UNIVERSITI PUTRA MALAYSIA

ENZYMATIC PRODUCTION OF MULTIFUNCTIONAL BIOACTIVE PEPTIDES FROM SEA CUCUMBER (Actinopyga lecanora Jaeger)

RAHELEH GHANBARI

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ENZYMATIC PRODUCTION OF MULTIFUNCTIONAL BIOACTIVE PEPTIDES FROM SEA CUCUMBER (*Actinopyga lecanora* Jaeger)

By

RAHELEH GHANBARI

Thesis Submitted to School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Doctor of Philosophy

December 2014

DEDICATION

To my beloved parents

Thank you for your patience, support and understanding

To my beloved brothers

Mojtaba and Mohsen Ghanbari

To my beloved grandfather

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Doctor of Philosophy

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Chairman: Professor Nazamid Saari, PhD

Faculty: Food Science and Technology

Food protein-generated bioactive peptides are natural products that have nutraceutical and pharmaceutical properties. They have been reported to have anti-oxidative, anti-bacterial, anti-hypertensive and anti-inflammatory properties. Due to their potential, there is an increasing interest in the use of these peptides for general health maintenance and well-being. Sea cucumbers have been utilized as a folk remedy to cure some diseases in some Asian countries for decades. Among the species, *Actinopyga lecanora*, commonly known as stone fish was chosen due to its relatively high protein content (58.30%). Moreover, it is considered as a by-catch of the Malaysia's fishing industry. This study was targeted to generate multifunctional bioactive peptides against cardiovascular diseases and its risk factors (hypertension and oxidative stress), inflammation and microbial infections, from an edible species of sea cucumber through enzymatic proteolysis. Thus, the protein was proteolysed using six proteases namely alkalase, papain, bromelain, flavourzyme, pepsin, and trypsin under their optimum conditions for 24 h. The degree of proteolysis and peptide content were evaluated using *O*-phthaldialdehyde based on a spectroscopic method. The amino acid compositions of *A. lecanora* and its generated proteolysates were evaluated. The multifunctional activities of the *A. lecanora* generated proteolysates including anti-hypertensive, anti-oxidative, anti-bacterial and inhibition of nitric oxide (NO) activities were determined. The anti-oxidative activity was measured using $DPPH^{\bullet}$ radical scavenging and ferrous-ion chelating activities. The anti-bacterial activity was measured as growth inhibition (%) against *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Escherichia coli* and *Busilus subtilis* and *Staphylococcus aureus.* The inhibition of NO-production was evaluated using Griess assay in RAW 264.7 cells. The bromelain-generated proteolysate showed the highest multifunctional activities after 1 h of proteolysis. The abilities of proteolysate to inhibit ACE, scavenge DPPH free radicals and chelate iron (Fe^{2+}) were 48.80%, 40.00% and 30.24%, respectively. The potency of proteolysate to inhibit NO-production in RAW 264.7 cell was 60.02%. Subsequently, this proteolysate showed the highest anti-bacterial activities against *Pseudomonas* sp., *P. aeruginosa*, *E. coli* and *S. aureus* of 51.85, 30.07, 30.00 and 24.30%, respectively. The proteolysate was

further profiled by fractionation methods based on hydrophobicity using RP-HPLC and isoelectric properties using isoelectric focusing technique. The best fraction in terms of multifunctional properties was selected for peptide identification and sequencing using an UPLC-QTOF-MS system, where a total of 12 peptides were identified. The multifunctional activities of the identified peptides were studied. Based on the results obtained 3 peptides namely LREMLSTMCTARGA, VAPAWGPWPKG and ATSFREALRCGAE showed the strongest ACE inhibitory activities of 98.10, 95.23 and 59.95%, radical scavenging activity of 93.30, 70.44 and 78.20% and ferrous ion chelating activity of 57.00, 43.50 and 54.00%, respectively. NO-production was inhibited by these peptides with values of 76.30, 69.90 and 30.00% and their NO scavenging activities were 51.14, 50.32 and 34.85%, respectively. These peptides exhibited growth inhibition against *P. aeruginosa, Pseudomonas* sp., *E. coli* and *S. aureus* with values ranging from 50.00 to 75.30%. The effect of the proteolysate and its derived peptides on the viability of RAW 264.7 cells were evaluated using MTT assay. Results showed that these samples had no cytotoxic effect and the cell viability was higher than 90%. Kinetic studies of ACE inhibition of multifunctional peptides demonstrated un-competitive and mixed-mode patterns. Results showed that *A. lecanora* could be used as an economical protein source to generate invaluable multifunctional proteolysate and bioactive peptides which could be exploited in the formulation of various functional foods or used as a source of nutraceuticals.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah Doktor Falsafah

