results showed that total ACE-inhibition rate of digests resulted from step 1, step 2, and step 3 were 55.96%, 80.09%, and 76.77%, respectively. In addition, the peptide sequence of each step was analyzed by MS/MS spectrophotometry and eleven types of peptide sequences were produced and conserved in each step, and new peptides including RLPTESVPEPK were identified in step 3**.**

Although research studies about antihypertensive activity of peptides derived from microbial protein sources are limited, but they were found to show ACE-inhibitory activity and antihypertensive effect in animal models comparable with that of peptides derived from major food sources. Therefore, further research for purpose of isolation and identification of peptides with biological activities from different microbial sources should be conducted.

CEREAL GRAIN AND SEED PROTEIN-DERIVED HYDROLYZATES AND PEPTIDES

Cereal grain foods are now receiving a specific attention by scientists and researchers to the importance in terms of their beneficial impact on health. There is strong epidemiological evidence that whole-grain cereals protect the body against age-related diseases such as diabetes, cardiovascular diseases and some cancers **(Fardet *et al.*, 2008).** It has been reported that numerous studies have suggested an inverse relation between daily servings of whole-grain foods

and incident hypertension among women (**Wang *et al*, 2007).** In addition, an independent inverse association between intake of whole grains and incident hypertension was also found in men. It has been suggested that bran may play an important role in this association (**Flint *et al*., 2009)**. Recently The US Department of Agriculture previous nutritional guidelines put grains and grain products at the base of the food guide pyramid to emphasise grains or grain product consumption as part of normal diet for optimal health **(USDA, 2000, 2005).**

From the literature data summarised in Table 3, it can be observed that several hydrolyzates and peptides with ACE-inhibitory activity have been isolated and identified from different types of cereal grains, pseudocereals, and oil seeds**.** Some of these hydrolyzates and peptides were also found to have antihypertensive effect in hypertensive rats. In addition, the antihypertensive effect of whole-grain foods consumption was investigated. In one study**,** **Saltzman *et al*., (2001)** found that a hypocaloric diet containing oat (*Avena sativa*) consumed over 6 weeks resulted in greater improvements in SBP and lipid profile than did a hypocaloric diet without oat in adults. In another study, **Pins *et al.*, (2002)** documented that consumption of whole-grain wheat cereal has been found to be associated with improved blood pressure control and decreased doses of antihypertensive medications in individuals with hypertension. More recent study by **Tighe *et al*. (2010)** assessed the effects of consumption of 3 daily portions of whole-grain foods (provided as only wheat or a mixture of wheat and oats) on markers of cardiovascular disease risk in relatively high-risk individuals. The results showed that SBP and pulse pressure were significantly reduced by 6 and 3 mm Hg, respectively, in the whole-grain foods groups compared with the control one.

Albumin 1 and globulin fractions of amaranth grains were found to have no ACE-inhibitory activity; however, their alcalase hydrolyzates showed activity **(Tovar-Pérez *et al.*, 2009).** In contrast, **Fritz *et al.* (2011)** found that the ACE-inhibitory activity of the non-hydrolyzed isolate of amaranth protein was higher than 0% ($P < 0.01$). They also found that there was a difference between the effects obtained with oral administration and those produced by direct intragastric administration of the amaranth grain protein hydrolyzate since a dose of 2.4 g/kg BW that reduced blood pressure was twice as effective when injected directly to the stomach of SHR.

Effect of processing and preparation conditions on cereal grain and seed protein-derived hydrolyzates and peptides

As for other food types, the antihypertensive activity of hydrolyzates and peptides derived from cereal grains and seeds was found to be affected be some treatments and conditions. **Kim, Whang, Suh (2004)** reported that the ultrafiltration treatment was an effective method for the enhancement of ACE-inhibitory activity of corn gluten hydrolyzate without increasing bitterness and with improvement of functional properties, such as emulsifying and foaming properties. In a recent study, **Jia *et al*. (2010)** investigated the effects of ultrasonic treatment during proteolysis on kinetic characterisation of the hydrolysis of defatted wheat germ protein, and on ACE-inhibitory activity of the hydrolyzate. Analysis of ACE-inhibitory activity indicated that ultrasound during enzyme treatments had less effect on the ACE-inhibitory activity, while ultrasonic pretreatment caused a 21.0–40.7% increase in ACE-inhibitory activity

of the hydrolyzate. In addition, amino acid composition showed that the hydrolyzate of ultrasound-pretreated defatted wheat germ protein had more hydrophobic amino acids and proline, which play important roles in the activities of ACE-inhibitory peptides, than that without ultrasonic pretreatment. Furthermore, **Yang *et al.* (2011)** reported that the peptides concentration and ACE-inhibitory activity in wheat germ were found to be affected by some factors such as incubation time, temperature, initial pH, and liquid to solid ratio of incubation medium.

ACE-inhibitory peptides present in rice protein hydrolyzate were found to be relatively resistant to digestion by gastrointestinal enzymes, as ACE-inhibitory activity of the hydrolyzate was changed slightly after treatment with digestive enzymes **(Li *et al.*, 2007).** However**,** the hydrolyzate produced by thermolysin from oat protein isolate under high E/S ratio (3%) and short time (20 min) conditions showed slightly stronger activity than hydrolyzate produced using a low E/S ratio (0.1%) and long time (120 min) process. In addition, the ACE-inhibitory activity was stable to prolonged hydrolysis time as well as simulated gastrointestinal digestion. Further fractionation of the hydrolyzates by size did not improve ACE-inhibition, implying that a simple process of hydrolysis would be sufficient to produce functional products **(Cheung *et al.*, 2009).**

The unhydrolyzed proteins of flaxseed were found to have no ACE-inhibitory activity and the activity was greater in hydrolyzates with lower degree of hydrolyzation (DH) and higher peptide chain length (PCL) than those with higher DH and lower PCL **(Marambe *et al.*, 2008).** However, the ACE-inhibitory activity and human recombinant rennin was found to be dependent on the ability of the different proteases used for hydrolysis to release bioactive peptide sequences

from flaxseed proteins rather than the protein yield of low-molecular-weight peptides **(Udenigwe *et al.*, 2009)**. In addition, it has been established that initial alcohol washing of the canola meal could be a useful step in increasing protein content of the ACE-inhibitory hydrolyzate **(Wu *et al.*, 2009).**

From the literature data reviewing above, it can be seen that cereal grains such as wheat, maize, rice, barley, sorghum, oats, and rye are important source of energy and protein for humanity, especially for people who are living in developing countries and rural poverty regions worldwide. In addition, several research studies found that whole grains and their fractions provide many compounds and nutrients with various biological activities and health benefits. Although antihypertensive activity of peptides derived from cereals and seeds is a slight lower than that of peptides derived from animal food sources such as milk and fish, but cereal grains could be considered as important source of bioactive peptides. However, it is not easy to isolate protein with high purity from cereals sources because of their high content of starch. Therefore, developed techniques and methods for isolation and purification of proteins and bioactive peptides from cereals are needed. As for other food derived peptides, these peptides also need to be tested in animal models and human trials.

