รายงานวิจัยฉบับสมบูรณ์

(ปีที่ 1)

เรื่อง

โปรตีนไฮโดรไลเสตจากเมล็ดผลไม้ไทยเพื่อการบำบัดโรค

นำเสนอโดย

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ABSTRACT

Blood pressure regulation is partially dependent on the renin-angiotensin system; renin acts on angiotensinogen to release angiotensin-I, which is further converted into the angiotensin II by the angiotensin I-converting enzyme (ACE). ACE plays a key physiological role in the regulation of blood pressure by virtue of two different reactions that it catalyzes: conversion of the inactive angiotensin I to the powerful vasoconstrictor angiotensin II, and inactivation of the vasodilator bradykinin. Crude extract and ammonium sulphate cut protein extracts, and their pepsin-pancreatin hydrolysates, from the seeds of 4 Thai fruits (i) *Carica papaya* L.; (papaya; unripen and ripen form), (ii) *Nephelium lappaceum* L. (rambutan) (iii) *Dimocarpus longan* Lour. subsp. (longan), and (iv) *Litchi chinensis* Sonn. (lychee) were screened for their in vitro angiotensin I-converting enzyme inhibitory (ACEI) activity. The protein hydrolysate of lychee seeds shows the highest potential of ACE inhibitory activity at IC_{50} value 0.22±0.010 mg protein/ml. The protein hydrolysate of unripen papaya seeds, longan seeds, and lychee seeds show uncompetitive and non-competitive inhibition with K_i values at 6.02, 2.82, and 5.62 mg protein/ml, with optimum pH in range of 6-8. After partial purification with ultrafiltration technique, UF-3 (below 5 kDa) of longan seeds show the highest inhibitory activity with IC_{50} values at 0.43±0.011 mg protein/ml. This fraction was subjected to RP-HPLC and five peaks were separated, and subjected to LC/MS/MS for amino acids sequences analysis. The P1-F1, P3-F1, and P3-F4 show the most inhibitory activity.

Keywords: angiotensin-I converting enzyme inhibitory activity, protein hydrolysate, Thai fruit seeds

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LIST OF ABBREVIATIONS

%	percentage
°C	degree Celsius
µg	microgram
µl	microliter
A	absorbance
BSA	Bovine serum albumin
CH_2CN	acetonitrile
cm	centimeter
Da	Dalton
g	gram
hr	hour
kDa	kilo Dalton
l	liter
LC/MS/MS	Liquid chromatography/Mass Spectrometry/Mass Spectrometry
M	molar
min	minute
ml	milliliter
mg	milligram
mM	milimolar
MW	molecular weight
NaCl	Sodium Chrloride
nm	nanometer
RP-HPLC	Reversed Phase-High Performance Liquid Chromatography
rpm	revolution per minute
TFA	trifluoroacetic acid

Tris	Tris(hydroxymethyl)aminomethane
U	unit activity
v/v	volume by volume
w/v	weight by volume

FULL TEXT

1. INTRODUCTION

Hypertension, one of the most common worldwide diseases, is a chronic medical condition in which the resultant elevated blood pressure can damage the health. There are many associated risk factors, such as strokes, heart disease, chronic renal failure or aneurysm disease. There are many predisposition factors, such as a sedentary lifestyle, stress and visceral obesity, of hypertension, which are not restricted to the aged and elderly. The angiotensin I-converting enzyme (ACE, EC.3.4.15.1) plays a key physiological role in the control of blood pressure, in the Renin-Angiotensin System (RAS), which mediates control of the extracellular volume (i.e. that of the blood plasma, lymph and interstitial fluid) and arterial vasoconstriction. ACE catalyses the conversion of the decapeptide angiotensin I to the potent vasoconstrictor angiotensin II and also degrades bradykinin, leading to the systematic dilation of the arteries and decrease in arterial blood pressure. Some of the ACE inhibitor (ACEI) peptides result in a decreased formation of angiotensin II and decreased blood pressure. For this reason, many studies have been directed towards the attempted synthesis of functional ACEIs without side-effects, such as captoprill or enalapril, which are currently used in the treatment of hypertensive patients. There is a strong trend towards developing natural ACE inhibitors (ACEI) for the treatment of hypertension.

Bioactive proteins and peptides have physiological properties and in recent times several studies have been done on identifying and optimizing the isolation of biopeptides from both plant and animal sources. These peptides are generated both *in vivo* and *in vitro* from the proteolytic hydrolysis of food proteins. Peptides with a wide range of regulatory effects have been discovered, including modulation of the immune defence, increased nutrient uptake, neuro-endocrine information transfer, antihypertensive, antithrombotic, antimicrobial, antigastric and opioid activity. These peptides have been discovered in a diverse array of sources, including snake venom, spinach, whey proteins and mushrooms. However, the only legumes that have been investigated for biopeptides

to the best of our knowledge are chickpeas, peas, cowpeas and soybeans. Thus, researchers consider seeds as new major sources of bioactive protein hydrolysate and interesting in distinction from previous report. Previous reports describe small molecule organic compounds but a few reports about bioactive protein hydrolysate. This is the good reason to find new bioactive protein hydrolysate from Thai fruits seeds for medical, pharmaceutical, industrial applications or higher research.

2. LITERATURE REVIEWS

2.1 Hypertension

Hypertension is a worldwide epidemic problem, affecting about 20 % of world's adult population. It is the one of the major risk for the development of cardiovascular disease and it often called a silent killed because the persons with hypertension are asymptomatic for years (Gao *et al*., 2010). Hypertension is the most common serious chronic health problem because it carries a high risk factor for arteriosclerosis, stroke, myocardial infarction and end-stage renal disease (Je *et al*., 2005). Therefore, the decreasing of blood pressure to normal levels is crucial for preventing cardiovascular and renal disease. In the human body, blood pressure is regulated by renin-angiotensin system (RAS). RAS has been found to be a coordinated peptidic hormonal cascade for the control of cardiovascular, renal, and adrenal function governing fluid and electrolyte balance and arterial blood pressure (Carey and Siragy, 2003). Renin catalyzes the inactivated form of angiotensinogen to form angiotensin I, which further cleaved by angiotensin I-converting enzyme (ACE) to vasoconstrictor angiotensin II and damage bradykinin into an inactive metabolite (Ahn *et al*., 2012; Gao *et al*., 2010). (Figure 2.1)

2.2 Angiotensin-I converting enzyme (ACE)

Angiotensin-I converting enzyme (ACE) is a key enzyme in the regulation of blood pressure and electrolyte homeostasis. ACE belongs to the class of zinc proteases and located in the vascular endothelial lining of lungs. ACE acts as an exopeptidasse that cleaves dipeptides from the C-terminus of various oligopeptides. (Curtiss *et. al.* , 1978; Yang *et.al*., 1970). ACE is an important enzyme of the renin-angiotensin system, major regulation of blood pressure in mammals (Tomatsu *et al*., 2013). A membrane-anchored

dipeptide-liberating carboxypeptidase (peptidyldipeptide hydrolase, kinase II, EC 3.4.15.1) converts angiotensin I (a decapeptide; Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu) to the highly potent vasoconstrictor octapeptide angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe + His-Leu), (Figure 2.2). The effects of angiotensin II including vasoconstriction, arterial constriction and blood pressure elevation are mediated by angiotensin type 1 receptors (AT1). Angiotensin I also bind to angiotensin type 2 receptor (AT2) which highly expressed in fortal mesenchymal tissues but poorly expressed in the adult. This enzyme also plays a key physiological role in the regulation of local levels of several endogenous bioactive peptides such as breaks down bradykinin, a vasodilator, further contributing to blood pressure elevation in the kinin-kallikrein system (Barbana and Boyce., 2010). The inhibition of ACE would be expected to prevent the formation of the hypertensive agent angiotensin II and to potentiate the hypotensive properties of bradykinin, leading to combined lowering of the blood pressure. Inhibitors of ACE are therefore widely used in therapy for hypertension, heart failure, myocardial infarction, and diabetic nephropathy.

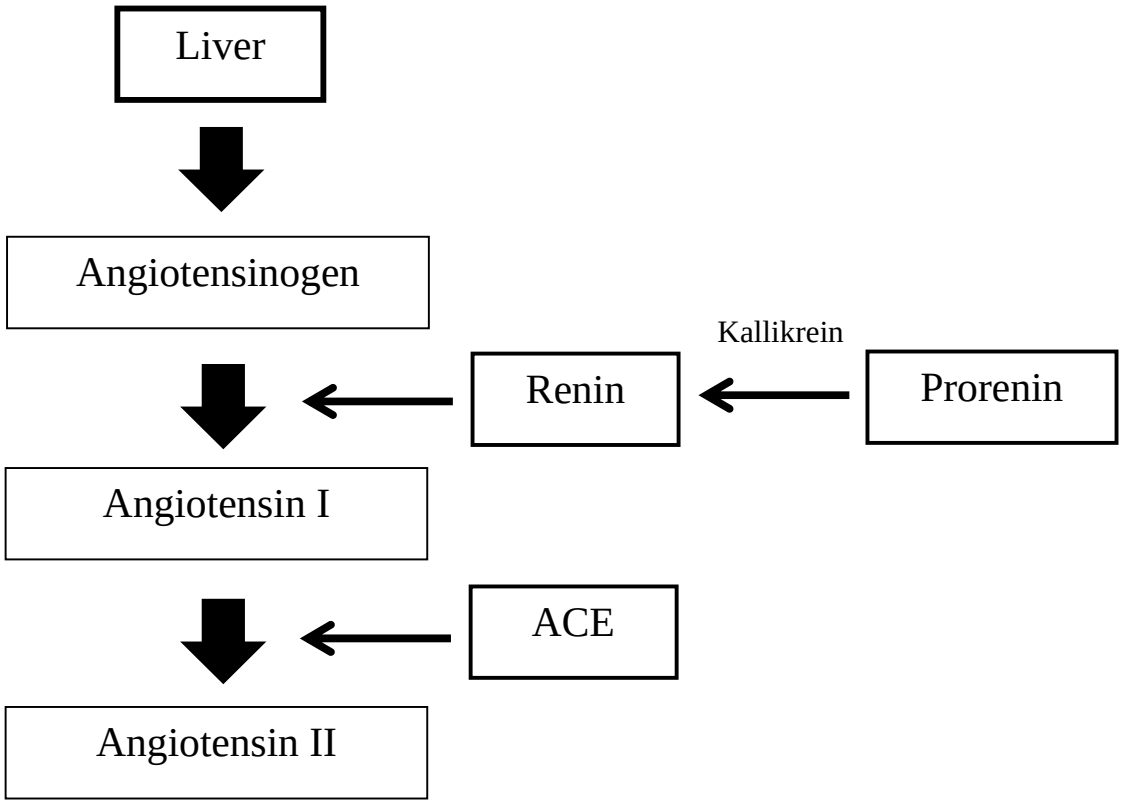


Figure 2.1 The renin-angiotensin system (RAS)

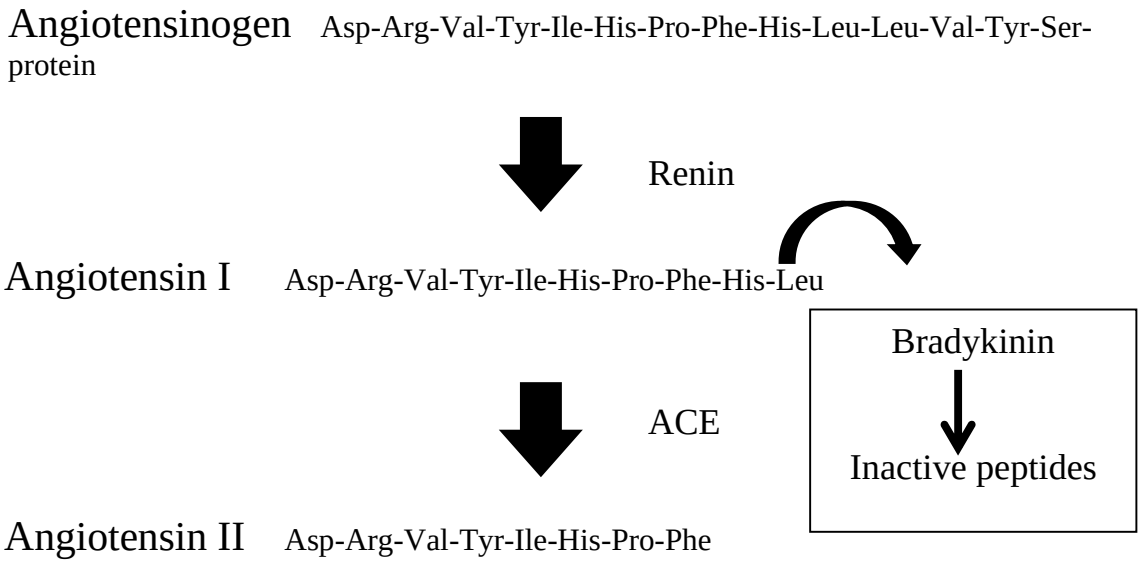


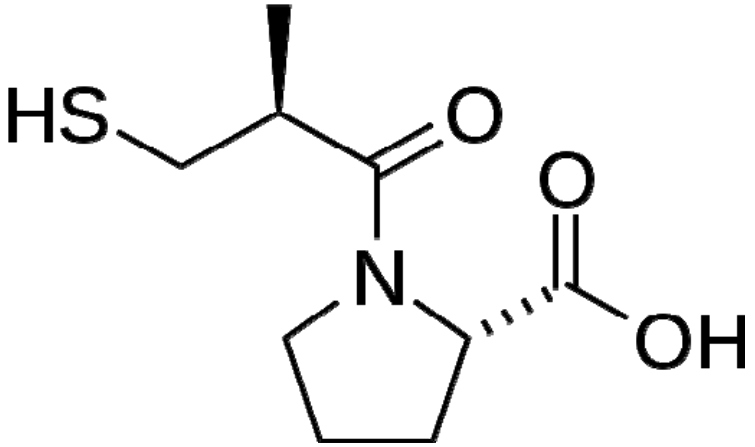
Figure 2.2 Angiotensin converting enzyme (ACE)

2.3 Angiotensin I-converting enzyme inhibitors (ACEI)

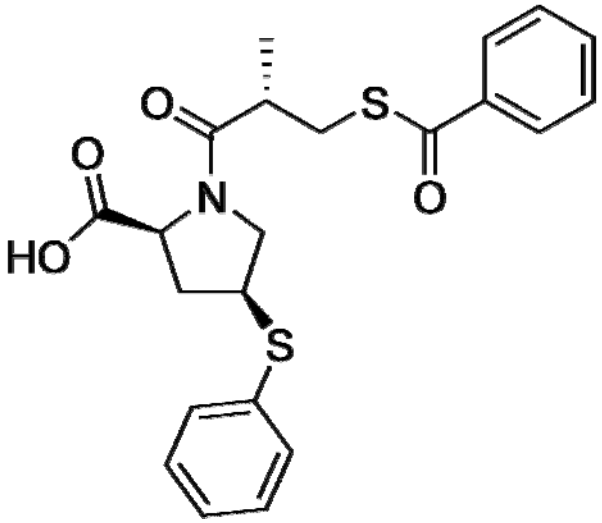
The inhibition of ACE is considered to be a useful therapeutic approach in the treatment of hypertension, the development of drugs to control high blood pressure, ACE inhibitor (ACEI) has become an important activity. The first anti-hypertensive effect *in vitro* was discovered in snake venom. Many studies have been attempted in the synthesis of ACEI such as captopril or D-3-mercapto-2-methylpropanoyl-L-proline is the first synthesis compound which an analog of Ala-Pro sequence, with sulfhydryl as a strong chelating group of zinc ion (Patchett *et al*., 1980). The ACEI can be divided into three group based on their molecular structure as sulfhydryl-containting agents such as captopril, zefenopril, fentiapril, and alacepril.

Decarboxylate-containing agents such as enalapril, remipril, quinapril, lisinopril, imidapril, perindopril, benazepril, and trandolapril. Fosinopril is the only phosphonate-containing agents in this group (Lawrie, 1991). (Figure 2.3) ACEI are current used in the treatment of hypertension and heart failure in humans (Ondetti *et al*., 1977). However,

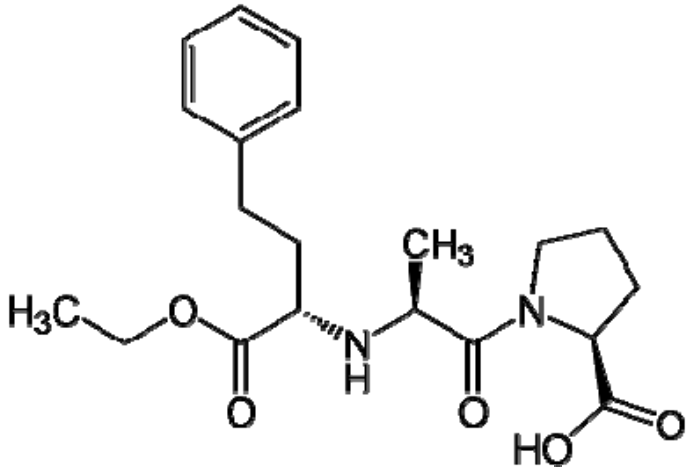
these synthetic drugs are believed to have certain side effects such as cough, taste disturbances, skin rashes or angioneurotic edema all of which might be intrinsically linked to synthetic ACEI (Kim & Wijesekara *et al*., 2010). Therefore, the research and development to find non-toxic and economical ACEI are necessary for the prevention and remedy of hypertension (Goretta *et al*., 2006).