PENGELUARAN ENZYMATIK BIO-AKTIF PEPTIDAMULTIFUNGSI DARI GAMAT (*Actinopyga lecanora* Jaeger)

Oleh

RAHELEH GHANBARI

Disember 2014

Pengerusi : Professor Nazamid Saari, PhD

Fakulti: Sains dan Teknologi Makanan

Peptida bioaktif makanan protein yang dihasilkan ialah produk semula jadi yang mempunyai ciri-ciri nutraseutikal dan farmaseutikal. Mereka telah dilaporkan mempunyai sifat anti-pengoksidaan, anti-bakteria, anti-hipertensi dan anti-radang. Oleh kerana potensi mereka, terdapat minat yang semakin meningkat dalam penggunaan peptida untuk mengekalkan kesihatan umum dan kesejahteraan. Timun laut telah digunakan sebagai ubat tradisional untuk mengubati beberapa penyakit di beberapa negara Asia selama beberapa dekad. Diantara spesies, *Actinopyga lecanora*, biasanya dikenali sebagai ikan batu dipilih kerana kandungan protein yang tinggi. Selain itu, ia dianggap sebagai mempunyai hasil buangan tangkapan industri perikanan Malaysia, dan didapati dengan banyaknya tanpa pada nilai komersial. Kajian ini disasarkan untuk menjana bio-aktif peptida pelbagai fungsi terhadap penyakit kardiovaskular dan faktor-faktor risikonya (tekanan darah tinggi dan tekanan oksidatif), keradangan, dan jangkitan mikrob dari pada satu spesies timun laut yang boleh dimakan melalui proteolisis enzim oleh itu, proteolysis protein dijalankan menggunakan enam protease iaitu alkalase, papain, bromelain, flavourzyme, pepsin dan trypsin, di bawah keadaan optimum selama 24 jam. Darjah proteolisis dan kandungan peptida telah dinilai menggunakan *O*-phthaldialdehyde (OPA) berdasarkan kaedah spektroskopi. Komposisi asid amino *A. lecanora* dan proteolisat yang dijana telah nilai. Aktiviti pelbagai-fungsi proteolisat *A. lecanora* termasuk aktiviti anti-hipertensi, anti-pengoksidaan, anti-bakteria dan perencatan aktiviti nitrik oksida (NO) telah ditentukan. Aktiviti anti-pengoksidaan diukur melalui pemerangkap radikal DPPH$^{\bullet}$ dan pengkelatan ion ferus. Aktiviti anti-bakteria diukur melalui perencatan pertumbuhan (%) *Pseudomonas aeruginosa*, *Pseudomonas* sp., *Escherichia coli* dan *Staphylococcus aureus*. Perencatan NO-penghasilan telah dinilai menggunakan kaedah Griess dalam sel RAW 264.7. Proteolisat yang dihasilkan oleh bromelain mencatatkan aktiviti pelbagai-fungsi yang tertinggi selepas 1 jam proteolisis. Keupayaan proteolisat untuk merencat ACE, mengskaveng radikal bebas dan mengkelat ion ferus masing-masing adalah 48.80%, 40.00% dan 30.24%. Potensi proteolisat untuk menghalang pengeluaran NO dalam sel RAW 264.7 adalah 60.02%. Proteolisat ini turut mempamerkan aktiviti anti-