LEGUMES, BEANS AND PEANNUT PROTEIN-DERIVED HYDROLYZATES AND PEPTIDES

Legumes are widely grown in the world following cereals, and they are known to be good sources of dietary protein. Therefore, health benefits of legumes were investigated in

experimental, epidemiological and clinical studies. **Yust *et al.*, (2003)** reported that treatment of Chickpea (*Cicer arietinum L.*) legumin with alcalase yielded a hydrolyzate with ACE-inhibitory activity (IC_{50} of 0.18 mg/ml). Further, fractionation of this hydrolyzate by reverse phase chromatography afforded six inhibitory peptides with IC_{50} values range 0.011-0.021 mg/ml. In addition, all these peptides were found to contain the amino acid methionine and also rich in other hydrophobic amino acids. **Barbana and Boye (2010)** documented that the non-hydrolyzed chickpea and pea protein concentrates showed no ACE- inhibitory activity, whereas all enzymatic hydrolyzates displayed activity and the type of enzyme used for hydrolysis affects the ACE-inhibitory activity. For example, chickpea protein digest hydrolyzed by *in vitro* gastrointestinal simulation showed higher ACE-inhibition (IC_{50} of 140 μg/ml) compared to its digests obtained by alcalase/flavourzyme (IC_{50} of 228 μg/ml) or papain (IC_{50} of 180 μg/ml). Once again, **Boye *et al.*, (2010**) found that ACE-inhibitory activity of red lentil protein hydrolyzates varied as a function of the protein fraction. The total lentil protein hydrolyzate possessed the highest activity (IC_{50} of 111 mM), followed by the enriched legumin (IC_{50} of 119 mM), albumin (IC_{50} of 127 mM) and vicilin (IC_{50} of 135 mM) fractions, respectively**.**

As for chickpea, also Pea protein is mainly composed of the seed storage globulins and the heterogeneous albumins that comprise proteins with a biological function in the seed. Globulins are products of a multi-gene family and include legumin, vicilin and convicilin **(Page and Duc, 1999; Guéguen, 2000)**. *In vitro* gastrointestinal digestion of pea protein produced a digest with high ACE-inhibitory activity (IC_{50} of 0.076 mg/ml). The high activity was observed after the simulated stomach phase and augmented slightly in the simulated small intestine phase

(Vermeirssen *et al.*, 2004). Further study was done on this digest and the results showed that ultrafiltration/centrifugation using a membrane with a molecular weight cut-off of 3kDa decreased the IC_{50} value to 0.055 mg/ml. However, further fractionation by reverse phase HPLC gave IC_{50} values as low as 0.016 mg/ml **(Vermeirssen *et al.*, 2005).** Furthermore, three dipeptides were identified as IR, KF and EF from pea protein and showed strong inhibitions (IC_{50} values <25 mM) of ACE and renin and the potency against ACE was higher than against rennin **(Li *and* Aluko, 2010).** In a recent study, the blood pressure lowering effect of a pea protein hydrolyzate (PPH) that contained <3 kDa peptides, isolated by membrane ultrafiltration from the thermolysin digest of pea protein isolate, was examined using different rat models of hypertension as well as hypertensive human subjects. The PPH showed weak *in vitro* activities against renin and ACE with inhibitory activities of 17 and 19%, respectively, at 1 mg/ml test concentration. However, oral administration of the PPH to SHR at doses of 100 and 200 mg/kg BW led to a lowering of hourly SBP, with a maximum reduction of 19 mm Hg at 4 h. In addition, orally administered unhydrolyzed pea protein isolate had no blood pressure reducing effect in SHR, suggesting that thermolysin hydrolysis may have been responsible for releasing bioactive peptides from the native protein. On the other hand, oral administration of the PPH to the Han:SPRD-cy rat (a model of chronic kidney disease) over an 8-week period led to 29 and 25 mm Hg reductions in SBP and DBP, respectively. However, in a 3-week randomized double blind placebo-controlled crossover human intervention trial (7 volunteers), significant ($p < 0.05$) reductions (over placebo) in SBP of 5 and 6 mm Hg were obtained in the second and third weeks, respectively, for the PPH group **(Li *et al.*, 2011).** From cowpea (*Vigna unguiculata L. walp*), **Segura-Campos *et al.* (2011)** isolated peptides with ACE-inhibitory activity after

modification by the food-grade microbial enzyme Flavourzyme. Further, purification of the peptides by ultrafiltration, gel filtration chromatography and RP-HPLC produced fractions with different activities, all of which were much higher than the source hydrolyzate and the <1 kDa ultrafiltered fraction exhibited the highest activity. In addition, the observed amino acid composition suggested a substantial contribution of hydrophobic residues to the peptides' inhibitory potency.

Mung bean protein isolates were also hydrolyzed by alcalase and neutrase and the highest ACE-inhibitory activity recorded was for a hydrolyzate generated by alcalase (IC_{50} of 0.64 mg/ml) after 2 h of hydrolysis. A significant decrease in SBP (-30.8 mm Hg) was observed in SHR after 6 h following a single oral administration of this hydrolyzate at a dose of 600 mg/kg BW (**Li *et al*., 2006)**. In a recent study, different dosages of mung bean raw sprout extract or dried sprout extracts and enzyme-digested sprout extracts were used in a single intragastric administration test to examine the short-term effect in SHR. The results indicated that high doses (600 mg peptide/kg BW) extracts significantly reduced SBP of the rats after administration for 6–9, 3–6 and 3–9 h, respectively **(Hsu *et al*., 2011).** Furthermore, crude protein extracts of common dry beans, dry pinto beans and green lentils were found to have range of 4.08–28.54% ACE-inhibition activities after heat treatment and *in vitro* digestion. In addition, 30-min heat treatment caused a decrease in ACE- inhibitory activity; however, 50-min heat treatment was observed to be beneficial for the release of ACE-inhibitory peptides from the three legume species. Further, *in vitro* digestion process increased ACE-inhibitory activities of the samples mainly green lentil digests (**Akıllıoglu and Karakaya, 2009)**.