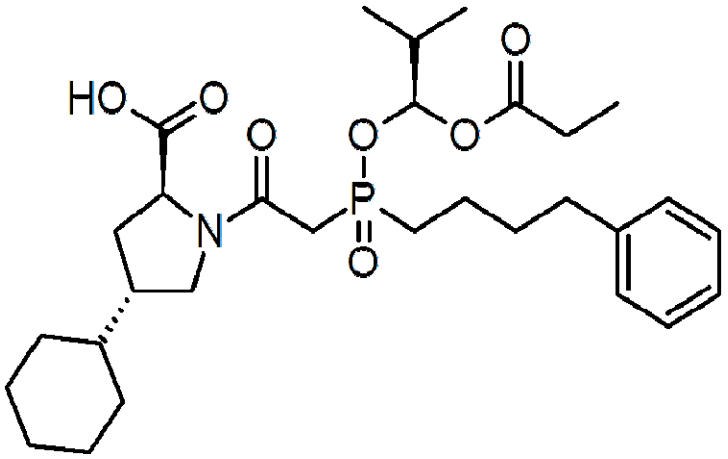
Captopril



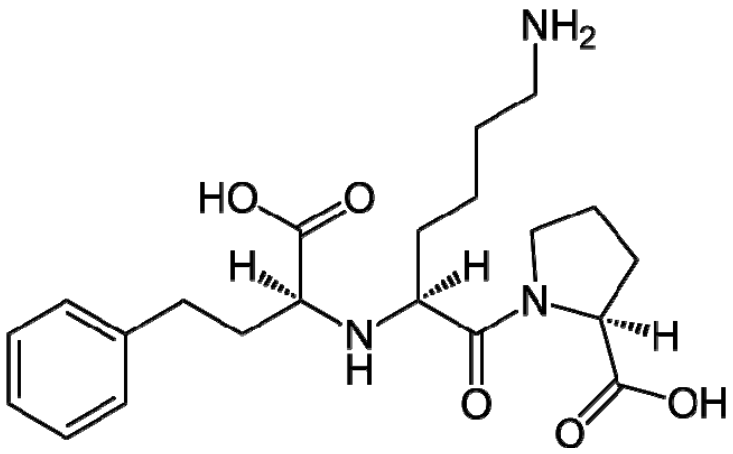
Zofenopril



Enalapril



Fosinopril



Lisinopril

Figure 2.3 Chemical structures of captopril, zofenopril, enalapril, fosinopril, and lisiopril.

ACE inhibitory peptides are also present in the amino acid sequences of several food proteins (Ariyoshi, 1993). The intrinsic bioactivities of the peptide encrypted in food proteins are latent until they are released ad activated by enzymatic hydrolysis, for example, during gastrointestinal digestion and food processing (Takano, 1998). Activated peptides are potential modulators of various regulatory processes in the living system. Therefore, food protein-derived inhibitors of ACE represent natural, physiologically active, food-grade components, which may provide health benefits beyond basic nutrition (Clare & Swaisgood, 2000). In particular, food protein-derived peptides may contribute to reducing the risk of developing cardiovascular disease through the consumption of ACE inhibitors as functional food ingredients (Meisel, 1993; FitzGerald & Meisel, 1999).

2.4 Source protein derives ACE inhibitory peptides

The procedures have generally been used in the identification and characterization of ACE-inhibitory peptides are (i) isolation from *in vitro* enzymatic hydrolysate (ii) *in vivo* gastrointestinal digestion , and (iii) chemical synthesis of peptides having identical or similar structures to those known to be bioactive. In some cases ACE-inhibitory

peptides may be isolated from a food source without prior enzymatic processing, for example, from garlic (Suetsuma, 1998).

A widely variety of ACE inhibitory peptides have been identified and characterized from milk, animal (non-milk), plant, and miscellaneous protein sources. The ability of an ACE-inhibitory peptide is usually revealed as an IC_{50} value, which is equivalent to the concentration of peptide mediating a 50% inhibition of activity (Holmquist *et al*., 1979; Vermeirssen *et al*., 2002). In the majority of cases, the most frequently used analytical method to determine IC_{50} is based on the hydrolysis of hippuryl-histidine-leucine (HHL) (Crushman and Cheng, 1971). With the creation of new artificial substrates for ACE, alternative methods have been developed to quantify the IC_{50} of ACE inhibitory peptides (Elbl *et al*., 1994; Mehanna *et al*., 1999). Unfortunately, the use of various modifications of the method of Crushman and Cheng has made the comparison of IC_{50} value from different studies difficult because some reports do not detail the number of enzyme units used in the inhibition analyses or include and IC_{50} value for and ACE-inhibitory standard such as Captopril. (FitzGerald and Meisel, 2000)

As can be seen in Table 2.1, the majority of peptides are short-chain peptides with low molecular mass. This agreement with the crystallography studies, the active site of ACE cannot bind with the large peptide molecules (Natesh *et al*., 2003)

Table 2.1 Potent of ACE inhibitory peptides

Source	Enzyme	Amino acid sequence	IC_{50} (μM)	Reference
Garlic	No enzyme	FY NY NF	3.74 32.6 46.3	Suetsuna *et al*., 1998
Wheat	Alcalase	TF LY YL AF IY VF IVY VFPS TAPY TVPY TVVPG DIGYY DYVGN TYLGS GGVIPN APGAGVY	17.8 6.4 16.4 15.2 2.1 9.2 0.48 0.46 13.6 2 2.2 3.4 0.72 0.86 0.74 1.7	Matsui *et al*., 1999
Sunflower	Pepsin Pancratin	FVNPQAGS	6.9	Megias *et al*., 2006
Rapeseed	Gastrointestinal simulation	RIY VWIS	20 30	Marczak *et al*., 2003
Buckwheat	No enzyme	GPP	0.00625[a]	Ma *et al*., 2006
Mungbean	Alcalase	KDYRL VYPALR KLPAGYLF	26.5 82.5 13.4	Li *et al*., 2006

Table 2.1 (Continued)

Source	Enzyme	Amino acid sequence	IC_{50} (µM)	Reference
Seaweed pipefish	Papain Alcalase Neutrase Pronase Pepsin Trypsin	YFPHGP HWYYQA	0.62[a] 1.44[a]	Wijesekara *et al*., 2011
Hen egg white lysozyme	Pepsin Chymotrypsin Trypsin	MKA RGY VAW	25.7 61.9 2.86	Rao *et al*., 2012
Salmon byproduct	Alcalase Flavorzyme Neutrase Pepsin Protamex Trypsin	VWDPPKFD FEDWVPLSCF FNVPLWE	9.10 10.77 7.72	Ahn *et al*., 2012
Cornucopia mushroom	No enzyme	RLPSEFDLSAF-LRA RLSGQTIEVTS-EYLFRH	0.46[a] 1.14[a]	Jang *et al*., 2011
Wheat gliadin	Clarex Alcalase Esperase	PVILF	0.02[a]	Thewissen *et at*., 2011
Grass carp	Alcalase	VAP	0.00534[a]	Chen *et al*., 2011
Potato	Trypsin	GFR FK IMVAEAR GPCSR CFCTKPC MCESASSK	94.25 265.43 84.12 61.67 1.31 75.93	Huang *et al*., 2011

[a] IC_{50} values quoted are expressed as mg protein/ml.

2.5 Characterization of ACE inhibitory peptides

Inhibitors of ACE were developed for therapy of human hypertension without knowledge of the structure of human ACE, designed on the basis of an assumed mechanistic homology with carboxypeptidase A. Recently; the analysis of structure of ACE has shown the resembles zinc metalopeptidase. (Natash *et al*., 2003). The somatic

form of ACE consists of two homologous domains (N- and C-domain) (Inagami, 1992), each of which contains an active site which catalyzes the hydrolysis of angiotensin I (Wei *et al*., 1992). ACE inhibitors may preferentially act on either ACE domain. However, the C-domain seems to be necessary for controlling blood pressure, suggesting that this domain is the dominant angiotensin-converting site. Although there is no known specific physiological substrate of the C-domain, the C-domain activity can be assessed specifically *in vitro* by use the synthetic substrate (HHL).

ACE prefer to have substrates or inhibitors that contain hydrophobic (aromatic or branched side chains) amino acid residues at the first of three C-terminal positions (Cheung, Wang, Ondetti, Sabo, & Crushman, 1980; Wu *et al*., 2006). Many naturally occurring peptidic inhibitors containing proline at C-terminus. This applies also for the highly active short-chain peptides. The majority of di- and tri-peptide inhibitors have a Tyr, Phe, Trp, or Pro residue at the C-terminal end, the Trp seems to be most effective in increasing in highly active inhibitors. Example, the result of Rao *et al*., the hen egg white lysozyme protein hydrolyzed by pepsin, α-chymotrypsin, and trypsin was purified to the tri-peptides, the peptide Val-Ala-Trp show the strongest ACE inhibitory activity with IC_{50} value of 2.86±0.08 µM (Rao *et al*., 2012). The sequence of the peptide has the structure-activity relationship described as above. Many similar tri-peptide sequences in other reports, such as Val-Ala-Pro (2 µM) (De Leo *et al*., 2009) and Ile-Met-Tyr (1.8 µM) (Matsui *et al*., 2002)

2.6 Purification and sequencing of ACE inhibitory peptides

ACE inhibitory peptides can be separated from the protein hydrolsate mixture by the various techniques of membrane-based reparation and chromatography techniques or various techniques in the research. For the example, the peptide Phe-Asn-Val-Pro-Leu-Tyr-Glu has been purified from salmon byproduct protein hydrolysate by alcalase hydrolysis. Ahn and his co-workers were loaded the protein hydrolysate to DEAE FF ion-exchange column. The active fraction was subjected to Sephadex G-25 gel filtration column. The active fraction was then subjected to reverse-phase HPLC on an ODS C_{18} column. The active peak which has the highest ACE inhibitory fraction was further

purified by using the same column. Accurate molecular mass and amino acid sequence were determined by Hybrid Quadrupole-TOF LC/MS/MS mass spectrometer, sequence information was obtained by tandem MS analysis. (Ahn *et al*., 2012)

Moreover, ACE inhibitory peptide can be purified by another technique such as ultrafiltration by using ultrafiltration membrane bioreactor system with 30, 10 and 5 kDa of molecular weight cut-offs (MWCO) (Gao *et al*., 2010). Size-exclusion chromatography is based on their molecular size, also called gel filtration chromatography when operated with aqueous mobile phase or gel permeation chromatography when performed in organic mobile phases. Ion-exchange chromatography, capillary focusing and capillary electrophoresis were based on their charge properties.

Amino acid composition was studied by reversed-phase high performance liquid chromatography (RP-HPLC) system (Rozan *et al*., 2000), reversed phase columns are usually packed with bonding of octadecylsilyl coated silica. Organic solvents such as acetonitrile, methanol, and propanol were usually used as gradient elution mobile phase. Trifluoroacetic acid (TFA), is often added for improve the chromatographic peak shape into eluting solvents. The detection of amino acid was monitored at wavelength of UV visible with UV detector (Mohtar *et al*., 2012). To determination of the unknown peptides, mass spectrometry chosen to determine the amino acid sequence and accurate molecular mass. Two main techniques are electrospray ionization (ESI) and matrix assisted laser desorption/ionization (MALDI) are adopted to determine the unknown peptides its call mass spectrometry techniques.

2.7 The Thai fruits seeds

Thai fruits are the unique identity and culture of Thailand for representing the fertility of the country. With the great physical geography and plentifully, the rich plant varities with good taste, nice quality of fruits are abundant. The processing of fruits into canned, dried fruit or other products produces many wastes from fruits including seeds. The remaining seeds are mostly discarded without value or sold very cheap to fuel production. Therefore, some kind of Thai fruits seeds such as papaya (*Carica papaya* L)

longan (*Dimocarpus longan* Lour. subsp.), rambutan (*Nephelium lappaceum* L.), and lychee (*Litchi chinensis* Sonn.) might be interesting to investigate for the new sources of bioactive protein hydrolysates. This study might be differently from that previously reported which reports a protein hydrolyzate containing bioactive in non-edible fruits seeds.

3. EXPERIMENTALS

3.1 Biological materials

The fresh 4 kinds of the Thai fruits seeds such as (i) *Carica papaya* L.; (papaya; unripen and ripen seeds), (ii) *Dimocarpus longan* Lour. subsp. (longan), (iii) *Litchi chinensis* Sonn. (lychee), and (iv) *Nephelium lappaceum* L. (rambutan) were obtained from canned fruit industry, Malee Sampran Public Co., Ltd. in Nakhon Pathom province and dried fruit industry, Kim Chua Group Co., Ltd. in Bangkok, Thailand. All the samples were quickly taken to the laboratory and kept in the dark and cold at 4 °C until used.

3.2 Chemical materials

Angiotensin Converting Enzyme ; ACE (E.C. 3.4.15.1) from rabbit lung, Bovine Serum Albumin (BSA), Hippuric acid as standard, Captopril as positive control, Hippuryl-L-Histidyl-L-Leucine (HHL) as substrate peptide, Other proteases, Pancreatin from porcine pancreas and Pepsin from porcine gastric mucosa were purchased from Sigma Chemicals Co. (USA). All other biochemicals and chemicals used in the investigation were of analytical grade.

3.3 Preparation of the crude extract from Thai fruits seeds

The preparation of the crude protein was carried out according to the method of Yodjun *et.al*., 2012. The fresh 4 kinds of Thai fruits seeds were cleaned, pared, and removed the impurities and damaged seeds, then weight at 1.5 kg (wet weight) and blended in 5 l of phosphate buffered saline (PBS; 20 mM phosphate buffer with 0.15 M NaCl pH 7.2) using a blender (Phillips, Indonesia) until homogenous texture and subsequently stirred overnight at 4 ^{o}C with a 4 fin propeller, using a low-speed agitator (IKA Labortechnik, Germany) at middle speed. The double-layered cheesecloth was used

as filter to separate the suspension from the fluid and then the filtrate was clarified by centrifuged at 15,000 × g for 30 min at 4 ^{o}C, and the supernatant was harvested. Ammonium sulfate was slowly added with stirring to 80% saturation and subsequently stirred for overnight at 4 ^{o}C prior. The suspension was centrifuged at 15,000 × g for 30 min at 4 ^{o}C to harvest the insoluble material (precipitate) as the crude extract. The crude extract was then dissolved in double-deionized water, dialyzed against excessive amounts of double-deionized water and lyophilized to yield the crude protein.

3.4 Preparation of the protein hydrolysate

The crude proteins produced from the seeds of each fruit species were used as a substrate for production of the protein hydrolysate, by treatment with pepsin and pancreatin following the method of Magias *et. al*., 2006 with slightly modified. In brief, each crude protein was incubated with gastric enzyme pepsin until the final substrate/enzyme (v/w) concentration ratio was 20:1 and adjusted to pH 1.5 – 2.5 by 1M HCl. The hydrolysis was carried out for 180 min with shaking at 180 rpm at 37 ^{o}C, and then inactivated the activity by adding 1 M NaOH to pH 7.0 - 8.0. The pancreatic enzyme, pancreatin was added to a 20:1 (v/w) substrate/enzyme ratio and shaken 180 rpm for 180 min at 37 ^{o}C. The hydrolysis (enzyme reaction) was stopped by heating at 80 ^{o}C for 20 min. Hydrolysates were clarified by centrifuged at 15,000 × g for 30 min at 4 ^{o}C to remove the insoluble meterials. The supernatant was tested for the ACE inhibitory activity. The choice of these two proteases was to crudely mimic that in the human gastrointestinal tract.

3.5 ACE inhibitory activity assay

ACE inhibitory activity was measured according to the method of Je *et. al*., 2005 with slightly modification. 50 µl of crude proteins solution of 4 kinds of Thai fruits seeds was mixed with 50 µl of ACE (25 mU/ml) and pre-incubated at 37 ^{o}C for 10 min, after that, the mixture was re-incubated with 150 µl of substrate (10 mM HHL in PBS) for 30 min at 37 ^{o}C. Then, the reaction was stopped by adding 250 ml of 1M HCl. The hippuric acid was extracted with 500 µl of ethylacetate. After centrifugation at 15,000 × g and 4 ^{o}C for 15 min, 200 µl of the upper layer was transferred into another new test tube, and

evaporated in a vacuum at room temperature. The hippuric acid was dissolved in 500 µl of double deionized water, and the absorbance at 230 nm was measured using an UV-spectrophotometer. A standard curve was constructed using a series of hippuric acid standards of known concentration to quantify the release of hippuric acid in the assay. Double deionized water was used as controlled non-inhibitor. The inhibition potential can be calculated by the equation below, and the concentration of ACE inhibitor required to inhibit 50% of the ACE activity under the above assay conditions was defined as IC_{50}.

$$\% \text{ inhibition} = \frac{\text{A of control - A of sample}}{\text{A of control - A of blank}} \times 100$$

3.6 Determination of the protein content

The protein content was determined following the standard Bradford assay (Bradford, 1976), with dilutions of a known concentration of bovine serum albumin (BSA) as the standard. The absorbance at 595 nm was monitored with a microplate reader.

3.7 Amino acid analysis

Whole seeds of fresh Thai fruits were crushed with liquid nitrogen to fine powder. The analysis methods according to Liu *et. al*., 1995 and Bosch *et. al*., 2006 was used. The sample preparation was conducted by weighting sample into the test tube and added 5 ml of 6N HCl and places the reaction mixture in heating block at 110 °C for 22 hrs. The internal standard was added into the hydrolysate and diluted with deionized water, mixed the filtrate with AccQ-fluor derivatization buffer and AccQ-fluor reagent to derivatized of the amino acid. Samples were heated at 55 °C for 10 min in heating block. Five µl of samples were subjected to HPLC analysis using a Hypersil Gold column C_{18} HPLC (4.6 x 150 mm, 3 µm, Waters Alliance) with 60% acetonitrile in sodium acetate buffer pH 4.90±0.05 as eluent.

3.8 Inhibitory kinetics study

To clarify the ACE inhibition pattern, the method of ACE inhibitory kinetics was used according to Yodjun *et. al*., 2012. Different concentrations of substrate (1, 2, 3, 4, and 5 mM) and inhibitors (undiluted, diluted 2 folds, and 4 folds) were added to each

reaction mixture and incubated with ACE at 37 °C. The inhibition kinetics of ACE in the presence of protein hydrolysate was determined with Lineweaver-Burk plot.