bakteria tertinggi terhadap *Pseudomonas* sp., *P. aeruginosa*, *E. coli* dan *S. aureus,* masing-masing mencatat nilai perencatan sebanyak 51.85, 30.07, 30.00 dan 24.30%. Proteolisat tersebut seterusnya diprofil menggunakan furutan dua kaedah fraksinasi berdasarkan kehidrofobian peptida dengan RP-HPLC dan cirian isoelektrik dengon teknik pernofokusan isoelectric. Fraksi terbaik dari segi aktiviti pelbagai-fungsi telah dipilih untuk pengenalpastian dan penjujukan peptida melalui sistem UPLC-QTOF-MS, di mana sebanyak 12 peptida telah dikenalpasti. Aktiviti pelbagai-fungsi peptida yang dikenalpasti telah dikaji. Berdasarkan keputusan yang diperolehi, tiga (3) peptida iaitu LREMLSTMCTARGA, VAPAWGPWPKG dan ATSFREALRCGAE, mencatat aktiviti perencatan ACE terkuat pada 98.10, 95.23 dan 59.95%, aktiviti pengskaveng radikal 93.30, 70.44 dan 78.20% dan aktiviti pengkelatan ion ferus sebanyak 57.00, 43.50 dan 54.00%. Pengeluaran nitrik oksida pula direncat oleh peptida-peptida tersebut dengan nilai perencatan 76.30, 69.90 dan 30.00% manakala aktiviti pemerangkap NO mencatat nilai masing-masing sebanyak 51,14, 50.32 dan 34.85% . Kesemua peptida ini mempamerkan perencatan pertumbuhan terhadap *P. aeruginosa, Pseudomonas* sp., *E. coli* dan *S. aureus* dengan nilai antara 50.00-75.30%. Kesan proteolisat dan peptida ke atas kebolehhidupan sel RAW 264.7 telah dikaji melalui asai MTT. Keputusan menunjukkan bahawa sampel-sampel ini tidak mempamerkan kesan sitotoksik dan kebolehhidupan sel adalah lebih daripada 90.00%. Kajian kinetik peptida pelbagai-fungsi ke atas perencatan ACE menunjukkan mod tidak kompetitif dan mod bercampurcorak. Keputusan ini menggambarkan bahawa *A. lecanora* boleh digunakan sebagai sumber protein yang ekonomi untuk penjanaan proteolisat pelbagai-fungsi yang tidak ternilai dan peptida bioaktif ini boleh dieksploitasi ke dalam formulasi pelbagai makanan fungsian atau digunakan sebagai sumber nutraseutikal.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Doctor of Philosophy. The members of the supervisory committee were as follows:

Nazamid Saari, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Chairman)

Azizah Abdul-Hamid, PhD
Professor
Faculty of Food Science and Technology
Universiti Putra Malaysia
(Member)

Amin Bin Ismail, PhD
Professor
Faculty of Nutrition and Health Science
Universiti Putra Malaysia
(Member)

BUJANG BIN KIM HUAT, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date

Declaration by graduate student

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Name and Matric No: Raheleh Ghanbari: GS25867

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

a.a	Amino acid
ABTS	2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)
ACN	Acetonitrile
ACE	Angiotensin I-converting enzyme
AOAC	Association of official analytical chemists
ANOVA	Analysis of variance
AT-I	Angiotensin I
AT-II	Angiotensin II
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytolune
DPPH	2,2-diphenyl-1-2-picryl hydrazyl
ºC	Degrees celsius
CID	Collision induced dissociation
CAT	Catalase
CVDs	Cardiovascular diseases
cGMP	Cyclic guanosine monophosphate
Da	Dalton
DNA	Deoxyribonucleic acid
DW	Dry weight
et al.	And others
E/S	Enzyme-substrate ratio
g	Gram
GI	Gastrointestinal
GPx	Glutathione peroxidase
GSH	Glutathione
h	Hour
HPLC	High performance liquid chromatogeraphy
HCL	Hydrochloric acid
H_2O_2	Hydrogen peroxide
IC_{50}	The half maximal inhibitory concentration
IEF	Isoelectric focusing
IPG	Immobilized pH gradient gel
iNOS	inducible nitric oxide synthase
IL-1α	Interleukin 1-alpha
KDa	Kilodalton
Km	Michaelis constant or enzyme-substrate dissociation constant
L	Liter
LC-MS/MS	Liquid chromatography tandem mass spectrometry
LDL	Low-density lipoprotein
L-NAME	L-NG-nitro arginine methyl ester
LPS	Lipopolysaccharide