Soybean and its products are promoted for their biological effects. **Lo and Li-Chan (2005**) produced many different fractionates and peptides with ACE-inhibitory activity after *in vitro* pepsin-pancreatin digestion of soy protein isolate (SPI). The soy peptides generated during pepsin digestion had a greater ACE-inhibitory activity than soy peptides after subsequent digestion with pancreatin. An IC_{50} value of 0.28 mg/ml was determined after 180 min of digestion, while no ACE-inhibitory activity was observed for the undigested SPI at 0.73 mg/ml. Chromatographic fractionation of the SPI digest resulted in IC_{50} values of active fractions ranging from 0.13 to 0.93 mg/ml. Although many of the fractions showed ACE-inhibition, peptides with lower molecular masses and higher hydrophobicities were most active. In addition, the peptides produced during the initial stages of digestion had a greater ACE-inhibitory activity than that of peptides produced during the later stages. **Chiang *et al*. (2006)** reported that when alcalase, flavourzyme, chymotrypsin, pepsin or trypsin were used as the enzyme source to produce protein hydrolyzate with ACE-inhibitory activity, the most active hydrolyzate was obtained by alcalase hydrolysis of soy protein isolate (SPI). Therefore, alcalase was selected for further study on optimization of hydrolysis conditions. The optimum conditions for hydrolysis of SPI by alcalase to produce the lowest IC_{50} value were: E/S = 0.01, hydrolysis temperature of 50ºC, pH 9.0 and hydrolysis time 6 h. Under these conditions, the IC_{50} value of SPI was significantly reduced from 66.4 to 0.67 mg/ml. In addition , ultrafiltration of alcalase hydroylsate by different membranes with molecular weight cut-offs of 1–30kDa resulted in a significant increase of the inhibitory activity**.** Furthermore, **Zhang *et al*. (2006**) concluded that Douchi, a soybean product originating in China, was found to produce ACE-inhibitors with the

potential to lower blood pressure and the ACE-inhibitory activities were improved following the fermentation**.** However, **Rho *et al.* (2009**) isolated a novel ACE-inhibitory peptide with amino acid sequence identified to be LVGGS with IC_{50} value of 22 mg/ml from a fermented soybean extract through a five-step purification procedure. A recent study done by **Fung and Liong (2010)** indicated that probiotic-fermented soy whey showed *in vitro* antihypertensive activity.

Peanut (*Arachis hypogaea*), an important oil and food crop, is currently grown on approximately 17 million hectares worldwide. It is the third major oil seed crop of the world next to soybean and cotton. Peanut valued for its oil content is also rich in protein (25–32%) **(Jimsheena and Gowda, 2011)**. Defatted raw and roasted peanut flour were hydrolyzed with alcalase or sequentially with pepsin and pancreatin, and then the hydrolyzates were fractionated by RP-HPLC and tested for hypotensive potential. The results showed that fractions from the alcalase digestion of raw peanut exhibited IC_{50} range of 8.7–122 mg/ml, and those from roasted flour exhibited range of 12–235 mg/ml. However IC_{50} range of 7.9–65.9 mg/ml, and 11–36 mg/ml for raw and roasted peanut, respectively, from the pepsin–pancreatin system were observed. Therefore, the raw peanuts digests possess higher inhibitory activity than the roasted and the alcalase system produces significantly more potent antihypertensive peptides than those from the pepsin–pancreatin one **(Quist *et al.*, 2009).** In a study, the peptide P7 with ACE-inhibitory activity and an amino acid sequence of CVTPALR was identified in peanut protein isolate and peanut polypeptides (**Liu *et al.*, 2009)**. However, it has been established that as degrees of hydrolysis increased, ACE-inhibitory activity of peanut protein hydrolyzate increased **(Jamdar *et al.*, 2010)**. Furthermore, in a recent study, physiological proteases pepsin, trypsin,

chymotrypsin and pancreatin were used to release peptides from arachin/peanut proteins. The results revealed that degree of proteolysis and ACE-inhibitory activities were higher with pepsin. In addition, three peptides purified from the simulated gastric fluid digests were synthesized. Among them, the pentapeptide, NAQRP was the most potent with an IC_{50} of 32 μM. Further, molecular docking simulation with human tACE indicated that in addition to a favourable C-terminal proline residue, the length of the peptides advocate ACE-inhibitor potency (**Jimsheena and Gowda, 2011)**.

Now we come to the conclusion that grain legumes, soybean, and peanut have had multiple uses in human diet and are considered as protein-rich source and that explain the interest of nutritionists and food scientists to investigate their biological activities and potential health benefits. Based on data mentioned above, the antihypertensive activity of peptides derived from legumes proteins could be comparable with that of other plant food sources such as cereal grains; therefore, they could be recommended as an important source of bioactive peptides and functional components.

FRUITS AND VEGETABLES PROTEIN-DERIVED HYDROLYZATES AND PEPTIDES

Fruits and vegetables are an important component of a healthy diet and if consumed daily in sufficient amounts could help prevent major diseases such as cardiovascular disease and certain cancers. It has been reported that low fruit and vegetable intake is estimated to cause about 31% of ischemic heart disease and 11% of stroke worldwide **(WHO, 2002).** In one study, the protease alcalase was found to be very well suited generate hydrolyzates enriched with ACE-

inhibitory peptides from apricot kernel protein. A 60-min alcalase protein hydrolyzate fraction with a molecular weight mainly ranged from 200 to 900 Da, showed the highest ACE-inhibitory activity (82 %). Moreover, ultrafltration through 1-kDa cutoff membranes enhanced the ACE-inhibitory activity (**Zhu, 2010)**. Once again, in another study, apricot almond meal was hydrolyzed simultaneously with Neutrase and N120P proteases. The hydrolyzate was fractionated into three ranges of molecular weight (AP-I, [5 kDa; AP-II, 1–5 kDa; AP-III, 1 kDa) using an ultrafiltration membrane bioreactor system. The AP-III brought a high ACE-inhibitory activity with IC_{50} value of 0.138 mg/ml and the content of hydrophobic amino acid of AP-III was 50.08%. In addition, multiple dose oral administration (100, 400, 800 mg/kg BW) to SHR led to a significant decrease in blood pressure for AP-III (**Wang *et al.*, 2011)**.