3.9 pH resistance determination

To determine the pH resistance stability of protein hydrolysate, the method according to Rungsaeng *et. al*., 2013 was used. The protein hydrolysates were incubated with broadly similar salinity levels by varies pH 2 -12 by buffers with ratio 1:4 (sample:buffer) at 37°C for 0, 30, 60, 90, and 120 min prior to assaying the ACE inhibitory activity. The buffers used in this experiment were 50 mM of glycine-HCl (pH 2.0, 3.0 and 4.0), sodium acetate (pH 4.0, 5.0 and 6.0), potassium phosphate (pH 6.0, 7.0 and 8.0), Tris-HCl (pH 8.0, 9.0 and 10.0) and glycine-NaOH (pH 10.0, 11.0 and 12.0). Control used as non-inhibitor was double deionized water instead with the same ratio of buffers.

3.10 Temperature resistance determination

The thermostability of the protein hydrolysate were determined by method of Rungsaeng *et.al.*, 2013 with slightly modification. Each 500 µl of protein hydrolysate was pipetted into 1.5 ml microfuge tubes and incubated at the designed temperatures as -20 °C (in ultra-low freezer), 0 °C (in freezer), 4 °C (in refrigerator), 10, 20 °C (in cooling chamber), 30, 40, 50, 60, 70, 80, and 90 °C (in water bath) for 0, 30, 60, 90, and 120 min. At every designed time, the sample was quickly taken to evaluate for ACE inhibitor activity assay.

3.11 Partial purification of the hydrolysate protein

The hydrolyzed protein was further applied to ultrafiltration (UF) according to method of Mohtar *et.al*., 2012, the protein hydrolysate was fractionated into 3 parts by the ultrafiltration membrane bioreactor system with 10 and 5 kDa molecular weight cut-offs (MWCO). The fractions were named as UF-1, UF-2, and UF-3, UF-1 (molecular weight > 10 kDa) was not passed through the 10 kDa membrane, UF-2 (molecular weight 10 - 5 kDa) was passed through the 10 kDa membrane but not passed through the 5 kDa membrane. UF-3 (molecular weight below 5 kDa) was passed through the 5 kDa. All of

UF fractions were lyophilized in a freeze-drier before subjected to ACE inhibitory activity assay and protein content was determined.

3.12 Isolation of ACEI peptides

After partial purified with ultrafiltration technique, UF-3 of protein hydrolsate of each species of Thai fruit seeds were fractionated by using reversed phase-high performance liquid chromatography (RP-HPLC; spectraSYSTEM, USA) on Shimpak C-18 column (250 x 46 mm). The solvent system and conditions were used according to Yodjun *et. al*., 2012. The linear gradient of acetonitrile (CH_3CN) from 0% to 70% containing 0.1% Trifluoroacetic acid (TFA) at flow rate 0.7 ml/min. 50 µl of fractionated sample (UF-3) were injected. The elution peaks were monitored at 280 nm and collected every minute, each of fraction was pooled and lyophilized.

3.13 Identification of ACEI peptides

The collected peaks of RP-HPLC fractions were re-suspended with 50% acetonitrile containing 0.1% formic acid and subjected to amaZon SL Ion Trap LC/MS/MS mass spectrometer (Bruker, MA, USA) coupled with ESI source. Instrumental control and the analysis of data were performed by using Bruker Daltonics trapControl version 7.0 and ESI campass 1.3 for amaZon DataAnalysis version 4.0. The spectra were reported by the mass/charge (m/z) ranges of 200 – 1200 in both of MS and MS/MS modes. The peptide sequencing module of the software calculations were used to process for the MS/MS data and blast with Mascot database.

3.14 Statistical analysis

All determinations, were done in triplicate, and the results are reported as the mean $\pm$ 1 standard error of the mean. Regression analyses and calculation of IC_{50} values was done using GraphPad Prism Version 6.00 for Windows (GraphPad Software Inc.)

4. RESULT AND DISCUSSION

4.1 Screening of ACEI in seed samples

The potassium phosphate buffer extracted protein (crude extract), ammonium sulphate precipitated protein (crude protein) and the protein hydrolysates were determined for ACE inhibitory activity assay. Four Thai fruits seeds from *Carica papaya* L.; (papaya; unripen and ripen seeds), *Nephelium lappaceum* L. (rambutan), *Dimocarpus longan* Lour. subsp. (longan), and *Litchi chinensis* Sonn. (lychee) were chosen the be observed in this experiment. The inhibition potential was reported as IC_{50} (the half maximal (50%) inhibition concentration (IC) of a substance) values which are calculated from regression equation derived from the percent inhibition versus protein concentration of sample (Table 2).

From Table 2, The protein hydrolysate from *L. chinensis* Sonn. (lychee) show the greatest ACE inhibitory activity with IC_{50} value at 0.22±0.010 mg protein / ml) follow by *D. longan* Lour. subsp. (longan) and *C. papaya* L. (in unripen form) with IC_{50} values at 0.74±0.006 and 1.04±0.002 mg protein / ml. The worst ACEI activities were *C. papaya* L. (in ripen form) and *N. lappaceum* L. (rambutan) with no detection of inhibition activity. Thus, the 3 kinds of fruit seeds with the IC_{50} measurable (unripen papaya seeds, longan seeds, and lychee seeds) were considered for further characterization.

Table 4.1 The *in vitro* ACE inhibitory activity of crude extract, crude protein and protein hydrolysate of 4 kinds of Thai fruits seeds.

Plant seed species	IC_{50} values		
	crude extract (mg protein/ml)	**crude protein (mg protein/ml)**	**protein hydrolysate (mg protein/ml)**
C. papaya L. (papaya)			
unripen	ND	1.43±0.012	1.04±0.002
ripen	ND	ND	ND
N. lappaceum L. (rambutan)	ND	ND	ND
D. longan Lour. subsp. (longan)	0.35±0.002	0.88±0.002	0.74±0.006
L. chinensis Sonn. (lychee)	ND	0.23±0.002	0.22±0.010

ND = Not detected
All data are shown as the average mean ± 1 standard error of the mean and are obtained from 3 replicated determinations.

4.2 Mechanism of the inhibition

The inhibition mode of ACEIs from Thai fruits seeds were evaluated by kinetic studies. Table 3, shows the inhibition mode of the protein hydrolysate of each fruit seeds samples. From the table, that indicating 2 types of inhibition mode were possible, the uncompetitive inhibition (unripen papaya, and lychee seeds) which binding to the ACE-substrate complex not to free enzyme (Plamer, 2001), and the non-competitive inhibition (longan seeds) which binding with an enzyme molecule to produce dead-end complex by

binding at the different sites from the substrate (Ahn *et al*., 2012). These inhibition mode obtained by Lineweaver-Burk plots, the K_i values were determined are 6.02, 2.82, and 5.62 mg protein / ml, respectively. The K_m value, settle the ACE as the active enzyme and HHL as the substrate, was 0.04 mM with V_{max} was 7.0042 mM / min.

Most of the ACEIs that were derived from food protein hydrolysates belong to the competitive type such as natto (Akiko, Hiroshi, and Eiko., 1994) and fermented oyster sauce (Je *et al*., 2004). The competitive inhibitors are able to enter to the active center of ACE and interact with the active sites and prevent substrate to binding (Katayama *et al.*, 2008), Ruiz, Ramos and Recio., 2004), Rao *et al*., 2011). Some of isolated peptides show the non-competitive inhibition such as Pacific cod skin gelatin protein hydrolysate by using gastrointestinal enzymatic hydrolysis (Himaya *et al*., 2012) and uncompetitive inhibition in F7 of hen egg white lysozyme (HEWL) hydrolyzed by trypsin and papain followed by RP-HPLC separation (Asoodeh *et al*., 2011).

Table 4.2 Inhibition modes with K_i of the 3 kinds of Thai fruits seeds protein hydrolysates

Plant seed species	**Inhibition mode**	**K_i (mg protein/ml)**
C. papaya L. (unripen papaya)	Uncompetitive	6.02
D. longan Lour. subsp. (longan)	Non-competitive	2.82
L. chinensis Sonn. (lychee)	Uncompetitive	5.62

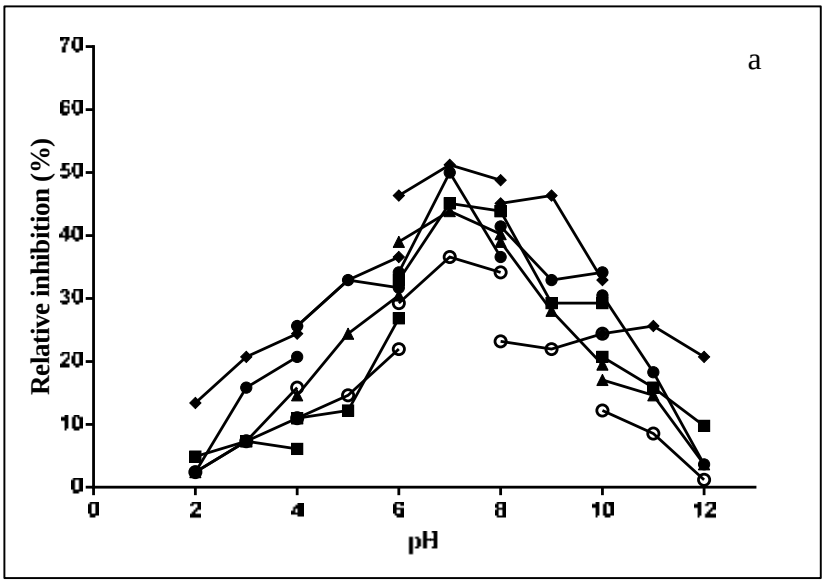
All data are shown as the average mean ± 1 standard error of the mean and are obtained from 3 replicated determinations.

4.3 pH resistance of the ACEIs

After incubated the protein hydrolysate with alter broad pH range of buffer from 2 – 12. Figure 4.1 showed the optimal ACEI activity of unripen papaya seeds (fig. 4.1a) at pH 6 – 8 in potassium phosphate buffer. The optimum pH of longan seeds (fig. 4.1b) was at pH 6 – 8, and the optimum pH of lychee seeds was at the range of 6 – 8 too (fig. 4.1c).

All of the samples had the excellent inhibition activity at 0 min after the incubation, an the activity was decreased after extensive incubation time (30, 60, 90, and 120 min). This broad pH range makes the potentially excellent of enzyme for pharmaceutical industry and food derived proteins. At the other pH which had poor inhibition activity may suggested some of ion in buffer might slow down or block the ACEI activity at each pH values or the high and low excessively pH might destroyed or degraded ACEI active peptides.

Yodjun et.al. (2012) reported the F75 of *Zingiber ottensii* rhizome showed the optimal ACEI activity at pH 4-5 and 8-11. Rungseang *et. al.* (2013) reported *Z. officinale* (post-DEAE cellulose unbound fraction) showed the optimal acetylcholinesterase inhibitory activity at pH 2-9 and 10-12.



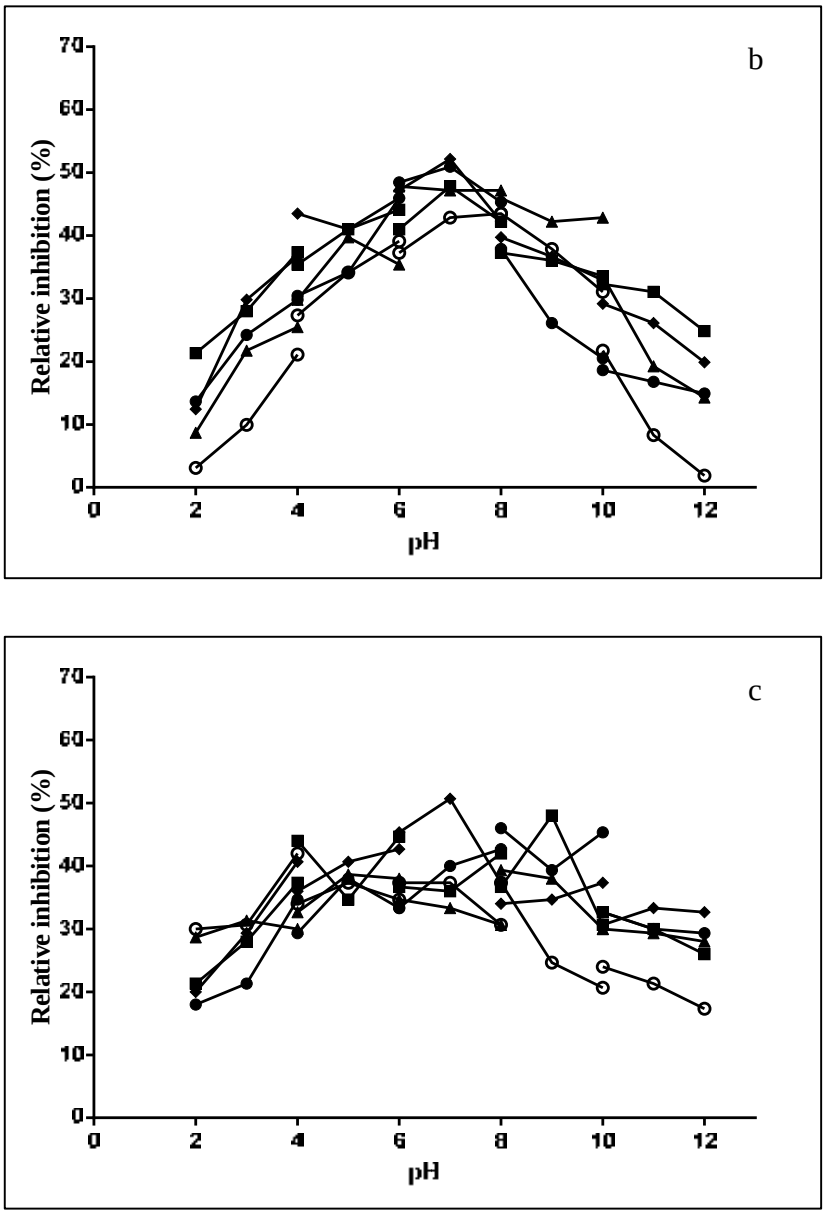
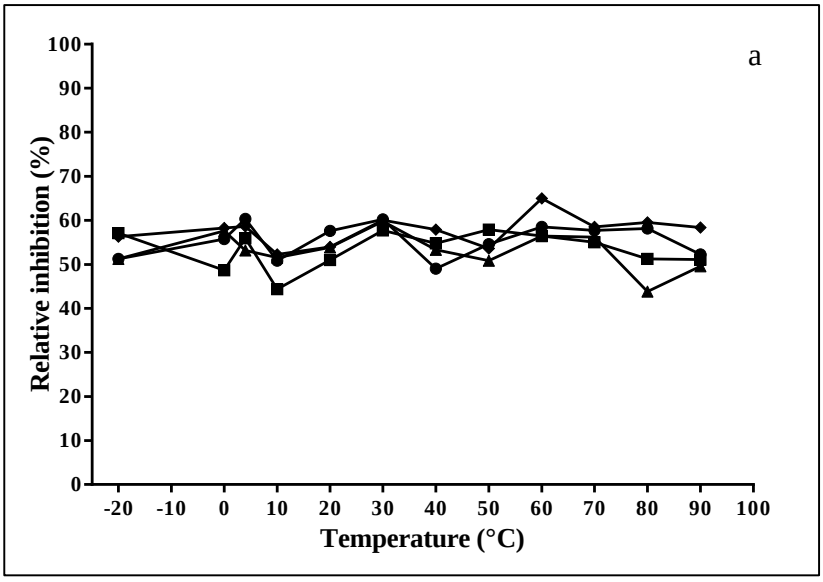


Figure 4.1 pH stability of ACEIs; a) unripen papaya seeds; b) longan seeds; and c) lychee seeds with various incubation times; 0 min (diamond); 30 min (close circle); 60 min (square); 90 min (triangle); and 120 min (open circle). All data are shown as the average mean ± 1 standard error of the mean and are obtained from 3 replicated determinations.

4.4 Temperature resistance of ACEI

The thermal stability of the ACEIs from various Thai fruits seeds protein hydrolysates are shown in Figure 4.2 (a – c). The relative inhibition activity of the ACEIs were wide range of temperature. Most of ACEIs gave the prominence relative percent inhibition at -20 – 80 °C and decreased the inhibition ability at 90 °C with various incubation times (30, 60, 90, and 120 min). The higher temperature and longer incubation cause the changing in the ability of ACEIs regions of the protein structure to bind enzyme.

From the previous studied, Yodjun *et.al*. (2012) reported the F75 of *Z. ottensii* rhizome showed the optimal ACEI activity at -20 - 60 °C. Rungseang *et. al.* (2013) reported that the post-DEAE cellulose unbound fraction of *Z. officinale* showed the optimal acetylcholinesterase inhibitory activity at -20 – 60 °C.