MALDI/TOF	Matrix-assisted laser desorption/ionization/time of flight
µg	Microgram
µM	Micromolar
mL	Mililiter
mg	Miligram
min	Minute
MW	Molecular weight
MWCO	Molecular weight cut off
NADPH	Nicotinamide adenine dinucleotid phosphate
NADH	Nicotinamide adenine dinucleotide
$NO_2^{\bullet}$	Nitrogen dioxide
NO	Nitric oxide
NOS	Nitric oxide synthase
$O_2^{\bullet-}$	Superoxide anion radical
$^1O_2$	Singlet oxygen
OD	Optical density
$OH^{\bullet}$	Hydroxyl radical
OPA	O-phthaldialdehyde
ORAC	Oxygen radical absorbance capacity
P	Probability
pI	Isoelectric point
Q-TOF	Quadrupole time-of-flight
RCS	Reactive chloride species
RNS	Reactive nitrogen species
SNP	Sodium nitroprusside
ROS	Reactive oxygen species
$ONOO^-$	Reactive peroxynitrite
$ROO^{\bullet}$	Peroxyl radical
RT	Retention time
RP-HPLC	Reverse-phase high performance liquid chromatography
rpm	Revolutions per minute
SD	Standard deviation
SOD	Superoxide dismutase
TAAs	Total amino acids
TFA	Trifluroacetic acid
TSB	Tryptone soy broth
TNF-α	Tumour necrosis factor-alpha
V_{max}	Maximum enzyme reaction rate
WHO	World Health Organization
UV	Ultraviolet
%	Pecentage

CHAPTER 1

INTRODUCTION

Bioactive peptides are considered as specific protein fragments that have positive impacts on human health (Kitts & Weiler, 2003). These peptides are inactive within the sequence of the parent protein, and may exert various physiological functions upon proteolysis (Sarmadi, Ismail, & Hamid, 2011). Food protein hydrolysates including bioactive peptides have the potential to provide numerous functional and health benefits based on their structural properties that is reflected by amino acid composition and sequences. They have shown a number of multiple biological activities, including anti-thrombotic and anti-hypertensive activities, anti-oxidant and hypocholesterolemic effects, anti-microbial function, opiate-like effects, immunomodulatory, anti-cancer activities, and mineral binding functions (specially for Ca^{2+}) (Kim & Wijesekara, 2010).