Proteins isolated from potato tubers (*Solanum tuberosum*) at different physiological states and by-products from the potato industry were used to evaluate their antihypertensive activity. The results showed that the ACE-inhibitory potencies of hydrolyzates were high with IC_{50} values range 0.018-0.086 mg/ml dray matter and the by-product fractions showed ACE-inhibition also before hydrolysis (**Pihlanto *et al.*, 2008)**. Another study documented that autolysis of protein isolates from vascular bundle and inner tuber tissues of potato enhanced the ACE-inhibitory activity. The highest inhibitory activity with IC_{50} value of 0.36 mg/ml was measured in tubers after 5-6 months of storage prior to sprouting. The rate of ACE-inhibition was positively correlated with protease activity in tuber tissues **(Makinen *et al.*, 2008).** From sweet potato protein, peptides with ACE-inhibitory activity were also purified by absorption chromatography and preparative HPLC after hydrolysis by different proteases. The amino acid sequences of isolated peptides were identified as ITP, IIP, GQY and STYQT with IC_{50} values of 9.5, 80.8,

52.3 and 300.4μM, respectively. Also the sweet potato peptides showed an anti-hypertensive effect in SHR. Significant differences between sweet potato peptide-administered rats and control rats were recognized 4 and 8 h after administration in the 500 mg/kg BW administered group and 8 h after administration in the 100 mg/kg BW administered group (**Ishiguro *et al.*, 2012)**.

In a study done in Japan**, Yang *et al*. (2003)** isolated four new ACE-inhibitory peptides identified as MRWRD, MRW, LRIPVA, and IAYKPAG from the pepsin-pancreatin digest of Spinach Rubisco with IC_{50} values of 2.1, 0.6, 0.38, and 4.2 μM, respectively. Further, MRW, MRWRD and IAYKPAG exhibited an antihypertensive effect after oral administration to SHR. Maximal reduction occurred 2 h after oral administration of MRW, whereas MRWRD showed maximal decrease 4 h after oral administration at doses of 20 and 30 mg/kg BW, respectively. IAYKPAG also exerted antihypertensive activity after oral administration at the dose of 100 mg/kg BW, giving a maximum decrease 4 h after oral administration. IAYKP, IAY, and KP, the fragment peptides of IAYKPAG, also exerted antihypertensive activity. However, LRPVIA did not show any antihypertensive effect at a dose of 100 mg/kg BW despite its potent ACE-inhibitory activity. **Yang *et al.*, (2004)** once again studied the antihypertensive activities of pepsin and pepsin pancreatin digests of spinach leaf protein as well as the undigested one. The results revealed that pepsin and pepsin pancreatin digests have potent ACE-inhibitory activity with IC_{50} values of 56 and 120 μg/ml. In addition, both digests of leaf protein have antihypertensive effects after oral administration to SHR with minimum effective doses of 250 and 500 mg/kg BW. The maximum antihypertensive effect for the pepsin digest (-11.1 mm Hg)

was observed 4 h after oral administration, while for the pepsin-pancreatin digest, the maximum effect (-7.2 mm Hg) was observed 2 h after oral administration. However, undigested spinach leaf protein did not exert any significant antihypertensive effect after oral administration to SHR at dose of 500 mg/kg BW, but 4 h after oral administration of undigested protein at the dosage of 1000 mg/kg BW there was a slight decrease in blood pressure (-5 mm Hg), meaning that a much higher dosage of undigested leaf protein may exert a significant antihypertensive effect.

Several mushroom species are known to have health benefits and therapeutic properties such as immunomodulating, anticancer, antioxidants, antihypertensive, cholesterol-lowering, liver protection, anti-inflammatory, antidiabetic, antiviral, antimicrobial. Therefore mushrooms have the potential to be used as dietary supplements or in the fortification of foods as functional compounds **(Shamtsyan, 2010)**. A number of ACE-inhibitory peptides have been isolated from edible mushrooms during the last decade including *Grifola frondosa (***Choi *et al.,* 2001)**, *Mycoleptodonoides aitchisonii (***Sakamoto *et al.,* 2001),** and *Tricholoma giganteum (***Lee *et al.,* 2004)**. Furthermore, in a recent study, **Jang *et al.,* (2011a)** described the characterisation of two purified ACE-inhibitors with IC_{50} values of 0.46 and 1.14 mg/ml obtained from the fruiting body of *Pleurotus cornucopiae*. In addition, water extracts of *P. cornucopiae* fruiting body showed a clear antihypertensive effect in SHR at a dose of 600 mg/kg BW (-50 mm Hg) after 2 h of administration.

It can be concluded that although fruits are well known to contain several nutrients and compounds with biological effects and health benefits such as minerals, vitamins, and antioxidants, but they are not considered as a good source of protein and that explain scarcity of

research carried about bioactive peptides from fruit sources. However, some kinds of vegetables such as mushroom, potato, spinach were found to have peptides with ACE-inhibitory activity and antihypertensive effect. Moreover, research studies that have been carried to isolate and identify peptides from vegetables are limited compared with other food kinds; thus, further studies are needed to identify new bioactive peptides. In addition, there is a need to human trials to verify efficiency of peptides that have been found to have activity *in vitro* and/or in animal models.