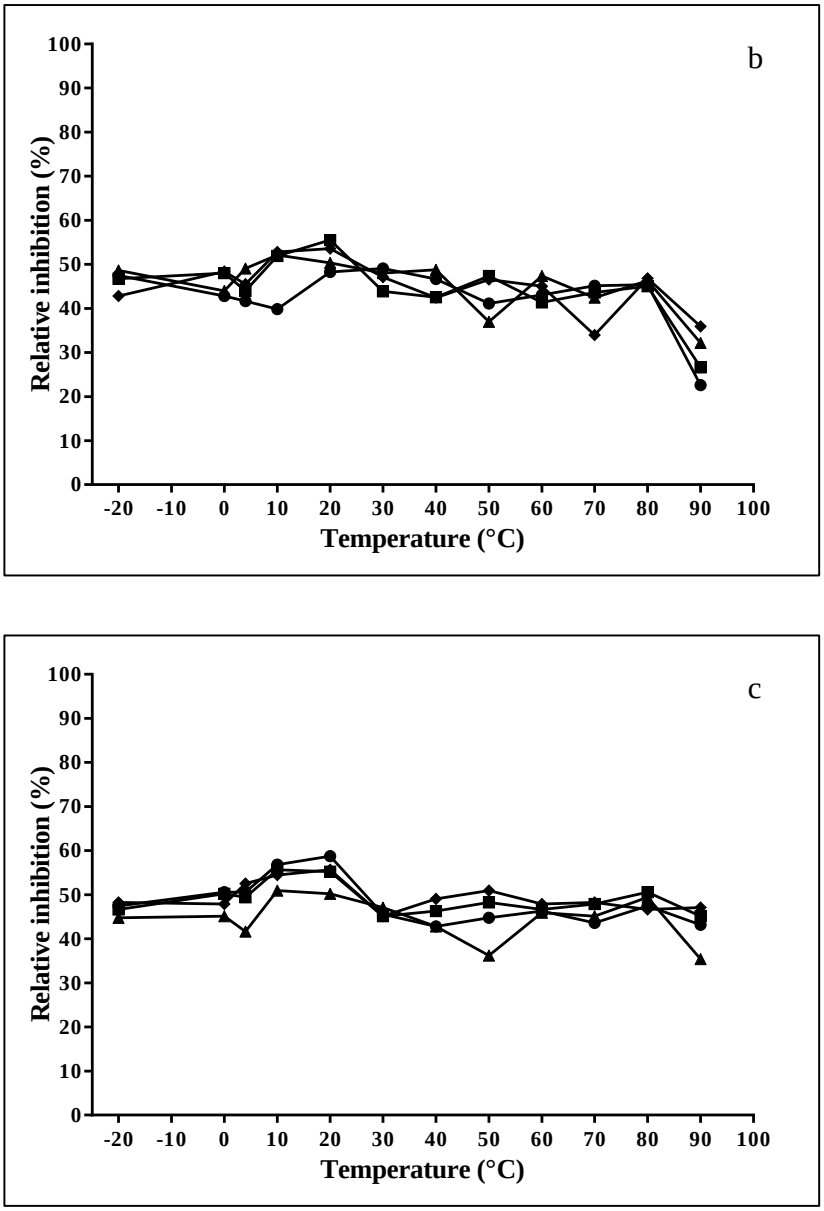


Figure 4.2 Thermostability of ACEIs; a) of unripen papaya seeds; b) longan seeds; and c) lychee seeds. With varies time; 30 min (diamond); 60 min (close circle); 90 min (square); and 120 min (triangle). All data are shown as the average mean ± 1 standard error of the mean and are obtained from 3 replicated determinations.

4.5 Partial purification of the hydrolysate proteins

Protein hydrolysates were prepared from the crude protein of 4 kinds of Thai fruits seeds with pepsin and pancreatin and were collected for further fractionation by ultrafiltration. Table 4.3 shows that the protein hydrolysates were fractionated into 3 parts as UF-1 (>10 kDa), UF-2 (5-10 kDa), and UF-3 (<5 kDa) by ultrafiltration membrane bioreactor system. Table 4.3 also shows that the ACEIs activity of ultrafiltration fractions varied with the molecular mass distribution, and the ACEIs activity were increased with decreasing of molecular weight cut-off. Thus, the low molecular weight peptides indicated the higher inhibition activity than the high molecular weight. The UF-3 (< 5 kDa) of longan seeds protein hydrolysate showed the most potent of ACEI activity with IC_{50} value at 0.43±0.011 mg protein / ml. This fraction was further subjected to high performance liquid chromatography (HPLC).

Won-Ko *et al*. (2006) reported that the yellowfine sole (*Limanda aspera*) frame protein hydrolysate were fractionated by ultrafiltration membrane bioreactor system into 3 ranges of MWCO as YFPH-I (30-10 kDa), YFPH-II (10-5 kDa), and YFPH-III (< 5 kDa) and the YFPH-III had the highest ACEI activity with an IC_{50} value of 0.883 mg protein / ml. Mohtar *et al*. (2012) reported that the winged bean (*Psophocarous tetragonolobus*) protein hydrolysate by four proteolytic enzymes as flavourzyme, alcalase, bromelain and papain was separated by ultrafiltration membrane bioreactor system with MWCO 10, 5 and 2 kDa and found that the 2 kDa had the highest ACEI activity with an IC_{50} value of 0.003 and 0.130 mg protein / ml.

Table 4.3 ACEIs activities of the protein hydrolysates fractionated by ultrafiltration.

Plant seed species	IC_{50} values		
	UF-1 (> 10 kDa) (mg protein/ml)	**UF-1 (10-5 kDa) (mg protein/ml)**	**UF-3 (< 5 kDa) (mg protein/ml)**
C papaya L. (unripen papaya)	19.77±0.011	4.68±0.007	ND
D. longan Lour. subsp. (longan)	9.25±0.017	1.95±0.006	0.43±0.011
L. chinensis Sonn. (lychee)	ND	1.47±0.005	ND

ND = Not detected

All data are shown as the average mean ± 1 standard error of the mean and are obtained from 3 replicated determinations.

4.6 Isolation of ACEI peptides

After partial purified with ultrafitration technique, UF-3 of longan seeds protein hydrolysate had the most ACEI activity potency. Thus, this fraction was further analyzed by RP-HPLC at 280 nm on a Shimpak C_{18} column using trifluoroacetic acid/acetonitrile solvent system to separate of the peptides. Figure 4.3 shows that 5 peaks were eluted. The fractions were separately collected and named as P1 - P5. Each fractions were collected by subsequently retention time. P1 was collected at 3 - 5 min; P2 was collected at 5 - 7 min; P3 was collected at 7 - 8 min; P4 was collected at 8 - 10 min; and P5 was collected at 15 - 17 min, respectively. After purification, P1 - P5 were further analyzed by LC/MS/MS to identify the amino acid sequences.

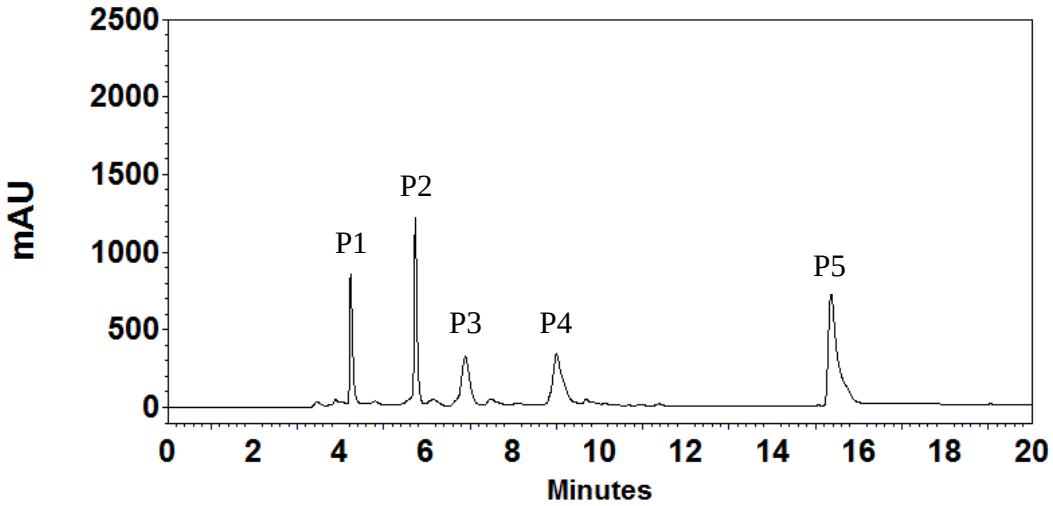


Figure 4.3 Preparative RP-HPLC profile of UF-3 longan seeds protein hydrolysate.

4.7 Identification of ACEI peptides

From RP-HPLC fractionation, five fractions were subjected to LC/MS/MS analysis for determination of the sequences of the peptides contained. The sequences of peptides identified are P1-F1 (Glu-Thr-Ser-Gly-Met-Lys-Pro-Thr-Glu-Leu) was related to Varicose-related protein in *Arabidopsis thaliana* and P1-F2 (His-Asp-Met-Arg-Ser-Cys-Cys-Val-Asp-Ile-Asp-His-Val-Ser-Leu-Tyr-Asn-Leu) was related to pentatricopeptide repeat-containing protein At2g39620 in *A. thaliana*. The peptides sequence of P2 is P2-F1 (Leu-Val-Ser-Seer-Asp-Pro-Asp-Ile-Ser-Gln-Arg-Met-Phe) was related to enzyme nicotianamine synthase in *Noccaea caerulescens*; the peptides sequences of P3 are P3-F1 (Ile-Ser-Ser-Met-Gly-Ile-Leu-Val-Cys-Leu) was related to enzyme vacuolar proton-pyrophosphatase in *Potamogeton distinctus*, P3-F2 (Thr-Asn-Gln-Val-Val-Ser-Glu-Met-Gly-Ile-Ala-Ala-Gly-Ala-Ala-Leu) was related to hypothetical protein OsI_04393 in *Oryza sativa* Indica Group, P3-F3 (Val-Arg-Ala-Met-Val-Ala-Glu-Cys-Leu) was related to hypothetical protein CARUB_v10000363mg in *Capsella rubella*, and P3-F4 (Ile-Ser-Tyr-Val-Val-Pro-Val-Tyr-Ile-Ala-Glu-Ile-Thr-Pro-Lys-Thr-Phe-Arg-Gly-Gly-Phe) was related to Beta integral membrane protein (gb|U43629) in *A. thaliana*. The peptides sequences of P4 are P4-F1 (Thr-Leu-Ala-Met-His-Tyr-Phe) was

related to ferric reductase-like transmembrane component family protein in *A. thaliana* and P4-F2 (Arg-Ser-Ile-Arg-Ile-Thr-Gly-Phe-Gly-Ser-Ser-Ser-Asp-Leu) was related to scarecrow transcription factor family protein in *A. lyrata* subsp. *lyrata*.

The previous report has been shown that the ACEIs properties of peptides contain the positive charged amino acids (arginine/lysine) at C-terminal is important for ACE inhibition (Meisel, 1998). Moreover, it has been reported about the positively charged amino acids in the middle position of tri-peptides had a stronger inhibition activity. For example, Ile-Arg-Tyr showed inhibitory activity five times stronger than that of Ile-Gln-Tyr (Majumder and Wu, 2010). From other studied, ACE might prefer to have substrates or inhibitors that contain tryptophan, tyrosine, phenylanine, proline, and a hydrophobic amino acid at the first three C-terminal position residues to contribute the inhibitory potency (Cheung *et al*., 1980; Wu *et al*., 2006)

From the experimental results, the peptides sequences of UF-3 longan seeds protein hydrolysate contained hydrophobic amino acid at the C-terminal and the positive charged amino acids in the middle of the peptides which were P1-F1 (Glu-Thr-Ser-Gly-Met-Lys-Pro-Thr-Glu-Leu), P3-F1 (Ile-Ser-Ser-Met-Gly-Ile-Leu-Val-Cys-Leu), and P3-F4 (Ile-Ser-Tyr-Val-Val-Pro-Val-Tyr-Ile-Ala-Glu-Ile-Thr-Pro-Lys-Thr-Phe-Arg-Gly-Gly-Phe). These peptides were possibility the most inhibitory activity peptides. Thus, these peptides were proposed to synthesize for further analysis.

4.8 Amino acid profile

The total amino acid contents of the crude protein of five kinds of Thai fruits seeds calculated on dry weight are shown in Table 4.4. The amino acid profile showed the amount of hydrophilic amino acids (unripen papaya seeds = 13.36, ripen papaya seeds = 12.87, rambutan seeds = 3.2, longan seeds = 2.63, and lychee seeds = 1.92 mg / 100mg protein) are higher than the hydrophobic amino acids (unripen papaya seeds = 8.48, ripen papaya seeds = 8.3, rambutan seeds = 2.69, longan seeds = 2.16, and lychee seeds = 1.75 mg / 100mg protein).

From the result, the high content of positive charge amino acids (arginine/lysine) are the two forms of papaya seeds, following by rambutan seeds, longan seeds, and lychee seeds, respectively. The high content of hydrophobic amino acids is unripen

papaya seeds following by ripen papaya seeds, the moderate contents are rambutan seeds and longan seeds, with lychee seeds had moderate hydrophobic amino acids content.

Table 4.4 Amino acids content of four kinds of Thai fruit seeds

Amino acids	Contents (mg / 100 mg protein dry weight)				
	unripen papaya seeds	**ripen papaya seeds**	**rambutan seeds**	**longan seeds**	**lychee seeds**
Hydrophilic					
Aspartic acid	3.52	2.93	0.57	0.49	0.39
Serine	0.88	0.89	0.37	0.30	0.24
Glutamic acid	3.55	3.28	0.89	0.55	0.43
Histidine	0.36	0.28	0.13	0.11	0.09
Arginine	1.66	1.56	0.54	0.54	0.29
Threonine	0.92	1.02	0.32	0.29	0.24
Lysine	2.47	2.91	0.38	0.35	0.24
Total	13.36	12.87	3.2	2.63	1.92
Hydrophobic					
Glycine	0.50	0.41	0.63	0.44	0.26
Alanine	0.72	0.37	0.26	0.20	0.24
Proline	1.98	2.96	0.28	0.25	0.20
Tyrosine	2.29	2.38	0.18	0.14	0.09
Valine	0.74	0.53	0.39	0.29	0.26
Isoleucine	0.62	0.46	0.24	0.20	0.18
Leucine	0.92	0.68	0.42	0.36	0.30
Phenylalanine	0.71	0.51	0.29	0.28	0.22
Total	8.48	8.3	2.69	2.16	1.75

5. CONCLUSSION

ACE inhibitory peptides were investigated from 4 kinds of the Thai fruits seeds, which were unripen and ripen papaya seeds, rambutan seeds, longan seeds, and lychee seeds. The crude proteins were hydrolyzed by stimulation of human digestion using gastric enzyme such as pepsin and pancreatin. The hydrolysates were partial purified by ultrafiltration technique with MWCO at 10 and 5 kDa. After fractionated, UF-3 of longan seeds protein hydrolysate (below 5 kDa) had the highest ACE inhibitory activity (IC_{50} with 0.43±0.011 mg protein / ml). Thus, this fraction was subjected to RP-HPLC, the collected peak named P1 - P5 were subjected to LC/MS/MS for identification of the peptides sequences. Mass spectra showed 9 peptide sequences, but only P1-F1, P3-F1, and P3-F4 showed the most inhibitory activity. This was the first study to show the production of antihypertensive peptides by enzymatic hydrolysis of protein from the seeds extract of Thai fruits. The result suggested that some Thai fruits could be a source of peptides that might be a potent source of ACEI bioactive compounds.

6. REFERENCES

Ahn, C.B., Jeon, Y.J., Kim, Y.T., and Je, J.Y. (2012). Angiotensin I converting enzyme (ACE) inhibitory peptides from salmon byproduct protein hydrolysate by Alcalase hydrolysis. *Process Biochemistry* 47: 2240-2245.

Akiko, O., Hiroshi, H., and Eiko, M. (1994). Antihypertensive substances in viscous material of fermented soybean (NATTO). *Food hydrocolloids: Structures, Properties, and functions* 497-502

Ariyoshi, Y. (1993). Angiotensin-converting enzyme inhibitors derived from food protein. *Trends Food Science Technol*ogy 4: 139-144.

Asoodeh, A., Yazdi, M., and Chamani, J.K. (2012). Purification and characterisation of angiotensin I converting enzyme inhibitory peptides from lysozyme hydrolysates. *Food Chemistry* 131: 291–295.

Barbana, C., and Boyce, J.I. (2010). Angiotensin I-converting enzyme inhibitory activity of chickpea and pea protein hydrolysates. *Food Research International* 43: 1642-1649.

Beldent, V., Michaud, A., Wei, L., Chauvet, M.T., Corvol, P. (1993), Proteolytic release of human angiotensin converting enzyme : localization of the cleavage site. *Journal of Biological Chemistry* 268: 26428-26434.

Bradford, M.M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of proteins utilizing the principle of protein-dye binding. *Analytical Chemistry* 72:248-254.

Braga, F.C., Serra, C.P., Viana, N.S. Jr., Oliveira, A.B., Côrtes, S.F., and Lombardi, J.A. (2007) Angiotensin-converting enzyme inhibition by Brazilian plants. *Fitoterapia* 78: 353-358.

Carey, R.M., and Siragy, H.M. (2003) Newly recognized components of the renin angiotensin system: Potential roles in cardiovascular and renal regulation. *Endocrine Reviews* 24(3): 261–271.

Cheung, H.S., Wang, F.L., Ondetti, M.A., Sabo, E.F. and Crushman, D.W. (1980). Binding of peptide substrates and inhibitors of angiotensin-converting enzyme, Importance of the COOH-terminal dipeptide sequence. *Journal of Biological Chemistry* 25: 401-407.

Cinqo-Mars, C.D., and Li-Chan, E.C.Y. (2007) Optimizing angiotensin I-converting enzyme inhibitory activity of Pacific Hake (*Merloccius productus*) fillet hydrolysate using response surface methodology and ultrafiltration. *Journal of Agricultural and Food Chemistry* 55: 9380-9388.

Clare, D.A., and Swaisgood, H.E. (2000). Bioactive milk peptides: a prospectus. *Journal of Dairy Science* 83: 1187-1195.

Compos, M.R.S., Guerrero, L.A.C., and Ancona, D.A.B. (2010) Angiotensin-I converting enzyme inhibitory and antioxidant activities of peptide fractions extracted by ultrafiltration of cowpea *Vigna unguiculata* hydrolysates. *Journal of the Science of Food and Agriculture* 90: 2512-2518.

Crushman, W., and Cheung, H.S. (1971). Spectrometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochemical Pharmacology* 20:1637-1648.

Curtiss C., Chon J.N., Vrobel T. and Francious J.A. (1978) Role of the rennin-angiotensin system in the systemic vasoconstriction of chronic congestive heart failure. *Circulation*., 58: 763-770.

De Leo, F., Panarese, S., Gallerani, R., and Ceci, L.R. (2009) Angiotensin converting enzyme (ACE) inhibitory peptides: Production and implementation of functional food. *Current Pharmaceutical Design* 15:3622-3543.

Edens, L., Hoevan, R.A.M., and vander Delest, V. (2004) Protein hydrolysates enriched in peptides having a carboxy terminal proline residues. *United States Patent Application* 0241791.