Hypertension is one of the major causes of disease worldwide, and mainly is identified as a cardiovascular risk factor. It is directly connected to stroke, heart attack, and kidney failure. Hypertension is ranked third as a cause of disability-adjusted life-years (Aleman, Gomez-Guillen, & Montero, 2013; Ezzati et al., 2002). The prevalence of hypertension is increasing. It has been projected that more than 1.56 billion people worldwide will suffer by 2025 (Kuo & Pu, 2011). In Malaysia, one out of three adults aged 30 and above suffers from hypertension, according to the National Health and Morbidity Survey conducted in 2011. Angiotensin-I-converting enzyme (ACE) plays a crucial role in the regulation of blood pressure via its two mechanisms on body systems, including the renin-angiotensin and the kinin-kallikrein systems. In fact, ACE promotes the conversion of angiotensin I into the potent vasoconstrictor angiotensin II as well as inactivating the bradykinin (Murray & FitzGerald, 2007). Bradykinin is a vasodilator that regulates vascular endothelial nitric oxide (NO) release. NO contributes in the regulation of blood pressure by promoting vascular relaxation, and angiogenesis (Fleming, 2006). The dual functions of ACE cause an increase in blood pressure and finally lead to the development of hypertension as a risk factor of cardiovascular diseases (Wijesekara & Kim, 2010). The effect of ACE inhibitors on hindering or reducing the formation of angiotensin II, and accordingly on reducing the occurrence of elevated blood pressure has been demonstrated (Pfeffer & Frohlich, 2006). In the condition of high blood pressure, angiotension II, increases the oxidative stress as it intervenes with several of its cellular actions through stimulating the formation of intracellular reactive oxygen species (ROS) (Schiffrin & Touyz, 2004). Therefore, apart from control of blood pressure, ACE inhibitors have been shown to increase the anti-oxidative defense system in animals and humans through inhibition of the formation of angiotensin II (Cavanagh et al., 2000). Moreover, hypertension can be considered as a proinflammatory activity (Schiffrin & Touyz, 2004). However, various ACE-inhibitory compounds that have been developed as anti-hypertensive drugs, have shown some undesirable side effects in humans such as coughing, dizziness, headaches, skin rashes and taste distortion. Apart from being sources of metabolic energy and essential amino acids, food protein-generated peptidesas natural ACE inhibitors are considered to be milder and safer without the side effects associated with the synthetic drugs (Wijesekara & Kim, 2010).

Oxidation is a very important process in aerobic organisms, particularly in vertebrates and humans; however, it contributes to the formation of free radicals (Pham-Huy, He, & Pham-Huy, 2008; Poli et al., 2004). When these unstable free radicals exist in excess or cellular defences are deficient due to absence of anti-oxidative molecules; bio-molecules are damaged by a process called oxidative stress. Oxidative stress would damage nucleic acids (DNA or RNA), lipids and proteins, thereby resulting in cell death and tissue damages (Kehrer, 1993). Oxidative stress leads to different kinds of human disease including cancer, cardiovascular diseases (Tain & Baylis, 2006), stroke, hypertension (Chan et al., 2009), cataracts, neurodegenerative disorders, inflammatory diseases and aging (Ames, Shigenaga, & Hagen, 1993). Although the human body has its own defence system against free radicals, it is not very effective in preventing damage completely (Kris-Etherton et al., 2002). Thus, food supplements containing anti-oxidative agents can be used to help and protect the human body against such oxidative damage (Makinen et al., 2012).

Inflammation is part of the complex biological response of vascular tissues to harmful stimuli, such as microbial infections, damaged cells, or irritants (Ferrero-Miliani et al., 2007). The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt to remove the injurious stimuli and to initiate the healing process. After the invasion of pathogens or tissue injuries, inflammation as a defense mechanism is initiated by activation of macrophages. The macrophages are ubiquitous cells that release inflammatory mediators such as nitric oxide (NO) and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6, and interleukin-1β (IL-1β), to enhance defense capacity (Je & Kim, 2012).

In addition, lipopolysaccharides (LPS), which are found in the outer membrane of gram-negative bacteria, acts as endotoxins to active macrophages to generate high amounts of NO (Ashino et al., 2008). Functions of NO are dependent on where it is located. In arterial endothelium cells, NO has been recognized as an important modulator of vascular diseases. NO has several intracellular effects that lead to vasorelaxation, endothelial regeneration and inhibition of platelet adherence and aggregation (Cannon, 1998). Over-production of NO has been shown to participate in human diseases such as arthritis, atherosclerosis, cancer, diabetes, inflammation, numerous degenerative neuronal diseases and stroke (Guslandi, 1998; Ritchlin et al., 2003). The cellular toxicity of NO has been related to its reaction with O_2^- in cellular tissues. Moreover, the reaction of NO derivatives such as $ONOO^-$ could lead to DNA fragmentation and protein structure modification (Pacher, Beckman, & Liaud et, 2007). Thus, NO scavengers could decrease the risk of cellular and tissue damages associated with over-production of NO.