FOOD PEPTIDES COMPARED WITH PHARMACEUTICAL DRUGS

In a number of studies, derived food hydrolyzates and peptides were compared with the activity of pharmaceutical drugs used for hypertension treatment. However most of studies showed that pharmaceutical drugs have higher ACE-inhibitory activity and antihypertensive effect at low doses than that of hydrolyzates and peptides derived from various foods such as milk **(Contreras *et al.*, 2009; Miguel *et al.*, 2009; Rousseau-Ralliard *et al.*, 2010)**, fish **(Lahogue *et al.*, 2010)**, chicken **(Terashima *et al.*, 2010)**, corn **(Huang *et al.*, 2011)**, soy bean **(Lo and Li-Chan, 2005)**. On the other hand, there are some few studies found that food hydrolyzates and peptides have higher or similar antihypertensive effect in SHR with that of pharmaceutical drugs used for hypertension treatment. For example, in one study it has been found that SBP and ACE- activity of SHR were significantly ameliorated in fermented milk peptides (10 mg/kg BW/day) group after oral administration compared to that of captopril (CAP, 50 mg/kg BW/day) one and with no side effects **(Kim *et al.*, (2010)**. In another study, a single

oral administration (10 mg/kg BW) of peptide with amino acid sequence identified as PTFP from tuna frame showed a strong suppressive effect on SBP of SHR and this antihypertensive activity was almost similar with that of captopril and no side effects were observed after administration (**Lee, Qian, Kim, 2010).** In addition, **Aihara *et al.* (2005)** reported that administration of supplementary tablets containing powdered *Lactobacillus helveticus*-fermented milk with two tripeptides (VPP and IPP), which have ACE-inhibitory activity, to subjects with high-normal blood pressure or mild hypertension resulted in a significant decrease in blood pressure without any adverse effects**.** However, although food hydrolyzates and peptides with ACE-inhibitory activity and antihypertensive effect and based on these observations are claimed to be safe and have no side effects compared with pharmaceutical drugs because they are derived naturally, but actually it should be noticed that they may have no observable side effects because of their low antihypertensive activity compared with pharmaceutical drugs. Therefore, efficiency, safety, and potential side effects of food derived peptides should be investigated extensively in animal model and human trials to clarify these observations. Moreover, it is well known that designing and development of an effective oral delivery system for peptide and protein drugs remain a challenge to pharmaceutical scientists and require a thorough understanding of their physicochemical properties, such as molecular weight, hydrophobicity, ionization constants, and pH stability, as well as biological barriers that restrict protein and peptide absorption from the gastrointestinal tract, including pH variability, enzymatic degradation, and membrane efflux **(Mahato *et al.*, 2003)**. Furthermore, there is no clear relation between the *in vitro* and *in vivo* antihypertensive activity of food peptides has been established and mechanism of action of their effect is not well understood. Therefore with respect to high bioavailability and effectiveness of

hypertension pharmaceutical drugs in human body at available doses and in easy administration forms, these findings need more clarifications through further research. In other words, it is early at present for claiming that food peptides could be safe and effective alternatives to synthetic pharmaceutical drugs used for hypertension treatment.

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

This review presents the recent studies carried out to date for purposes of isolation, identification, purification and evaluation of hydrolyzates and peptides with antihypertensive activity from food proteins. Based on the research studies carried out to date, it can be observed that bioactive hydrolyzates and peptides from food including milk, marine food, egg, royal jelly, cereal grains, legumes, and fruits and vegetables protein are appropriate candidates for functional food products that can be used as part of diet-related measures for the prevention or treatment of hypertension. However, milk and milk products are being the most important source of bioactive peptides with antihypertensive activity. On the other hand, there is increasing interest in bioactive peptides with antihypertensive activity from fish and shellfish and their products were observed during the recent years and they have been found to have antihypertensive peptides can be comparable with that of milk. In addition, proteins of plant food such as cereal grains, legumes, and vegetables were also found to possess bioactive peptides with antihypertensive activity. Plant foods are available to larger population and high consumed in daily diet of human compared with animal food, especially in developing countries and poverty regions worldwide. Therefore, much more attention should be paid to research in bioactive peptides from plant food

in future studies. Measurement of ACE-inhibitory activity is the most common strategy followed in the selection of antihypertensive hydrolyzates and peptides derived from food sources. However, the antihypertensive effect of the *in vitro* ACE-inhibitory peptides has not always been evaluated. In addition, some hydrolyzates and peptides were found to have potent ACE-inhibitory activity, but did not exert antihypertensive effect or showed weak effect. Thus, further research is needed to understand and verifying the efficiency of the discovered peptides in animal models and human trials. In addition, mechanisms other than ACE-inhibitory activity mechanism need to be investigated and the relation between the activity of peptides and their amino acids structure need more clarifications. Moreover, studies that have been found that food peptides showed no side effects after administration in animal models or hypertensive subjects are limited. Thus, further studies are needed to test the safety and potential adverse effects of food peptides in animal models and human trials. In addition, a potential relation between the activity of these peptides compared with pharmaceutical drugs and their potential side effects need to be clarified. In some cases parent protein isolates, other food extracts, and whole food were found to show antihypertensive activity. Therefore, studying the antihypertensive properties of whole foods and other non-protein food components is needed. Further studies should also be performed to explain the effect of interaction of peptides with other food components, as well as the effect of processing conditions on peptide formation, bioactive stability and efficiency. More human clinical trails should be conducted to obtain consistent evidence for the health effects of the antihypertensive hydrolyzates and peptides derived from food proteins. Research for purposes of designing and development of an effective oral delivery system such as encapsulation for bioactive peptides and proteins derived from foodstuffs is very important.

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Tables

Table 1. Studies on milk protein-derived hydrolyzates and peptides with ACE-inhibitory activity and/or antihypertensive effect.

Table 2. Studies on fish and shellfish protein-derived hydrolyzates and peptides with ACE-inhibitory activity and/or antihypertensive effect.

Table 3. Studies on cereal grain and seed protein-derived hydrolyzates and peptides with ACE-inhibitory activity and/or antihypertensive effect.

Table 1 Studies on milk protein-derived hydrolyzates and peptides with ACE-inhibitory activity and/or antihypertensive effect.