Ehlers, R.W., Riordan, J.F. (1989) Angiotensin-converting enzyme: new concepts concerning its biological role. *Biochemistry*, 28: 5311-5318.

Elbl, G., Wagner, H. (1994). New method for the in vitro screening of inhibitors of angiotensin converting enzyme (ACE), using the chromophore- and fluorophore labelled substrate, dansyltriglycine. *Planta Medica*., 57: 137-141.

FitzGeralk, R.J., Meusel, H. (1999) Lactokinins: whey protein-derived ACE inhibitory peptides. *Nahrung/Food*, 165-167.

Franek, F., Hohenwarter, O., and Katinger, H. (2000) Plant protein hydrolysates: Preparation of defined peptide fractions promoting growth and production in animal cells cultures. *Biotechnology Progress* 16: 688-692.

Gao, D., Chang, T., Li, H., and Cao, Y. (2010) Angiotensin I-converting enzyme inhibitor derived from cottonseed protein hydrolysate. *African Journal Biotechnology* 9: 8977-8983.

Ganten, D., Unger, T., and Lang, R.E. (1984) Pharmacological interferences with the renin-angiotensin system. *Arzneimittelforschung* 34: 1391-1398.

Goretta, L.A., Ottaviani, J.I., and Fraga, C.G. (2006). Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. *Journal of Agricultural and Food Chemistry*., 54:229-234.

Holmquist, B., Bunning, P., Riordan, J.F. (1979) A continuous spectrophotometric assay for the angiotensin converting enzyme. *Analytical Biochemistry* 95: 540-548.

Inagami, T. (1992) The renin-angiotensin system. *Biochemical Society Essays in Biochemistry* 28: 147-164.

Je, J.Y., Park, J.Y., Jung, W.K., Park, P.J., and Kim, S.K. (2005) Isolation of angiotensin I-converting enzyme (ACE) inhibitor from fermented oyster sauce, *Crassostrea gigas*. *Food Chemistry*., 90: 809-814.

Katayama, K. Anggraeni, H.E., Mori, T., Ahhmed, A.A., Kawahara, S., Sugiyama, M., et.al., (2008) Porcine skeletal muscle troponin is good source of peptides with angiotensin-converting enzyme inhibitory activity and antihypertensive effects in spontaneously hypertensive rats. *Journal of Agricultural and Food Chemistry* 56: 355-360.

Kim, S.K., and Wijesekara, I. (2010) Development and biological activities of marine-derived bioactive peptide: A review. *Journal of Functional Foods* 2: 1-9.

Li, G.H., Wan, J.Z., Le, G.W., and Shi, Y.H. (2006) Novel angiotensin I-converting enzyme inhibitory peptides isolated from alcalase hydrolysate of mung bean protein. *Journal of Peptide Science* 12: 509-514.

Ma, M.S., Bae, I.Y., Lee, H.G., and Yang, C.B. (2006) Purification and identification of angiotensin I-converting enzyme inhibitory peptide from buckwheat (*Fagopyrum esculentum* Moench). *Food Chemistry* 96: 36-42.

Mahmaod, M.I. (1994) Physicochemical and functional properties of protein hydrolysates in nutritional products. *Journal of Food Science* 59: 89-95.

Matsui, T., Yukiyoshi, A., Doi, S., Sugimoto, H., Yamada, H. and Matsumoto, K. (2002). Gastrointestinal enzyme production of bioactive peptides from royal jelly protein and their antihypertensive ability in SHR. *The Journal of Nutritional Biochemistry* 13: 80-86.

Marczak, E.D., Usui, H., FuJita, H., Yang, Y., Megumi Yokoo, Lipkowski, A.W., and Yoshikawa, M. (2003) New antihypertensive peptides isolated from rapeseed. *Peptides* 24: 791-798.

Medina-Gody, S., Ambriz-perez, D.L., Fuentes-Gutierrez, C.I., German-Baez, L.J., Gutierrez-Dorodo, R., Reyes-Moreno, C., and Valdez-Ortiz, A. (2011) Angiotensin-

converting enzyme inhibitory and antioxidative activities and functional characterization of protein hydrolysate of hard-to-cook chickpeas. *Journal of Agricultural and Food Chemistry* 92:1974-1981.

Mehanna, A.S., Dowling, M. (1999) Liquid chromatographic determination of hippuric acid for the evaluation of ethacrynic acid as angiotensin converting enzyme inhibitor. *Journal of Pharmaceutical and Biomedical Analysis* 19: 967-973.

Meisel, H. (1993) Casokinins as inhibitors of angiotensin-converting enzyme. In: Sawatzki, G., Renner, B. (Eds). New Perspectives in Infant Nutrition. *Stuttgart, New York: Thieme,* 153-159.

Möller, N.P., Scholz-Ahrens, K.E, Roos, N., and Schrezenmeir, J. (2008) Bioactive peptides and proteins from foods: indication for health effects. *European Journal of Nutrition* 47, 171-182.

Natesh, R., Schwager, S.L.U., Sturrock, E.D., Acharya, R. (2003) Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. *Nature* 421: 551-554.

Ondetti, M.A., Rubin, B., and Cushman, D.W. (1977). Design of specific inhibitors of angiotensin-converting enzyme: new class of orally active antihypertensive agents. *Science* 196: 441-444.

Oudit, G.Y., Crackower, M.A., Backx, P.H., Penninger, J.M. (2003) The role of ACE2 on cardiovascular physiology. *Trends in Cardiovascular Medicine* 13: 93-101.

Pihlanto, A., Akkanen, S., and Korhonen, H.J. (2008) ACE-inhibitory and antioxidant properties of potato (*Solanum tuberosum*). *Food Chemistry* 109.1044-112.

Quitain, A.T., Sato, N., Daimon, H., and Fujie, K. (2001) Production of valuable materials by hydrothermal treatment of shrimp shells. *Industrial and Engineering Chemistry Research* 40: 5885-5888.

Rao, S.Q., Ju, T., Sun, J., Su, Y.J., Xu, R.R., and Yang, Y.J. (2012) Purification and characterization of angiotensin I-converting enzyme inhibitory peptides from enzymatic hydrolysate of hen egg white lysozyme. *Food research International* 46: 127-134.

Ruiz, J.A., ramos, M., and Recio, I. (2004). Angiotensin converting enzyme-inhibitory activity of peptides isolated from Manchego cheese. Stability under simulated gastrointestinal digestion. *International Dairy Journal* 14: 1075-1080.

Rungsaeng, P., Sangvanich. P., and Karnchanatat, A. (2013) Zingipain, a Ginger Protease with Acetylcholinesterase Inhibitory Activity. *Applied Biochemistry and Biotechnology* 170:934–950

Suetsuna, K. (1998). Purification and identification of angiotensin I-converting enzyme inhibitors from red alga *Porphyra yezoensis*. *Journal of Marine Biotechnology* 6: 163-167.

Takano, T. (1998). Milk derived peptides and hypertension reduction. *International Dairy Journal*., 8:375-381.

Tomatsu, M., Shimakage, A., Shinbo, M., Yamada, S., and Takahashi, S. (2013) Novel angiotensin I-converting enzyme inhibitory peptides derived from soya milk. *Food Chemistry* 136: 612-616.

Torres-Fuentes, C., Alaiz, M., and Vioque, J. (2011) Affinity purification and characterization of chelating peptides from chickpea protein hydrolysates. *Food Chemistry* 129: 485-490.

Turner, A.J., Hooper, N.M. (2002) The angiotensin-converting enzyme gene family, genomics and pharmacology. *Trends in Pharmacological Sciences* 23: 177-183.

Vermeirssen, V., Van Camp, J., and Verstrate, W. (2002) Optimization and validation of an angiotensin-converting enzyme inhibition assay for the screening of bioactive peptides. *Journal of Biochemical and Biophysical Methods* 51: 75-87.

Wan Mohtar, W.A.A.-Q.I., Hamid, A.A., Muhamad, S.K.S., and Saari, N. (2013) Preparation of bioactive peptides with high angiotensin converting enzyme inhibitory activity from winged bean (*Psophocarpus tetragonolobus* (L.) DC.) seed. *Journal of Food Science and Technology* 51: 3658-3668.

Wei, L., Clauser, E., Alhenc-Gelas, F., and Corvol, P. (1992) The two homologous domains of the human angiotensin I-converting enzyme interact differently with competitive inhibitors. *The Journal of Biological Chemistry* 267: 13398-13405.

Wijesekara, I., Qian, Z.J., Ryu, B., Ngo, D.H., and Kim, S.K. (2011). Purification and identification of antihypertensive peptides from seaweed pipefish (*Syngnathus schlegeli*) muscle protein hydrolysate. *Food Research International* 44: 703-707.

Wu, J., Aluko, A.E., and Muir, A.D. (2009). Production of angiotensin I-Converting enzyme inhibitory peptides from defatted canola meal. *Bioresource Technology*., 100: 5283-5287.

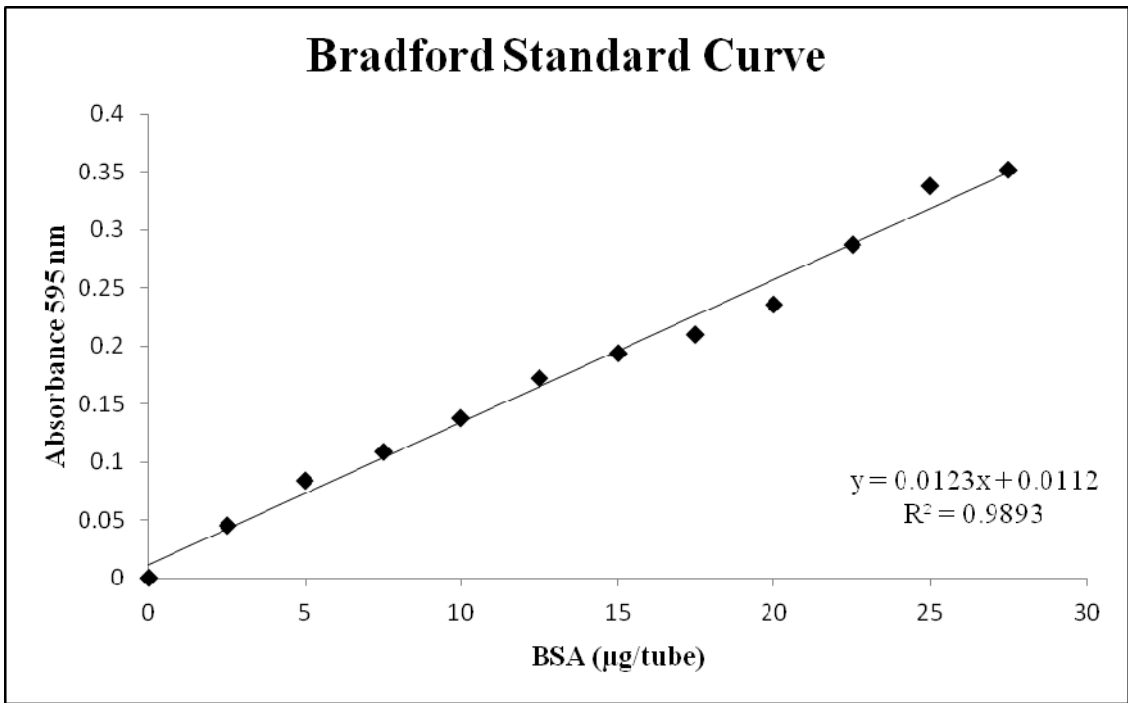
Yang, H.Y.T., Erdos, E.G., and Levin, Y. (1970). A dipeptidyl carboxypeptidase that converts angiotensin I and inactivates bradykinin. *Biochimica et Biophysica Acta (BBA) - Protein Structure* 214: 374–376.

Yodjun, M., Karnchanatat, A., and Sangvanich, P. (2012) Angiotensin I-converting enzyme inhibitory proteins and peptides from the rhizomes of Zingiberaceae plants. *Applied Biochemistry and Biotechnology* 166: 2037-2050.

APPENDICES

APPENDIX A

Calibration curve for protein determination by Bradford method



APPENDIX B

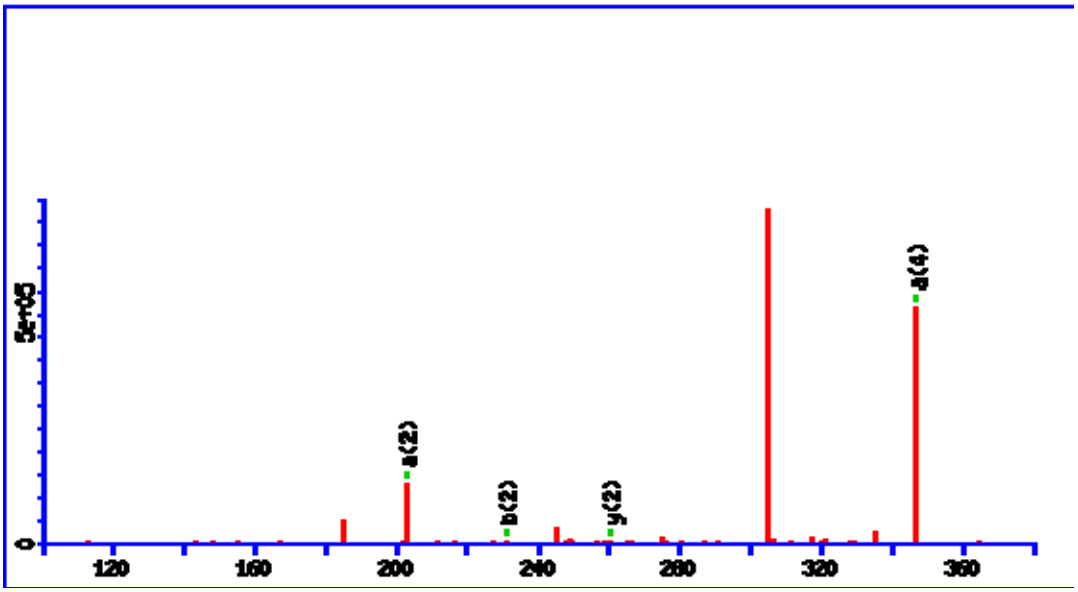
Amino acid abbreviations

Amino acid	Three-letter	One-letter
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V

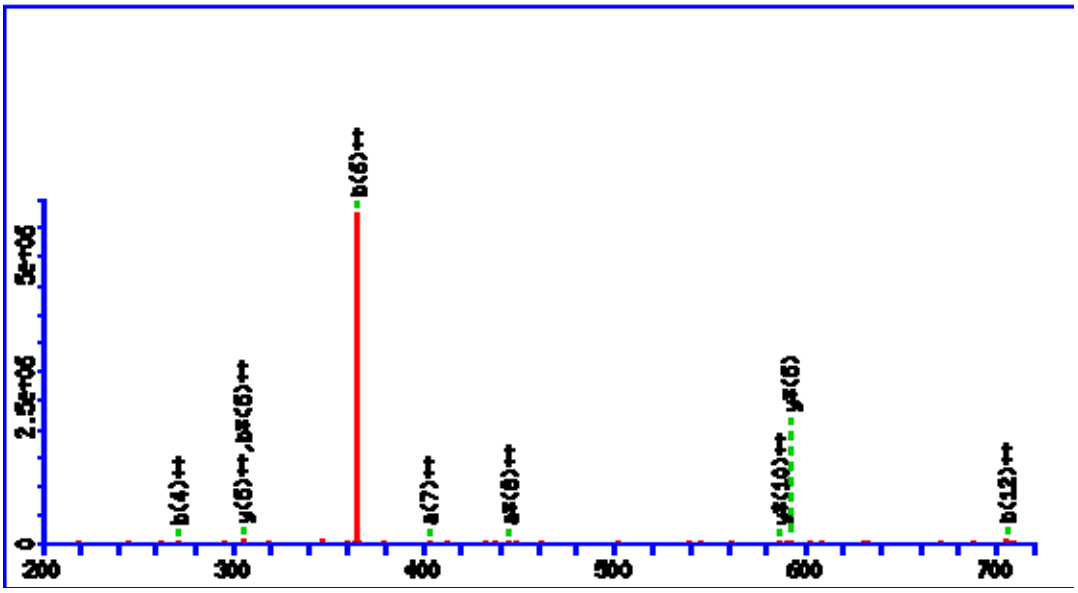
APPENDIX C

Molecular weight of 4 peaks from MS/MS spectrum of the UF-3 from longan seeds protein hydrolyste by RP-HPLC.