Protein source	Preparation	Final product	ACE-inhibitory activity	Antihypertensive effect	References
Whey	Enzymatic hydrolyzation	Hydrolyzate	N/A	Reduced SBP (-8.0 mm Hg) and DBP (-5.5 mmHg) of hypertensive subjects at dose of 20g /kg BW after oral administration for 4 weeks	Pins and Keenan (2006)
α -lactalbumin	Thermolytic digest or thermolysin catalysed	Peptides	IC_{50} rang of 1–5 μM	N/A	Otte et al. (2007)
Casein	Alcalase	Hydrolyzates	IC_{50}[a] of 47 μg/ml	N/A[d]	Zhao and Li (2009)
Casein	Pepsin	Hydrolyzates > 3000 Da and < 3000 Da	IC_{50} of 242 and 5.5 μg/ml	Reduced SBP of SHR[c] at a dose of 400 and 200 mg/kg BW after different times	Miguel et al. (2009)
Casein	Porcine pepsin A	Novel peptides	IC_{50} of 0.71, 6.58, and 20.08 μM	Reduced SBP[e] of SHR at a dose of 5 mg/ kg BW (Maximum reduction of -25 mm Hg after 6 hour of oral administration)	Contreras et al. (2009)
Whey	Fermentation by lactobacillus species	Purified fractions	IC_{50} range of 2637.8 to 5.3 μg/ml	N/A	Ahn et al. (2009)

Whey	Lactic-Fermentation with a protease(Prozyme 6) addition	Fermented whey powder	IC_{50} range of 1.18 to 0.24 mg /ml (based on fermentation time)	Reduced SBP and DBP of SHR (-22.6 and - 21.5 mm Hg) after 8 weeks of oral administration with diluted whey (12.5 mg /ml)	Chen et al. (2009)
Casein	Chemical synthesis	Synthetic peptides	IC_{50} range of 10.10 to 77.10 mM	Reduced SBP of (rang of -11.5 to -23.5 mm Hg) and DBP[f] (rang of -9.7 to -24.6 mm Hg) after oral administration at different doses and after different times	Miguel et al. (2010)
Casein	AS1.398 neutral protease	Novel peptides	IC_{50} of 54 and 21 μg/ml	Reduced SBP of SHR at doses of 10,100, and 300 mg/kg BW after different times of oral administration	Jiang et al. (2010)
αS1- and αS2-casein	Trypsin	Hydrolyzates $CH_1$and CH_2	IC_{50} of 94.8 and 64.3 μg/ml	Showed a decrease in some blood pressure parameters that was too weak to be considered	Rousseau-Ralliard et al. (2010)
Whey	Various proteases	Hydrolyzates	Highest ACE-inhibition of 54.30%	N/A	Wang et al. (2010)
Casein	Chemical synthesis	A novel peptide	IC_{50} of 20.85 μM	N/A	Zhan-li et al. (2011)
Commercial casein	Peptic hydrolysis	Peptides	IC_{50} of 0.71 and 6.58 μM	Reduced SBP of SHR at dose of 200-800 mg/ kg BW (Maximum decrease of -25 mm Hg at 6 hour after oral	Contreras et al. (2011)

				administration)	
Donkeys' milk	Gastrointestinal digest	Hydrolyzate and identified peptide	IC_{50} of 273.0 µg/ml, and 48.8 µM	N/A	Bidasolo et al. (2011)
Synthetic based on αS1 casein and ß-lactoglobulin	Chemical synthesis	Tripeptides	ACE[b] Inhibition range of 20 to 70%, the most potent peptide IC_{50} of 0.85 µM	N/A	Ren et al. (2011)
Whey	Incubation with a commercial crude aqueous extract of *C. cardunculus*	Novel Peptides	IC_{50} of 0.80, 25.2 and 13.0 µg/ml	N/A	Tavares et al. (2011b)
Whey	Incubation with a commercial crude aqueous extract of *C. cardunculus*	Peptides concentrate and its fraction (PepC and PepCF)	IC_{50} of 52.9 and 23.6 mg/ml	Reduced SBP of SHR (-17 and -21 mm Hg) at dose of 400 mg/kg BW after 4 hours of oral administration	Tavares et al. (2011c)
lactoferrin	Porcine pepsin	3 novel peptides	IC_{50} of 0.47, 56.5 and 105.3 µM	Reduced SBP of SHR at dose of 10 mg/kg BW at 1 h of administration (-18.9, -25.3, and -15.3 mm Hg)	Ruiz-Giménez et al. (2012)
Whey	Trypsin	Fractions	ACE inhibition, 64.26, 51.54, and 40.46 % At 10 mg/ml	N/A	Pan et al. (2012)

[a] $IC_{50:}$ the concentration of ACE inhibitor required to inhibit 50% of the ACE.

[b] ACE: Angiotensin-converting enzyme

[c] SHR: spontaneously hypertensive rats.

[d] N/A: not available

[e]SBP: systolic blood pressure

[f]DBP: diastolic blood pressure

Table 2 Studies on fish and shellfish protein-derived hydrolyzates and peptides with ACE-inhibitory activity and/or antihypertensive effect.

Protein source	Preparation	Final product	ACE-inhibitory activity	Antihypertensive effect	References
Salmon	Thermolysin	Hydrolyzate	IC_{50} of 27.9 µg/ml	Reduced SBP of SHR at dose of 500 and 2000 mg/kg BW (-28 and -38 mm Hg) within 4h of administration	Ono et al. (2003)
Bonito	Trypsin and/or chymotrypsin	Peptides	IC_{50}[a] of 4.5 and 8.0 mM	N/A[d]	Hasan et al. (2006)
Freshwater clam	Hot water, Protamex and Flavourzyme	Hydrolyzates	IC_{50} of 3.7 and 1045 mM	Reduced SBP and DBP[f] of SHR (-22 and -13.2 mm Hg) at dose of 5 mg/ml after 8 weeks of administration	Tsai et al. (2006)
Hard clam	Hot water extraction/ Protamex	Hot water extract , hydrlysate and identified peptide	IC_{50} of 1.090 , 0.036 mg/ml and 51 mM	N/A	Tsai et al. (2008)
Oyster	Pepsin	Peptide	IC_{50} of 66 µM	Reduced SBP of SHR[c] at doses of 20 and 100 mg/kg BW after different times of administration	Wang, Hu, Cui et al. (2008)
Pacific Hake	Incubation of fillet with 3.00% Protamex	Hydrolystae	IC_{50} of 165 µg/ml	N/A	Cinq-Mars et al. (2008)
Shark meat	Trypsin	Novel peptides	IC_{50} of 1.96, 2.68 and 1.45 µM	N/A	Wu et al. (2008)