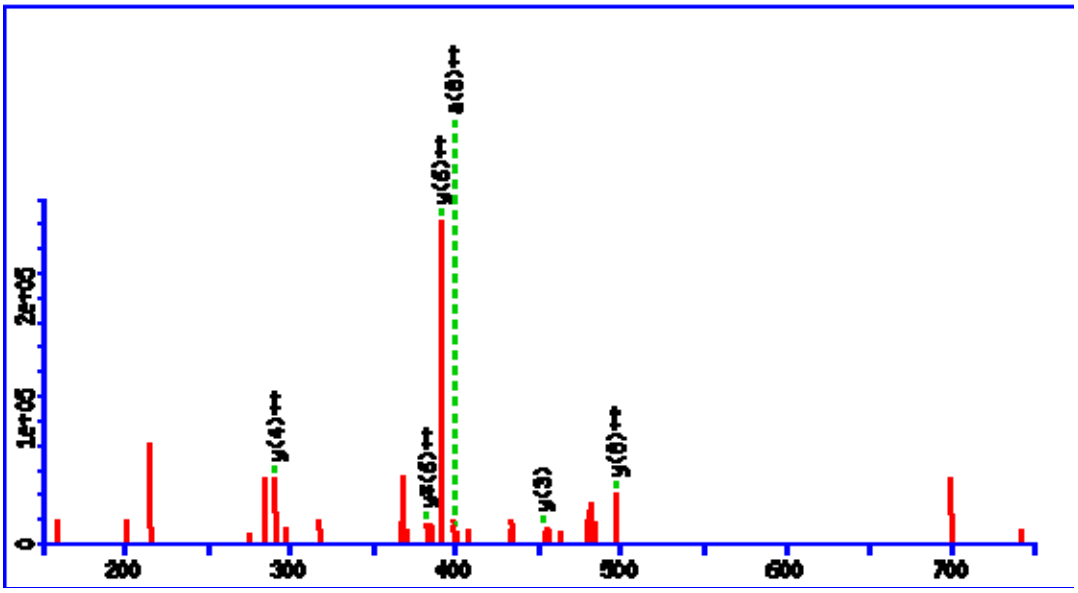
P1-F1
Observed: 365.0000
Mr(expt); 1091.9782
Mr(calc); 1091.5169
Unique Peptide: ETSGMKPTEL



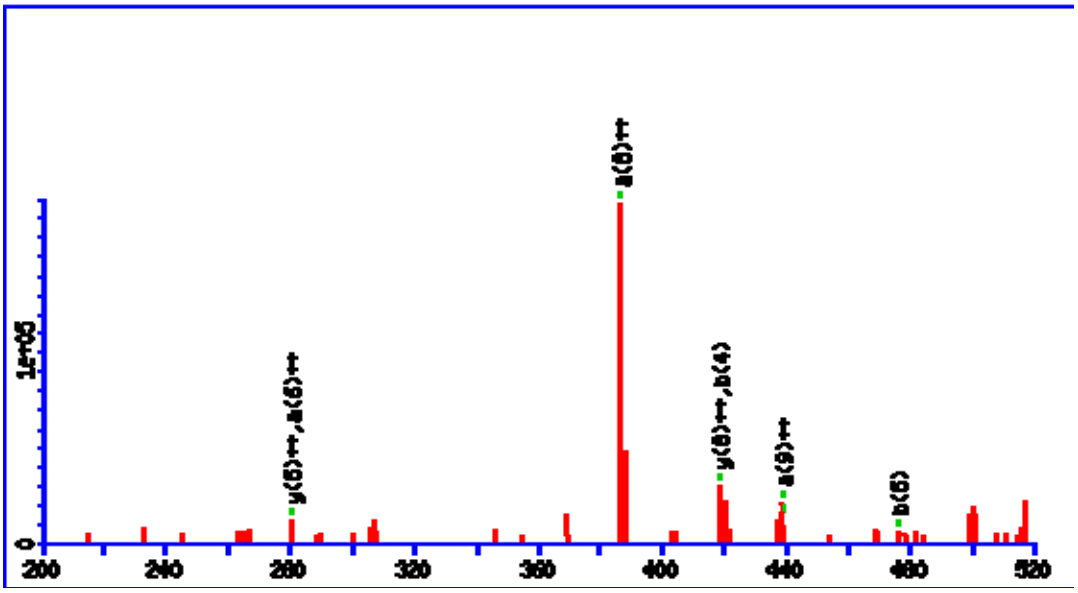
P1-F2
Observed: 707.0000
Mr(expt): 2117.9782
Mr(calc): 2118.9285
Unique Peptide: HDMRSCCVDIDHVSLYNL



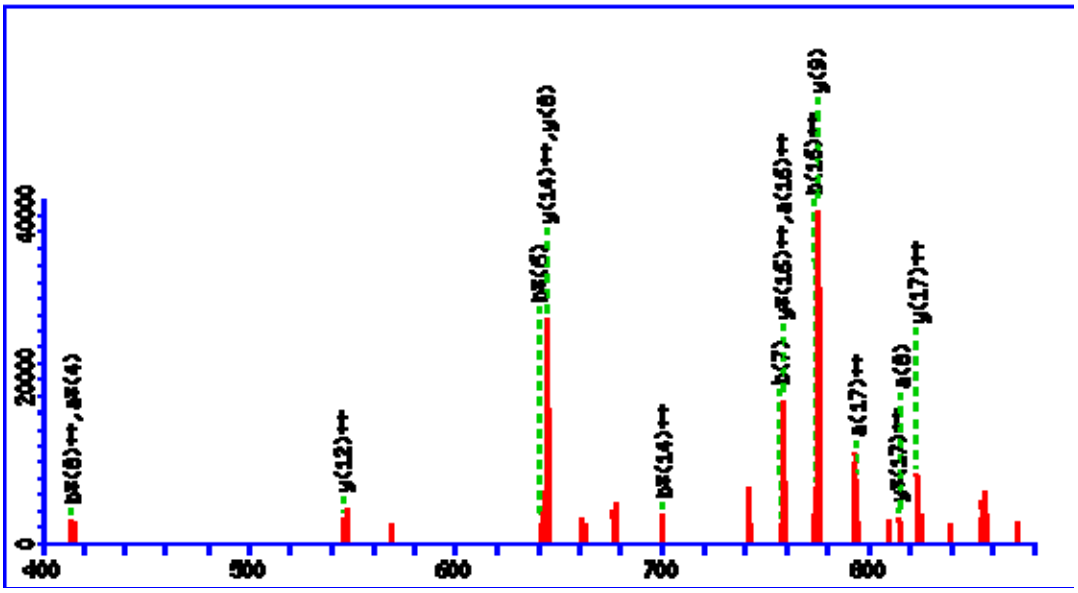
P2-F1
Observed: 498.7000
Mr(expt): 1493.0782
Mr(calc): 1493.7184
Unique Peptide: LVSSDPDISQRMF



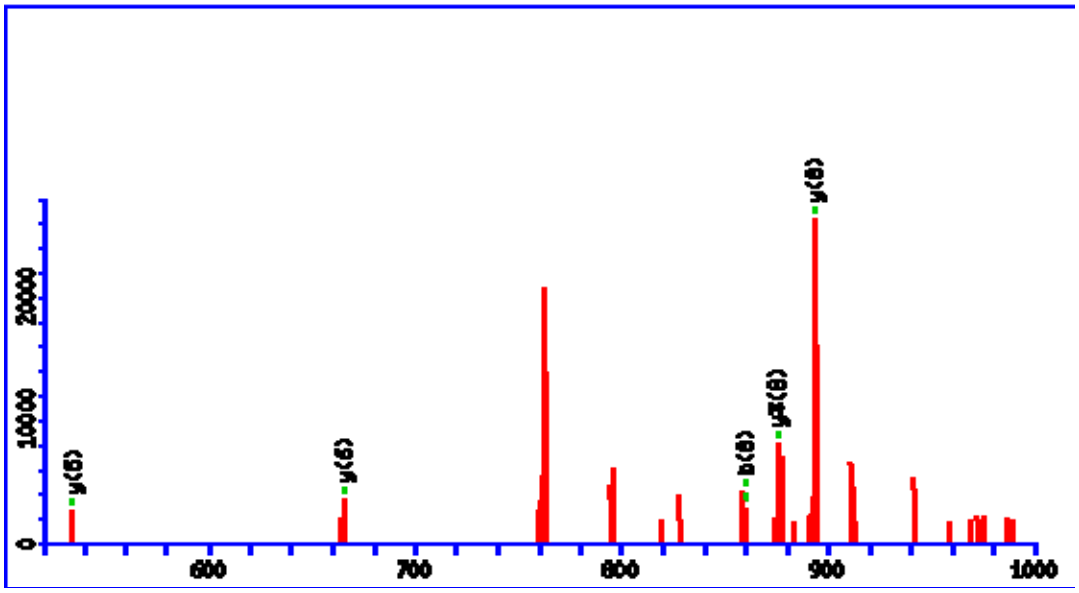
P3-F1
Observed: 518.1000
Mr(expt): 1034.1854
Mr(calc): 1034.5504
Unique Peptide: ISSMGILVCL



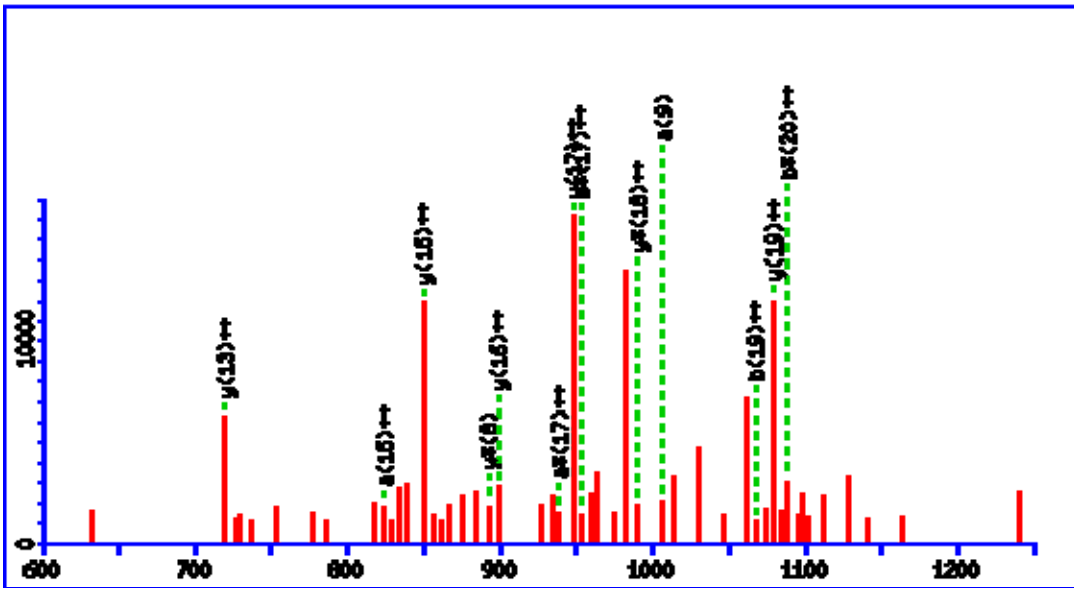
P3-F2
Observed: 874.0000
Mr(expt): 1745.9854
Mr(calc): 1744.8665
Unique Peptide: TNQDVVVSEMGIAAGAAL



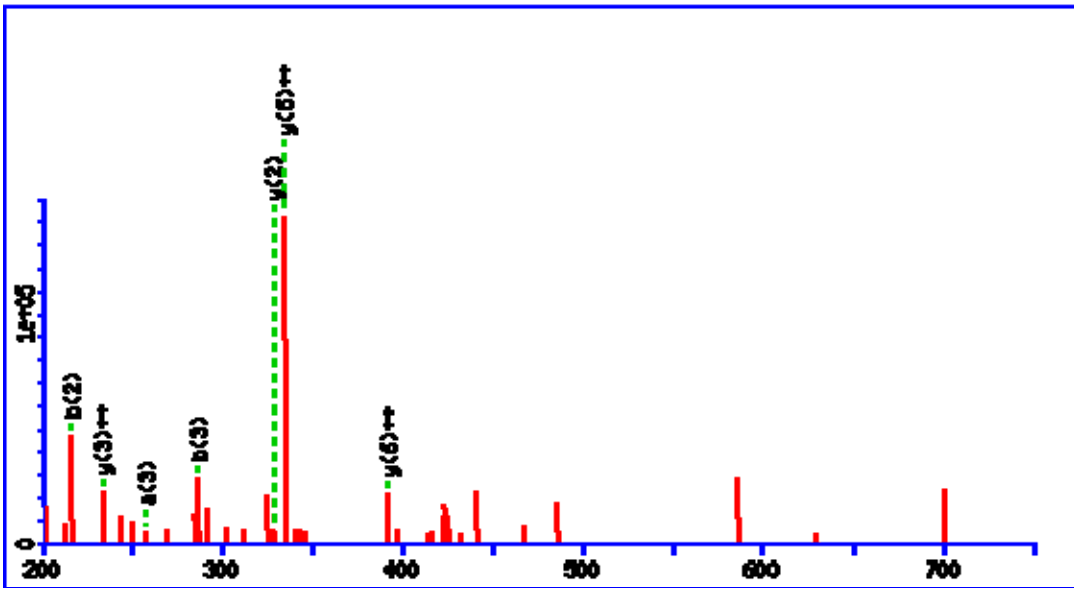
P3-F3
Observed: 992.0000
Mr(expt): 990.9927
Mr(calc): 990.4990
Unique Peptide: VRAMVAECL



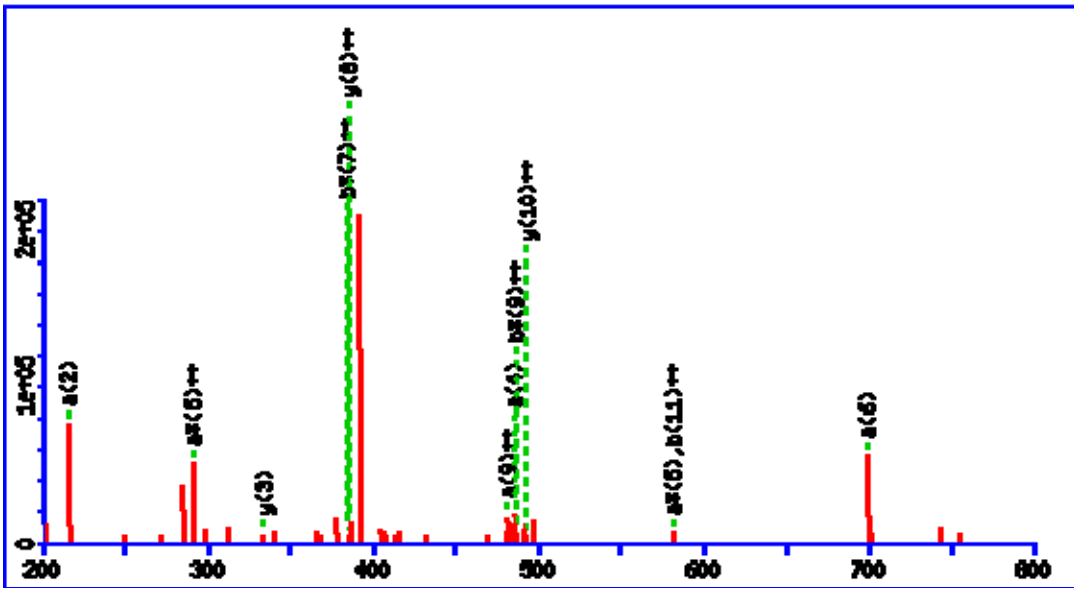
P3-F4
Observed: 1179.0000
Mr(expt): 2355.9854
Mr(calc): 2356.2831
Unique Peptide: ISYVVPVYIAEITPKTFRGGF



P4-F1
Observed: 442.0000
Mr(expt): 881.9854
Mr(calc): 881.4106
Unique Peptide: TLAMHYF



P4-F2
Observed: 499.0000
Mr(expt): 1493.9782
Mr(calc): 1494.7791
Unique Peptide: RSIRITGFGSSSDL



Curriculum Vitae

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4. Education

University	Degree	Field	Year
Chulalongkorn University	Ph.D.	Biotechnology	2006
Chulalongkorn University	M.Sc.	Biochemistry	2001
Ramkhamhaeng University	B.Sc.	Chemistry	1998

5. Research interest
   - 5.1 Enzyme biotechnology
   - 5.2 Protein and peptide chemistry: Structure and function
   - 5.3 Chemical natural products
   - 5.4 Fungal bioremediation
6. Career
   - 6.1 Assistance Director of The Institute of Biotechnology and Genetic Engineering, Chulalongkorn University (2012-present)
   - 6.2 Quality Assurance Manager of The Institute of Biotechnology and Genetic Engineering, Chulalongkorn University (2012-present)
   - 6.3 Deputy Director of The Institute of Biotechnology and Genetic Engineering, Chulalongkorn University (2014-present)

7. Award and honors
    7.1 Research Award, Office of the National Research Council of Thailand, Uptake of inorganic and organic nitrogen compounds in the cyanobacterium *Aphanothece halophytica* under osmotic stress, 2008.
    7.2 Fibrinolytic enzyme from sand warm *Perinereis nuntia*, Thailand Toray Science Foundation 2010.
    7.3 The third prize for poster presentation, 14th Food Innovation Asia Conference 2012; "Green and Sustainable Food Technology for All", Antioxidation of polysaccharide-protein complex extracted from *Phaeogyroporus portentosus* (Berk. & Broome) McNabb, 2012
    7.4 Development of therapeutic leads from protein hydrolysate: A case study of Thai fruit seeds, Thailand Toray Science Foundation 2014.
8. Grants and fellowships
    8.1 Production, purification and biochemical characterization of lignin degrading enzymes from Psilocybe mushroom and its application in decolorization of synthetic dyes, Ratchadaphiseksomphot Endowment Fund, 2008-2009.
    8.2 Structure analysis and antitumor activity of polysaccharide from *Phaeogyroporus portentosus* (Berk. & Broome McNabb), The Thailand Research Fund, 2008-2010.
    8.3 Purification and characterization of lectin from rhizomes of *Curcuma amarissima* Roscoe. TRF-MAG Window II Co-funding, 2008-2010.
    8.4 L-Asparaginase from xylariaceous fungi and application in antitumor activity, Office of the National Research Council of Thailand, 2009-2011.
    8.5 Amino acid sequences and biological activities of proteins from xylariaceous fungi, The Institute of Biotechnology and Genetic Engineering, 2009-2010.
    8.6 Amino acid sequences and biological activities of proteins from *Sterculia monosperma* Vent., The Institute of Biotechnology and Genetic Engineering, 2009-2010.

8.7 Purification and characterization of xylanase from endophytic fungi isolated from thai medicinal plants, TRF-MAG Window II Co-funding, 2009-2011.

8.8 Purification and characterization of lipase from endophytic fungi isolated from thai medicinal plants, TRF-MAG Window II Co-funding, 2008-2010.

8.9 Alpha-glucosidase inhibitor from *Archidendron jiringa* Nielsen. and Parkia speciosa *Hassk*. seeds, TRF-MAG Window II Co-funding, 2008-2010

8.10 Protein and peptide with *acetylcholinesterase* inhibitory activity from the rhizomes of Zingiberaceae plants, TRF-MAG Window II Co-funding, 2010-2012.

8.11 Protein and peptide with *antiproliferative* activity of *macrophage RAW 264.7* from the rhizomes of Zingiberaceae plants, TRF-MAG Window II Co-funding, 2010-2012.