Atlantic salmon, Coho salmon, Alaska pollack, and Southern blue whiting	Pepsin, pancreatin or thermolysin	Hydrolyzates	Various ACE inhibitory activities (dependent on source and preparation enzyme)	N/A	Nakajima et al. (2009)
Grass carp fish scale	Neutral protease	Identified peptides	IC_{50} of 0.13 mg/ml	N/A	Zhang et al. (2009)
Tilapia	Cryotin-F or Flavourzyme	Hydrolyzates and its corresponding fractions >30 kDa, 10–30 kDa, and <10 kDa fractions	ACE[b]-inhibition 62 to 73%, depend on degree of hydrolysation and type of enzyme	N/A	Raghavan and Kristinsson (2009)
Tuna liver	Various proteases	Hydrolyzates	ACE-inhibition 36%	N/A	Je et al. (2009)
Cuttlefish	Various digestive proteases	Novel peptides	IC_{50} of 6.1, 8.7, and 16.32 μM	N/A	Balti et al. (2010)
Oyster	Trypsin	Peptide	IC_{50} of 143 nM/ml	Reduced SBP[e] of SHR at a dose of 8 mg/ Kg BW after 3 and 6h of administration	Shiozaki et al. (2010)
Haruan (Channa striatus)	Proteinase K and thermolysin	Peptides	IC_{50} of 0.45 and 0.63 μM	N/A	Ghassem et al. (2011)
Northern shrimp	Comercial protein hydrolyzate	Hydrolyzate	2 methods IC_{50} of 0.075 and 0.035 mg/ml	Reduced SBP of SHR at dose of 60 mg /kg BW	Gildberg et al. (2011)
Pacific cod skin	Various proteases	Peptides	ACE-inhibition 81% and 68% at 500	N/A	Ngo et al. (2011)

			μg/ml		
Salmon skin	Alcalase and papain	Peptides	IC_{50} of 0.060 and 0.332 mg/ml	N/A	Gu et al. (2011)
Squid skin	Pepsin	Hydrolyzates	IC_{50} of 0.33 mg/ml	Reduced the arterial blood pressure of Renovascular Hypertensive Rats at dose of 200 and 50 mg/kg BW	Lin et al. (2012)

[a] $IC_{50:}$ the concentration of ACE inhibitor required to inhibit 50% of the ACE.

[b] ACE: Angiotensin-converting enzyme

[c] SHR: spontaneously hypertensive rats.

[d] N/A: not available

[e]SBP: systolic blood pressure

[f]DBP: diastolic blood pressure

Table 3. Studies on cereal grain and seed protein-derived hydrolyzates and peptides with ACE-inhibitory activity and/or antihypertensive effect.

Protein source	Preparation	Final product	ACE-inhibitory activity	Antihypertensive effect	References
Quinoa	Alcalase and ultrafiltration	Hydrolyzate	IC_{50} of 0.075 mg/ml	N/A	Aluko and Muno (2003)
Rapeseed	Various proteases	Peptides	IC_{50} of 28 and 30μM	Reduced SBP of SHR (−12.5 and −10.8 mm Hg) at doses of 12.5 and 7.5 mg/kg BW after 2h of administration	Marczak (2003)
Wheat gliadin	Acid protease	Peptide	IC_{50}[a] of 2.7 μM	Reduced SBP[e] of SHR[c] at doses of 50 and 150 mg/kg BW after 1.5, 3 and 5h of injection intraperitoneally	Motoi and Kodama (2003)
Corn	Flavourzyme	Hydrolyzate	IC_{50} of 0.18 mg/ ml	N/A	Kim, Whang, and Suh (2004)
A wholemeal wheat flour	Dough Fermentation under different conditions	Peptides fractions and identified peptide	IC_{50} of 2.9, 2.33, 2.10 mg/ml, and 336 μM.	N/A[d]	Nakamura et al. (2007)
Corn	Protease	A novel peptide	IC_{50} of 14.2 μM	Reduced SBP of SHR[c]	Yang et al. (2007)

				(-9.5 mm Hg) at doses of 50 mg/kg BW after 2h of administration	
Rice	Alcalase	Hydrolyzate and peptide	IC_{50} of 0.14 mg/ml and 18.2 μM	Reduced SBP of SHR at doses of 600 and 30 mg/kg BW (-25.6 mm Hg) after 6h of administration	(Li et al. (2007).
Rice dregs	Trypsin	Hydrolyzates	N/A	Reduced SBP of SHR (-11, -17, -26 mm Hg) at a dose of 1, 10, and 50 mg/kg BW after 1h of administration	Chen et al. (2007)
Flaxseed	Flavourzyme	Hydrolyzates	ACE^- inhibition range 71.59–88.29%, the maximum IC_{50} of 0.07 mg/ml	N/A	Marambe et al. (2008)
Amaranth	Chemical synthesis	Tetrapeptides	IC_{50} of 6.32 mM and 175 μM	N/A	Vecchi and Anon (2009)
Amaranth	Gastrointestinal digestion and alcalase	Hydrolystaes	IC_{50} range of 0.475 to 0.118 mg/ml (different	N/A	Tiengo et al. (2009)

			treatments)		
Amaranth	Alcalase and gel filtration	Fractions	IC_{50} of 0.15 and 0.35 mg/ml	N/A	Tovar-Pérez et al. (2009)
Canola meal	Alcalase 2.4L and protease M ‘‘Amano”	Hydrolyzates	IC_{50} range of 18.1 to 82.5 µg/ml	N/A	Wu et al. (2009)
Flaxseed	Various proteases	Hydrolystates	IC_{50} range of 0.0275 to 0.151 mg/ml	N/A	Udenigwea et al. (2009)
Oat	Various proteases	Hydrolyzates	IC_{50} of 30 and 50 µg/ml	N/A	Cheung et al. (2009)
Wheat milling byproduct (bran + shorts)	Autolysis reactions	Peptides	IC_{50} range of 0.21 to 21 µM	N/A	Nogata et al. (2009)
Amaranth	Trypsin	Hydrolyzate	IC_{50} of 200 µg/ml	N/A	Rosa et al. (2010)
Amaranth	Trypsin and chymotrypsin	Hydrolyzate	IC_{50} of 0.064 mg/ml	N/A	Luna-Suarez et al. (2010)
Barley and its byproducts	Extraction by 70% ethanol solution	Various extracts	ACE^{b}-inhibition of 61.73, 54.23, and 39.86%	N/A	Lee et al. (2010)
Wheat germ	Alcalase	Hydrolyzate	IC_{50} of 0.55 mg/ml	N/A	Jia et al. (2010).
Amaranth	Various proteases	Hydrolyzates	% ACE-inhibition ranged from 0.2 to 74.5	Different effects in SHR depending on the administration method, dose, and time after administration	Fritz et al. (2011)

Corn	Crude alkaline and neutral proteases	Oligopeptide	IC_{50} of 1.020 mg/ml	Reduced SBP of SHR (-40 mm Hg) at dose of 0.45 g/kg BW from the sixth week onwards of administration	Lin et al. (2011)
Corn	Alcalase (enzymatic membrane reactor)	Fractions Mw < 1, Mw < 3 , and Mw < 5 kDa	IC_{50} of 0.44, 0.29, and 1.27 mg/ml	Fraction Mw < 3 kDa reduced SBP of SHR at different doses (-26.57, -19.57, and -17.91 mm Hg)	Huang et al. (2011)
Rye malt supplemented with wheat gluten	Fermentation with Lactobacillus reuteriTMW 1.106 with protease addition	Tripeptides	IC_{50} of 0.23, 0.71, 1.09, and 0.09 mmol (kg DM) $^{-1}$	N/A	Hu et al. (2011)
Transgenic rice	Pepsin and pancreatin	Hydrolyzate	N/A	Reduced SBP of SHR (-15.6 mm Hg) at dose of 1000 mg/kg BW after 4 h of oral administration	Wakasa et al. (2011)
Wheat germ	Incubation under different conditions (wheat germ endogenous	5 Peptides	IC_{50} of 115.20, 94.87, 40.56, 26.82, and 5.86 μM	N/A	Yang et al. (2011)

	proteases)				
Wheat gliadin	Cereal digesition by Various proteases	Fractions	IC_{50} of 0.33 and 0.02 mg/ml	N/A	Thewissen et al. (2011)
Amaranth (7S globulin)	Gastrointestinal digestion	Hydrolyzate	IC_{50} of 170 μg/ml	N/A	Quiroga et al. (2012)

[a] $IC_{50:}$ the concentration of ACE inhibitor required to inhibit 50% of the ACE.

[b] ACE: Angiotensin-converting enzyme

[c] SHR: spontaneously hypertensive rats.

[d] N/A: not available

[e] SBP: systolic blood pressure

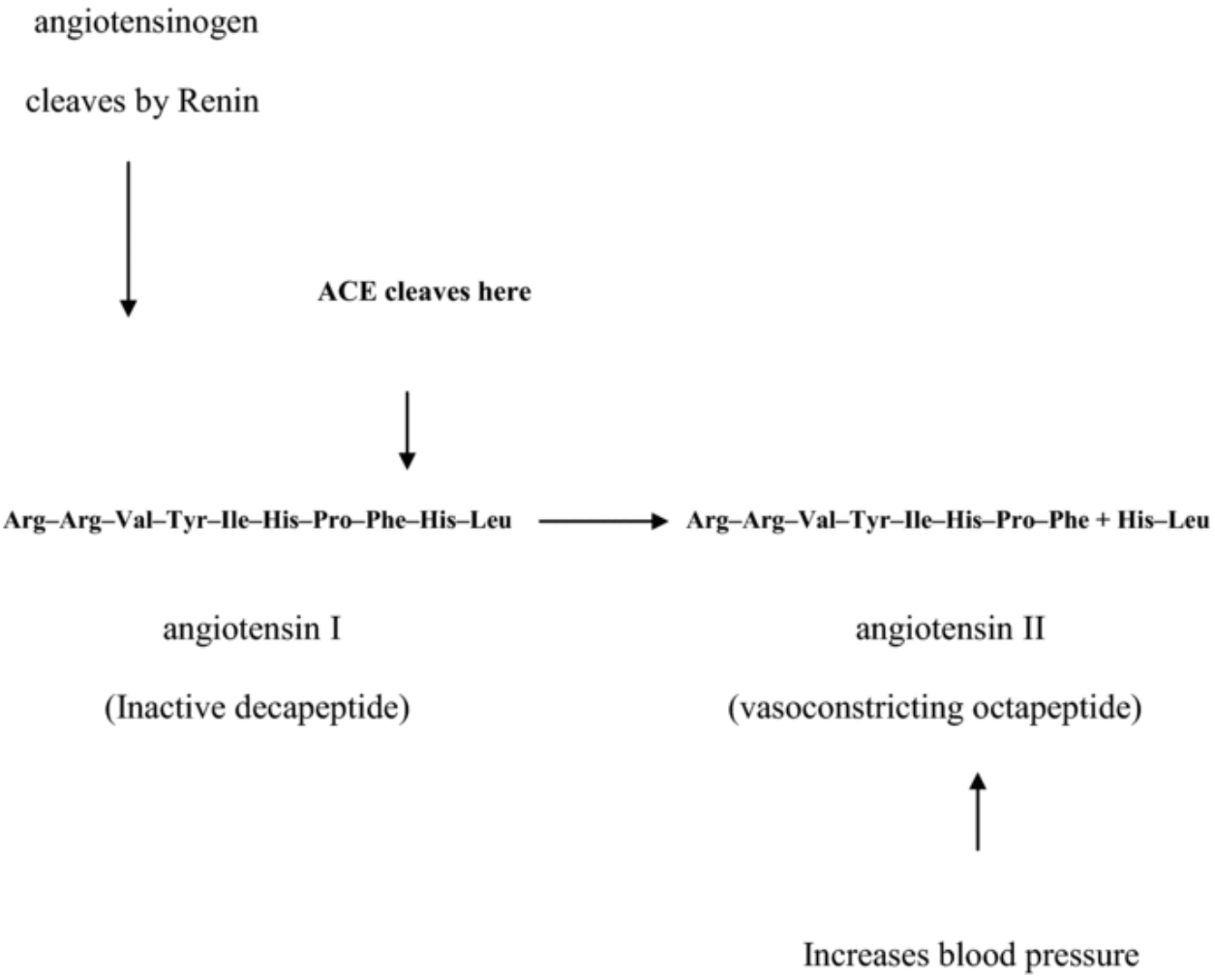

Fig 1 Conversion of angiotensin I to angiotensin II **(Smith, 2010).**