8.12 Smart biopolymer from Thai medicinal plants for therapeutic use, National Research University, 2010-2012.

8.13 Fibrinolytic enzyme from sand warm *Perinereis nuntia*, National Research Council, 2011-2012.

8.14 *Tyrosinase* inhibitory activity of the protein hydrolysate from the seeds of Thai fruits, TRF-MAG Window II Co-funding, 2011-2013.

8.15 Protein hydrolysate from from Thai fruit seeds for therapeutic use, National Research University, 2013-2014.

8.16 Protein hydrolysate from Thai fruit seeds for therapeutic use, National Research University, 2013-2014.

8.17 Development of therapeutic leads for cardiovascular diseases: A case study of fibrinolytic enzyme from sand warm, National Research University, 2014.

8.18 Preparation of protein hydrolysate from chicken feather meal for applications in health products and cosmetics, Researchers and Research for Industry Grants: Master Sci. & Tech Grants (RRI Grants-MAG), 2014-2015.

8.19 The use of alkaline protease to produced protein hydrolysate witth biological activities from chicken feather meal, Researchers and Research for Industry Grants: Master Sci. & Tech Grants (RRI Grants-MAG), 2014-2015.

8.20 Development of therapeutic leads for cancer treatment: A case study of bioactive peptide from spotted Babylon, Researchers and Research for Industry Grants: Master Sci. & Tech Grants (RRI Grants-MAG), 2014-2015.

9. Publications

9.1 Incharoensakdi, A.***** and **Karnchanatat, A.** (2003) Salt stress enhances choline uptake in the halotolelant cyanobacterium *Aphanothece halophytica*. *Biochimica et Biophysica Acta* 1621: 102-109.

9.2 **Karnchanatat, A.,** Petsom, A., Sangvanich, P., Piaphukiew, J., Whalley, A.J.S., Reynolds, C.D., and Sihanonth, P.***** (2007) Purification and biochemical characterization of an extracellular β-glucosidase from wood-decaying fungus *Daldinia eschscholzii* (Ehrenb.:Fr.) Rehm. *FEMS Microbiology Letters* 270:162-170.

9.3 **Karnchanatat, A.,** Petsom, A., Sangvanich, P., Piapukiew, J., Whalley, A.J.S., Reynolds, C.D., and Sihanonth, P.* (2008) A novel thermostable endoglucanase from the wood-decaying fungus *Daldinia eschscholzii* (Ehrenb.:Fr.) Rehm. *Enzyme and Microbial Technology* 2008; 42: 404-413.

9.4 Kheeree, N., Sangvanich, P., Puthong, S., and **Karnchanatat, A.*** (2010) Antifungal and antiproliferative activities of lectin from the rhizomes of *Curcuma amarissima* Roscoe. *Applied Biochemistry and Biotechnology* 162: 912-925.

9.5 Niyomploy, P., Thunyakitpisal, P., **Karnchanatat, A.**, and Sangvanich, P.* (2010) Cell proliferative effect of polyxyloses extracted from the rhizomes of wild tumeric, *Curcuma aromatic* Salisb. *Pharmaceutical Biology* 48: 932-937.

9.6 Konkumnerd, W., **Karnchanatat, A.**, and Sangvanich, P.* (2010) A thermostable lectin from the rhizomes of *Kaempferia parviflora*. *Journal of the Science of Food and Agriculture* 90: 1920-1925.

9.7 Petnual, P., Sangvanich, P., and **Karnchanatat, A.*** (2010) A lectin from the rhizomes of turmeric (*Curcuma longa* L.) and its antifungal, antibacterial and alpha-glucosidase inhibitory activities. *Food Science and Biotechnology* 19: 907-916.

9.8 Tiengburanatam, N., Sangvanich, P., Boonmee, A and **Karnchanatat, A.*** (2010) A novel α-glucosidase inhibitor protein from the rhizomes of *Zingiber ottensii* Valeton. *Applied Biochemistry and Biotechnology* 2010; 2010; 162: 1938-1951.

9.9 Boonmee, A., Srisomsap, C., **Karnchanatat, A.**, and Sangvanich, P.* (2011) An antioxidant protein in *Curcuma comosa* Roxb. rhizomes. *Food Chemistry* 124: 476-480.

9.10 Charungchitrak, S., Petsom, A., Sangvanich, P., and **Karnchanatat, A.*** (2011) Antifungal and antibacterial activities of lectin from the seeds of *Archidendron jiringa* Neilson. *Food Chemistry* 126: 1025-1032.

9.11 **Karnchanatat, A.***, Tiengburanatam, N., Boonmee, A., Puthong, S., and Sangvanich, P. (2011) Zingipain, A cysteine protease from *Zingiber ottensii* Valeton rhizomes with antiproliferative activities against fungi and human malignant cell lines. *Preparative biochemistry and biotechnology* 41: 201-217.

9.12 Tangngamsakul, P., **Karnchanatat, A.**, Sihanonth, P. and Sangvanich, P.* (2011) An extracellular glucoamylase produced by endophytic fungus EF6. *Applied Biochemistry and Microbiology* 47: 412-418.

9.13 Sawaengsak, W., Saisavoey, T., Chuntaratin, P., and **Karnchanatat, A.*** (2011) Micropropagation of the medicinal herb *Glycyrrhiza glabra* L., through shoot tip explant culture and glycyrrhizin detection. *International Research Journal of Plant Science* 2:129-136.

9.14 Baebprasert, W., **Karnchanatat, A.**, Linblad, P., and Incharoensakdi A.* (2011) Na^+-stimulated nitrate uptake with increased activity under osmotic upshift in *Synechocystis* sp. strain PCC 6803. *World Journal of Microbiology and Biotechnology* 27: 2467-2473.

9.15 Kilaso, M., Kaewmuangmoon, J., **Karnchanatat, A.**, Sangvanich P., and Chanchao, C.* (2011) Expression and characterization of *Apis dorsata* α-glucosidase III. *Journal of Asia-Pacific Entomology* 14: 479-488.

9.16 Boonmee, A., Srisomsap, C., Chokchaichamnankit, D., **Karnchanatat, A.**, and Sangvanich P.* (2011) A proteomic analysis of *Curcuma comosa* Roxb. rhizomes. *Proteome Science* 9: 43.

9.17 Boonmee, A., Srisomsap, C., **Karnchanatat, A.**, and Sangvanich P.* Biologically active proteins from *Curcuma comosa* Roxb. Rhizomes. *Journal of Medicinal Plants Research* 5: 5208-5215.

9.18 Wipusaree, N., Sihanonth, P., Piapukiew, J., Sangvanich, P., and **Karnchanatat, A.*** (2011) Purification and characterization of a xylanase from the endophytic fungus *Alternaria alternata* isolated from the Thai medicinal plant, *Croton oblongifolius* Roxb. *African Journal of Microbiology Research* 5: 5697-5712.

9.19 Intrama, V., **Karnchanatat, A.**, Bunaprasert, T., and Vadhanasindhu, P.* Critical effects of regulation on Thailand's cosmeceutical development process: human placenta extract *International Journal of Management and Business and Studies* 1: 96-99.

9.20 Songserm, P., Sihanonth, P., Sangvanich, P., and **Karnchanatat, A.*** (2012) Decolorization of textile dyes by *Polyporus seudobetulinus* and extracellular laccase. *African Journal of Microbiology Research* 6: 779-792.

9.21 Panuthai, T., Sihanonth, P., Piapukiew, J., Sooksai, S., Sangvanich, P., and **Karnchanatat, A.*** (2012) An extracellular lipase from the endophytic fungi *Fusarium oxysporum* isolated from the Thai medicinal plant, *Croton oblongifolius* Roxb. *African Journal of Microbiology Research* 6: 2622-2638.

9.22 Moon-ai, W., Niyomploy, P., Boonsombat, R., Sangvanich, P., and **Karnchanatat, A.*** (2012) A Superoxide dismutase purified from the rhizome of *Curcuma aeruginosa* Roxb. as inhibitor of nitric oxide production in the

Macrophage-like RAW 264.7 cell line. *Applied Biochemistry and Biotechnology* 166: 2138-2155.

9.23 Yodjun, M., **Karnchanatat, A.,** and Sangvanich, P.* (2012) Angiotensin I-converting enzyme inhibitory proteins and peptides from the rhizomes of Zingiberaceae plants. *Applied Biochemistry and Biotechnology* 166: 2037-2050.

9.24 Virounbounyapat, P., **Karnchanatat, A.** and Sangvanich, P.* (2012) An alpha-glucosidase inhibitory activity of thermostable lectin protein from *Archidendron jiringa* Nielsen seeds. *African Journal of Biotechnology* 11: 10026-10040.

9.25 **Karnchanatat, A.*** and Sangvanich, P. (2012) A chitinase-like protein with α-amylase inhibitory activity from Kluai Hom Thong banana Fruit: Musa (AAA group)**.** *Food Biotechnology* 26: 218-238.

9.26 Chantaranothai, C., Palaga, T., **Karnchanatat, A.**, and Sangvanich, P.* (2013) Inhibition of nitric oxide production in the Macrophage-like Raw 264.7 cell line by protein from the rhizomes of Zingberaceae plants. *Preparative biochemistry and biotechnology* 43: 60-78.

9.27 Rungsaeng, P., Sangvanich, P., and **Karnchanatat, A.*** (2013) Zingipain, a ginger protease with acetylcholinesterase inhibitory activity. *Applied Biochemistry and Biotechnology* 170: 934-950.

9.28 **Karnchanatat, A.***, Sihanonth, P., Piapukiew, J., and Sangvanich, P. (2013) An antioxidation and antiproliferation of polysaccharide-protein complex extracted from *Phaeogyroporus portentosus* (Berk. & Broome) McNabb. *African Journal of Microbiology Research* 7: 1668-1680.

9.29 Saisavoey, T., Thongchul, N., Sangvanich, P. and **Karnchanatat, A.*** (2014) Effect of methyl jasmonate on isoflavonoid accumulation and antioxidant enzymes in *Pueraria mirifica* cell suspension culture. *Journal of Medicinal Plants Research* 8: 401-407.

9.30 Niyomploy, P., Srisomsap, C., Chokchaichamnankit, D., Vinayavekhin, N., **Karnchanatat, A.**, and Sangvanich, P.* (2014) Superoxide dismutase isozyme

detection using two-dimensional gel electrophoresis zymograms. *Journal of Pharmaceutical and Biomedical Analysis* 90: 72-77.

9.31 Niyomploy, P., Boonsombat, R., **Karnchanatat, A.**, and Sangvanich, P.***** (2014) A Superoxide dismutase purified from the roots *Stemona tuberosa*. *Preparative biochemistry and biotechnology* 44: 663-679.

9.32 Saisavoey, T., *Palaga, T., Malaivijitnond, S., Jaroenporn*, S., Thongchul, N., Sangvanich, P. and **Karnchanatat, A.*** (2014) Anti-osteoclastogenic, estrogenic and antioxidant activities of cell suspension culture and tuber roots extract from *Pueraria mirifica*. *Food Science and Biotechnology* 23: 1253-1259.

9.33 Srinieng, K., Saisavoey T., and **Karnchanatat, A.*** (2015) Effect of salinity stress on antioxidative enzyme activities in tomato cultured *in vitro*. *Pakistan Journal of Botany* 47: 1-10.

10. Books and research articles

10.1 **Karnchanatat, A.*** and Tiengburanatam, N. (2010) Antimicrobial peptides. *Thaksin University Journal* 13: 101-108.

10.2 **Karnchanatat, A.*** (2012) Antimicrobial activity of lectins from plants**,** Antibacterial Agents / Book 1, ISBN 979-953-307-281-3. p. 145-178.

11. Research conferences

11.1 Incharoensakdi*, A., **Karnchanatat, A.** Effect of salinity on the uptake of choline by *Aphanothece halophytica*. *In* "American Society of Plant Biologists Annual Meeting 2003". University of Hawaii, Honolulu, Hawaii, USA. (Abstract book)

11.2 Incharoensakdi*, A., Wangsupa, J., Laloknum, S., **Karnchanatat, A.,** Jantaro, S., and Maenpaa, P. Biochemical adaptation of cyanobacteria to high salinity environments: changes in nitrogen metabolism. *In* "17th FAOBMB Symposium/2nd IUBMB Special Meeting/7thA-IMBN Conference 2004". Chulalongkorn University, Bangkok, Thailand.

11.3 **Karnchanatat, A.**, Petsom, A., Sangvanich, P., Piaphukiew, J., Whalley, A.J.S., Reynolds, C.D., and Sihanonth, P*. Purification and biochemical

characterization of an extracellular β-glucosidase from wood-decaying fungus *Daldinia eschscholzii*. *In* "50th Anniversary of Annual Meeting of the Mycological Society of Japan". 3-4 June, 2006, Aoba-no-mori Park Arts and Culture Hall, Chiba, Japan. (Abstract book)

11.4 **Karnchanatat, A.***, Petsom, A., Sangvanich, P., Piaphukiew, J., Whalley, A.J.S., Reynolds, C.D., and Sihanonth, P. Purification and biochemical characterization of an extracellular β-glucosidase from wood-decaying fungus *Daldinia eschscholzii*. *In* "II International Conference on Environmental, Industrail and Applied Microbiology (BioMicroWorld 2007)". 28 November-1 December 2007, Seville, Spain. (Proceeding book)

11.5 Kheeree, N., Sangvanich, P., Puthong, S., and **Karnchanatat, A.*** A Lectin from the rhizomes of *Curcuma amarissima* Roscoe and its role as anticancer activity. *In* "The 2nd Biochemistry and Molecular Biology Conference: Biochemistry and Molecular Biology for Sustainable Development" 7-8 May 2009, Khon Kaen University, Khon Kaen, THAILAND, p. 81-85. (Proceeding book)

11.6 Petnual, P., **Karnchanatat, A.** and Sangvanich, P.* Isolation of lectin from rhizomes of *Cucuma longa* L. with antifungal activity. *In* "The 2nd Biochemistry and Molecular Biology Conference: Biochemistry and Molecular Biology for Sustainable Development" 7-8 May 2009, Khon Kaen University, Khon Kaen, THAILAND, p. 91-95. (Proceeding book)

11.7 Konkumnerd, W., **Karnchanatat, A.** and Sangvanich, P.* A newly thermostable lectin from *Keampferia parviflora* Wall. Ex Baker. *In* "The 2nd Biochemistry and Molecular Biology Conference: Biochemistry and Molecular Biology for Sustainable Development" 7-8 May 2009, Khon Kaen University, Khon Kaen, THAILAND, p. 182-186. (Proceeding book)

11.8 Charungchitrak, S., **Karnchanatat, A.**, and Petsom, A.* Purification and characterization of lectin from *Archidendron jiringa* Neilson seeds. *In* "4th BUU

Grad. Research Conference" 13 March 2009, Burapha University, Chonburi, THAILAND, F-P004 (1-8). (Proceeding book)

11.9 Konkumnerd, W., **Karnchanatat, A.** and Sangvanich, P.* A newly thermostable lectin from *Keampferia parviflora* Wall. Ex Baker. *In* "4th Annual Symposium of Protein Society of Thailand: Protein Research: from basic studies to applications in health sciences" 26-28 August 2009, Chulabhorn Research Institute Conference Center, THAILAND. p. 127-130. (Proceeding book)

11.10 Petnual, P., **Karnchanatat, A.** and Sangvanich, P.* Isolation of lectin from rhizomes of *Cucuma longa* L. with antifungal activity. *In* "4th Annual Symposium of Protein Society of Thailand: Protein Research: from basic studies to applications in health sciences" 26-28 August 2009, Chulabhorn Research Institute Conference Center, THAILAND. p. 132-136. (Proceeding book)

11.11 Kheeree, N., Sangvanich, P., Puthong, S., and **Karnchanatat, A.*** A Lectin from the rhizomes of *Curcuma amarissima* Roscoe and its role as anticancer activity. *In* "4th Annual Symposium of Protein Society of Thailand: Protein Research: from basic studies to applications in health sciences" 26-28 August 2009, Chulabhorn Research Institute Conference Center, THAILAND. p. 137-142. (Proceeding book)

11.12 Sawangsak, W., **Karnchanatat, A.**, and Chuntaratin, P.* Micropropagation of *Glycyrrhiza glabra* Linn. And medicinal herb through shoot tips culture. *In* "*Graduate Research Conference* King Mongkut's Institute of Technology Ladkrabang 2009" 31 August-2 September 2009, King Mongkut's Institute of Technology Ladkrabang, Bangkok, THAILAND. p. 441-446. (Proceeding book)

11.13 Tiengburanatam, N., Sangvanich, P., and **Karnchanatat, A.*** A novel α-glucosidase inhibitor protein from the rhizomes of *Zingiber ottensii* Valeton. *In* "The 3rd Technology and Innovation for Sustainable Development

Conference (TISD2010)" 4-6 March 2010, Nong Khai, Thailand. p. 355-360. (Proceeding book)

11.14 **Karnchanatat, A.***, Tiengburanatam, N., and Sangvanich, P. A cysteine protease with antifungal activity from *Zingiber ottensii* Valeton rhizomes. *In* "The 5rd Annual Symposium of Protein Society of Thailand: From Basic Approaches to Modern Technologies". 23-25 June 2010, Bangkok, Thailand. p. 213-218. (Proceeding book)

11.15 Wipusaree, N., Sihanonth, P., Piapukiew, J., Sangvanich, P., and **Karnchanatat, A.*** Screening and production of xylanase from endophytic fungi. *In* "The 22nd Annual Meeting of the Thai Society for Biotechnology, International Conference on Biotechnology for Healthy Living" 20-22 October 2010, Prince of Songkla University, Trang Campus, Thailand. p. 485-491. (Proceeding book)

11.16 Panuthai, T., Sihanonth, P., Piapukiew, J., Sangvanich, P., and **Karnchanatat, A.*** Screening and production of lipase from endophytic fungi. *In* "The 22nd Annual Meeting of the Thai Society for Biotechnology, International Conference on Biotechnology for Healthy Living" 20-22 October 2010, Prince of Songkla University, Trang Campus, Thailand. p. 619-626. (Proceeding book)

11.17 Songserm, P., Sihanonth, P., Piapukiew, J., Sangvanich, P., and **Karnchanatat, A.*** Decolorization of synthetic dyes by selected white-rot fungi. *In* "The 22nd Annual Meeting of the Thai Society for Biotechnology, International Conference on Biotechnology for Healthy Living" 20-22 October 2010, Prince of Songkla University, Trang Campus, Thailand. p. 758-762. (Proceeding book)

11.18 Saisavoey, T., **Karnchanatat, A.**, Thongchul, N., and Chuntaratin, P.* Enhancement of puerarin accumulation in *Pueraria mirifica* cell suspension culture using methyl jasmonate. *In* "The 22nd Annual Meeting of the Thai Society for Biotechnology, International Conference on Biotechnology for

Healthy Living" 20-22 October 2010, Prince of Songkla University, Trang Campus, Thailand. p. 1076-1083. (Proceeding book)

11.19 Virounbounyapat, P. **Karnchanatat, A.** and Sangvanich, P.* Protein from seeds of Djenkol Bean *Archidendron Jiringa* Nielsen. with alpha-glucosidase inhibitory activity. *In* "The 22nd Annual Meeting of the Thai Society for Biotechnology, International Conference on Biotechnology for Healthy Living" 20-22 October 2010, Prince of Songkla University, Trang Campus, Thailand. p. 1200-1205. (Proceeding book)

11.20 Panuthai, T., Sihanonth, P., Piapukiew, J., Sangvanich, P., and **Karnchanatat, A.*** Screening and production of lipase from endophytic fungi. *In* "The 11th *Graduate Research Conference Khon Kaen University* 2011" 28 January 2011, *Khon Kaen,* Thailand. p. 635-642. (Proceeding book)

11.21 Wipusaree, N., Sihanonth, P., Piapukiew, J., Sangvanich, P., and **Karnchanatat, A.*** Screening and production of xylanase from endophytic fungi. *In* "The 11th *Graduate Research Conference Khon Kaen University* 2011" 28 January 2011, *Khon Kaen,* Thailand. p. 643-648. (Proceeding book)

11.22 Yodjun, M., **Karnchanatat, A.***, and Sangvanich, P. Angiotensin I-converting enzyme inhibitory activity from the peptides of the rhizomes of Zingiberaceae plants. *In* "The 11th *Graduate Research Conference Khon Kaen University* 2011" 28 January 2011, *Khon Kaen,* Thailand. p. 649-653. (Proceeding book)

11.23 Rungsaeng, P., Sangvanich, P., and **Karnchanatat, A.*** Protein with acetylcholinesterase inhibitory activity from the rhizomes of Zingiberaceae plants. *In* "The 11th *Graduate Research Conference Khon Kaen University* 2011" 28 January 2011, *Khon Kaen,* Thailand. p. 654-658. (Proceeding book)

11.24 Moon-ai, W., **Karnchanatat, A.***, and Sangvanich, P. Purification and characterization of superoxide dismutase from the rhizome of *Curcuma aeruginosa* Roxb. *In* "The 11th *Graduate Research Conference Khon Kaen*

University 2011" 28 January 2011, *Khon Kaen,* Thailand. p. 659-665. (Proceeding book)

11.25 Chantaranothai, C., Palaga, T., **Karnchanatat, A.*,** and Sangvanich, P. Inhibitory activity against nitric oxide production in Macrophage RAW 264.7 from the protein of the rhizomes of Zingiberaceae plants. *In* "The 11th *Graduate Research Conference Khon Kaen University* 2011" 28 January 2011, *Khon Kaen,* Thailand. p. 666-672. (Proceeding book)

11.26 Songserm, P., Sihanonth, P., Piapukiew, J., Sangvanich, P., and **Karnchanatat, A.*** Decolorization of ynthetic dyes by selected white-rot fungi. *In* "The 11th *Graduate Research Conference Khon Kaen University* 2011" 28 January 2011, *Khon Kaen,* Thailand. p. 715-719. (Proceeding book)

11.27 Kheeree, N., Sangvanich, P., Puthong, S., and **Karnchanatat, A.*** Antifungal and antiproliferative activities of lectin from the rhizomes of *Curcuma amarissima* Roscoe. *In* "TRF-Master Research Congress V" 30 March-1April 2011, Jomtien Palm Beach Hotel and Resort, Pattaya City, Chonburi, Thailand. p. 156. (Abstract book)

11.28 Virounbounyapat, P. **Karnchanatat, A.** and Sangvanich, P.* Protein from seeds of Djenkol Bean *Archidendron Jiringa* Nielsen. with alpha-glucosidase inhibitory activity. *In* "TRF-Master Research Congress V" 30 March-1April 2011, Jomtien Palm Beach Hotel and Resort, Pattaya City, Chonburi, Thailand. p. 156. (Abstract book)

11.29 Wipusaree, N., Sihanonth, P., Piapukiew, J., Sangvanich, P., and **Karnchanatat, A.*** Screening and production of xylanase from endophytic fungi. *In* "TRF-Master Research Congress V" 30 March-1April 2011, Jomtien Palm Beach Hotel and Resort, Pattaya City, Chonburi, Thailand. p. 399. (Abstract book)

11.30 Panuthai, T., Sihanonth, P., Piapukiew, J., Sangvanich, P., and **Karnchanatat, A.*** Screening and production of lipase from endophytic fungi. *In* "TRF-

Master Research Congress V" 30 March-1April 2011, Jomtien Palm Beach Hotel and Resort, Pattaya City, Chonburi, Thailand. p. 459. (Abstract book)

11.31 Chantaranothai, C., Sangvanich, P., Palaga, T., and **Karnchanatat, A.*** Inhibitory activity against nitric oxide production in Macrophage RAW 264.7 from the protein of the rhizomes of Zingiberaceae plants. *Journal of Srinakharinwirot University* 2011; 3(supplement 1): 44-48.

11.32 Rungsaeng, P., Sangvanich, P., and **Karnchanatat, A.*** Screening for acetylcholinesterase inhibitory activity from the extract of the rhizomes of Zingiberaceae plants. *Journal of Srinakharinwirot University* 2011; 3(supplement 1): 234-238.

11.33 Yodjun, M., Sangvanich, P., and **Karnchanatat, A.*** Angiotensin I-converting enzyme inhibitory activity from the peptides of the rhizomes of Zingiberaceae plants. *Journal of Srinakharinwirot University* 2011; 3(supplement 1): 362-366

11.34 **Karnchanatat, A.***, Moon-ai, W., Niyomploy, P., and Sangvanich, P. A superoxide dismutase purified from the rhizome of *Curcuma aeruginosa* Roxb. *In* "The 6th International Symposium of the Protein Society of Thailand" 30 August-2 September 2011, Chulabhorn Research Institute Conference Center, THAILAND. p. 186-193. (Proceeding book)

11.35 **Karnchanatat, A.***, Yodjun, M., and Sangvanich, P. Angiotensin I-converting enzyme inhibitory protein from the rhizomes of Zingiberaceae plants. *In* "The 6th International Symposium of the Protein Society of Thailand" 30 August-2 September 2011, Chulabhorn Research Institute Conference Center, THAILAND. p. 194-199. (Proceeding book)

11.36 **Karnchanatat, A.***, Chantaranothai, C., Palaga, T., and Sangvanich, P. Inhibition of nitric oxide production by Zingiberaceae rhizome proteins. *In* "The 6th International Symposium of the Protein Society of Thailand" 30 August-2 September 2011, Chulabhorn Research Institute Conference Center, THAILAND. p. 200-207. (Proceeding book)

11.37 **Karnchanatat, A.***, Rungsaeng, P., and Sangvanich, P. A Ginger protease with acetylcholinesterase inhibitory activity. *In* "The 6th International Symposium of the Protein Society of Thailand" 30 August-2 September 2011, Chulabhorn Research Institute Conference Center, THAILAND. p. 208-216. (Proceeding book)

11.38 Inthuwanarud K., Sangvanich, P., and **Karnchanatat, A.*** Antioxidation activity of protein protein hydrolysate from the rhizome of Zingiberaceae plants. *In* "Pure and Applied Chemistry International Conference 2012 (PACCON 2012)" 11-13 January 2012, Department of Chemistry, Faculty of Science, Chiang Mai University, Chiang Mai, THAILAND, p. 888-891. (Proceeding book)

11.39 **Karnchanatat, A.***, and Sangvanich, P. A chitinase-like protein with α-amylase inhibitory activity from Kluai Hom Thong banana fruit: Musa (AAA group). *In* "The 14th Food Innovation Asia Conference 2012" 14-15 June 2012, Bangkok International Trade & Exhibition Centre (BITEC) Bangna, Bangkok, THAILAND, p. 190-200. (Proceeding book)

11.40 **Karnchanatat, A.***, Inthuwanarud, K., and Sangvanich, P. Antioxidant and antiproliferative activities of protein hydrolysate from the rhizomes of Zingiberaceae plants. *In* "The 14th Food Innovation Asia Conference 2012" 14-15 June 2012, Bangkok International Trade & Exhibition Centre (BITEC) Bangna, Bangkok, THAILAND, p. 201-208. (Proceeding book)

11.41 **Karnchanatat, A.***, Sihanonth, P., Piapukiewand, J., and Sangvanich, P. Antioxidation of polysaccharide-protein complex extracted from *Phaeogyroporus portentosus* (Berk. & Broome) McNabb. *In* "The 14th Food Innovation Asia Conference 2012" 14-15 June 2012, Bangkok International Trade & Exhibition Centre (BITEC) Bangna, Bangkok, THAILAND, p. 390-401. (Proceeding book)

11.42 Srinieng, K., Saisavoey, T., Sangvanich, P. and **Karnchanatat, A.*** Effects of salinity stress on antioxidative enzyme activities in tomato cultured *in vitro*. *In*

"The 7th International Symposium of the Protein Society of Thailand" 29-31 August 2012, Chulabhorn Research Institute Conference Center, THAILAND. p. 136-141. (Proceeding book)

11.43 Shinabhuthonsri, P., Saisavoey, T., Sangvanich, P. and **Karnchanatat, A.*** Salt stress enhance choline dehydrogenase activity in tomato cultured *in vitro*. *In* "The 7th International Symposium of the Protein Society of Thailand" 29-31 August 2012, Chulabhorn Research Institute Conference Center, THAILAND. p. 143-147. (Proceeding book)

11.44 **Karnchanatat, A.***, Sihanonth, P., Piapukiewand, J., and Sangvanich, P. A polysaccharide-protein complex extracted from *Phaeogyroporus portentosus* (Berk. & Broome) McNabb with antiprokiferative activity. *In* "The 7th International Symposium of the Protein Society of Thailand" 29-31 August 2012, Chulabhorn Research Institute Conference Center, THAILAND. p. 154-159. (Proceeding book)

11.45 Charoenchai, M., **Karnchanatat, A.***, and Saisavoey, T. Effect of salinity stress on nitrite reductase activity in tomato cultured *in vitro*. *In* "The 13th FAOBMB Congress 2012" 25-29 November 2012, Bangkok International Trade & Exhibition Centre (BITEC) Bangna, Bangkok, THAILAND. P-F-04. (Proceeding book)

11.46 Nuchprapha, A., and **Karnchanatat, A.*** Angiotensin I converting enzyme inhibitory activity of the protein hydrolysate from the seeds of Thai fruits. *In* "The 13th FAOBMB Congress 2012" 25-29 November 2012, Bangkok International Trade & Exhibition Centre (BITEC) Bangna, Bangkok, THAILAND. P-I-24. (Proceeding book)

11.47 Phetruantong, J., and **Karnchanatat, A.*** Tyrosinase inhibitory activity of the protein hydrolysate from the seeds of Thai fruits. *In* "The 13th FAOBMB Congress 2012" 25-29 November 2012, Bangkok International Trade & Exhibition Centre (BITEC) Bangna, Bangkok, THAILAND. P-I-25. (Proceeding book)

11.48 Sodsroy, S., and **Karnchanatat, A.*** Antioxidant activity of the protein hydrolysate from the seeds of Thai fruits. *In* "The 13th FAOBMB Congress 2012" 25-29 November 2012, Bangkok International Trade & Exhibition Centre (BITEC) Bangna, Bangkok, THAILAND. P-I-26. (Proceeding book)

11.49 Nuchprapha, A., Petsom, A., and **Karnchanatat, A.*** Angiotensin I converting enzyme inhibitory activity of the protein hydrolysate from the seeds of Thai fruits. *In* "The 4th Regional AFOB Syposium 2013" 17-19 January 2013, Chiangmai Grandview Hotel and Convention Center Chiang Mai, THAILAND. p. 40-42. (Proceeding book)

11.50 Sodsroy, S., Sangvanich, P., and **Karnchanatat, A.*** Antioxidant activity of the protein hydrolysate from the seeds of Thai fruits. *In* "The 4th Regional AFOB Syposium 2013" 17-19 January 2013, Chiangmai Grandview Hotel and Convention Center Chiang Mai, THAILAND. p. 43-46. (Proceeding book)

11.51 **Karnchanatat, A.***, Charoenchai, M., and Saisavoey, T. Effect of salinity stress on nitrite reductase activity in tomato cultured *in vitro*. *In* "The 4th Regional AFOB Syposium 2013" 17-19 January 2013, Chiangmai Grandview Hotel and Convention Center Chiang Mai, THAILAND. p. 47-51. (Proceeding book)

11.52 **Karnchanatat, A.***, Shinabhuthonsri, P., and Saisavoey, T. Salt stress enhance choline dehydrogenase activity in tomato cultured *in vitro*. *In* "The 4th Regional AFOB Syposium 2013" 17-19 January 2013, Chiangmai Grandview Hotel and Convention Center Chiang Mai, THAILAND. p. 67-71. (Proceeding book)

11.53 Phetruantong, J., Sangvanich, P., and **Karnchanatat, A.*** Tyrosinase inhibitory activity of the protein hydrolysate from the seeds of Thai fruits. *In* "The 4th Regional AFOB Syposium 2013" 17-19 January 2013, Chiangmai Grandview Hotel and Convention Center Chiang Mai, THAILAND. p. 76-79. (Proceeding book)

11.54 Saisavoey, T., Thongchul, N., *Malaivijitnond, S.*, *Jaroenporn*, S., **Karnchanatat, A.*** The estrogenic activity of *Pueraria mirifica* tuber and cell suspension culture in ovariectomized rats. *In* "Pharma-Nutrition 2013" 15-17 April 2013, Singapore Expo, SINGAPORE. (Abstract book)

11.55 Prakot, P., Chaitanawisuti, N., and **Karnchanatat, A.*** *In vitro* anti-tyrosinase activity of the protein hydrolysate from spotted babylon (*Babylonia areolata*). *In* "International Conference on Food and Applied Bioscience" 6-7 February 2014, The Empress Hotel, Chiang Mai, THAILAND (Abstract book)

11.56 Semanit, K., Piapukiew, J., Noitang, S., and **Karnchanatat, A.*** *In vitro* antioxidant of the protein hydrolysate isolated from the seeds of hoary basil (*Ocimum basilicum*). *In* "International Conference on Food and Applied Bioscience" 6-7 February 2014, The Empress Hotel, Chiang Mai, THAILAND (Abstract book)

11.57 **Karnchanatat, A.*,** and Sangvanich, P. Purification and Characterization of a novel protease from *Perinereis nuntia*. *In* "Th 4th International Biochemistry and Molecular Biology Conference" 2-3 April 2014, Rama Gardens Hotel & Resort, Bangkok, THAILAND (Abstract book)

11.58 Phakhum, T., Khongchareonporn, N., Noitang, S., **Karnchanatat, A.**, Sermsuvitwong, K., and Sooksai, S. *In* "Th 4th International Biochemistry and Molecular Biology Conference" 2-3 April 2014, Rama Gardens Hotel & Resort, Bangkok, THAILAND (Abstract book)

11.59 **Karnchanatat, A.,*** and Sangvanich, P. A glucose/mannose-specific lectin with alpha-glucosidase inhibitory activity from *Sterculia monosperma* Vent seeds. *In* "The 5th International Conference on Natural Products for Health and Beauty (NATPRO 5)" 6-8 May 2014, Moevenpick Resort & Spa Karon Beach, Phuket, THAILAND (Abstract book)

11.60 **Karnchanatat, A.,*** Noitang, S., Semanit, K., and Piapukiew, J., Comparative study on antioxidative activity of the seeds of hoary basil (*Ocimum basilicum*) protein hydrolysates produced by papain, pepsin and Protease G6 (alcalase).

In "The 5 th International Conference on Natural Products for Health and Beauty (NATPRO 5)" 6-8 May 2014, Moevenpick Resort & Spa Karon Beach, Phuket, THAILAND (Abstract book)

11.61 **Karnchanatat, A.,*** and Sangvanich, P. A Novel antitumor activity of L-asparaginase from *Xylaria feejeensis* strain XL001: Purification and characterization. *In* "The 10th International Mycological Congress (IMC10)" 3-8 August 2014, Queen Sirikit National Convention Center (QSNCC), Bangkok, THAILAND (Abstract book)

11.62 Prakot, P., Chaitanawisuti, N., and **Karnchanatat, A.*** Melanogenesis and antityrosinase activity of protein hydrolysate from spotted Babylon (*Babylonia areolata*). *In* "7th Asia Oceania Human Proteome Organization (AOHUPO) Congress and the 9th International Symposium of the Protein Society of Thailand" 6-8 August 2014, Miracle Grand Convention Hotel, Bangkok, THAILAND (Proceeding book)

11.63 Petsantat, P., Chaitanawisuti, N., and **Karnchanatat, A.*** Antioxidation and antiproliferative activities of protein hydrolysate from spotted babylon (*Babylonia areolata*). *In* "7th Asia Oceania Human Proteome Organization (AOHUPO) Congress and the 9th International Symposium of the Protein Society of Thailand" 6-8 August 2014, Miracle Grand Convention Hotel, Bangkok, THAILAND (Proceeding book)

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