Anti-microbial peptides (AMPs) have the ability to kill a wide range of microorganisms (Jenssen, Hamill, & Hancock, 2006). They mainly participate in the innate immune system and are used as a first line of immune defense (Gallo et al., 2002) in higher organisms such as plants, insects, and mammals (Zhang et al., 2008). In addition to their anti-microbial activity, these peptides have been considered to play a greater role in regulating host immune responses to infections. Some AMP speptides have been shown to be involved in the neutralization of lipopolysaccharides (LPS)-induced endotoxic effects (Bowdish, Davidson, &

Hancock, 2005; Yang et al., 2004). Recently, the demands for AMPs generated from edible protein sources has become more important since they are effective, non-toxic and they are unaffected by antibiotic-resistance mechanisms (Shahidi & Zhong, 2008).

Lipopolysaccharides (LPS) are released from the outer membrane of gram-negative bacteria during bacterial killing. LPS is an endotoxin that causes to release of pro-inflammatory cytokines and NOin many cells (Correa et al., 2014), thereby resulting in increased inflammation. Owing to the role of LPS in septic shock and inflammation, neutralizing the bacterial endotoxin must be considered during the killing of bacteria by anti-bacterial peptides. Thus, generating of AMPsthat simultaneously kill the bacteria, neutralize the LPS or reduce the NO production is of interest.

NO can react with $O_2^{\bullet-}$ and lead to the formation of highly reactive peroxynitrite ($ONOO^-$). The $ONOO^-$ is readily soluble in lipids and has the potential to cause lipid peroxidation. This is the main cause of cellular damages that is attributed to NO (Pacher et al., 2007). It is accepted that the reactive oxygen species (ROS) is produced becauseof hypertension and contributes to hypertension (Harrison & Gongora, 2009). Cardiovascular risk factors such as hypertension increase vascular reactive oxygen species (ROS) production through the action of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, which is activated by angiotensin II. Although, NO acts as the vasodilator, increase reactive oxygen species (ROS) can quench endothelial-derived nitric oxide resulting in reduced bio-availability of NO in the vascular endothelium and consequently decreased vasodilation (Wilcox et al., 1997). Therefore, hypertension, oxidative stress, inflammation and anti-bacterial activity are interrelated (Figure 1.1). This suggests that prevention or control of any one of these conditions can lead to the prevention or control of the others. Thus, peptides with more than one bioactive property, which are defined as multi-functional bioactive peptide (Meisel, 1997) such as ACE inhibition, anti-oxidative, anti-inflammation and anti-bacterial activities can be beneficial in diseases with multiple symptoms such as cardiovascular diseases.

Food protein-generated bioactive peptides are considered as natural products that have nutraceutical and pharmaceutical properties. Due to their potential, there is an increasing interest in the utilized of food protein-generated peptides against human diseases and for maintenance of general well-being. Marine organisms are rich sources of various bioactive compounds with valuable nutraceutical, pharmaceutical and cosmeceutical potentials (Shahidi & Zhong, 2008). Sea cucumbers are of those marine organisms, which are distributed around the world, with the highest diversity in shallow tropical waters. Sea cucumbers have been used as part of the diet in some East Asian countries such as the Philippines, Malaysia, Japan, Korea and China for hundreds of years (Venugopal et al., 2010), where extensive commercial fisheries operate (FAO, 2008). The biological and medicinal activities of sea cucumber species such as anti-angiogenic (Tian et al., 2005), anti-cancer (Roginsky et al., 2004), anti-coagulant (Chen et al., 2011), anti-hypertension (Forghani et al., 2012), anti-inflammatory (Collin, 2004), anti-microbial (Beauregard et al., 2001; Hing et al., 2007), anti-oxidative (Althunibat et al., 2009), anti-thrombotic (Pacheco et al., 2000), anti-tumor (Tong et al., 2005), and wound healing (Miguel-Ruiz & Garcia-Arraras,

2007) have been reported which is related to the presence of different bioactive compounds.

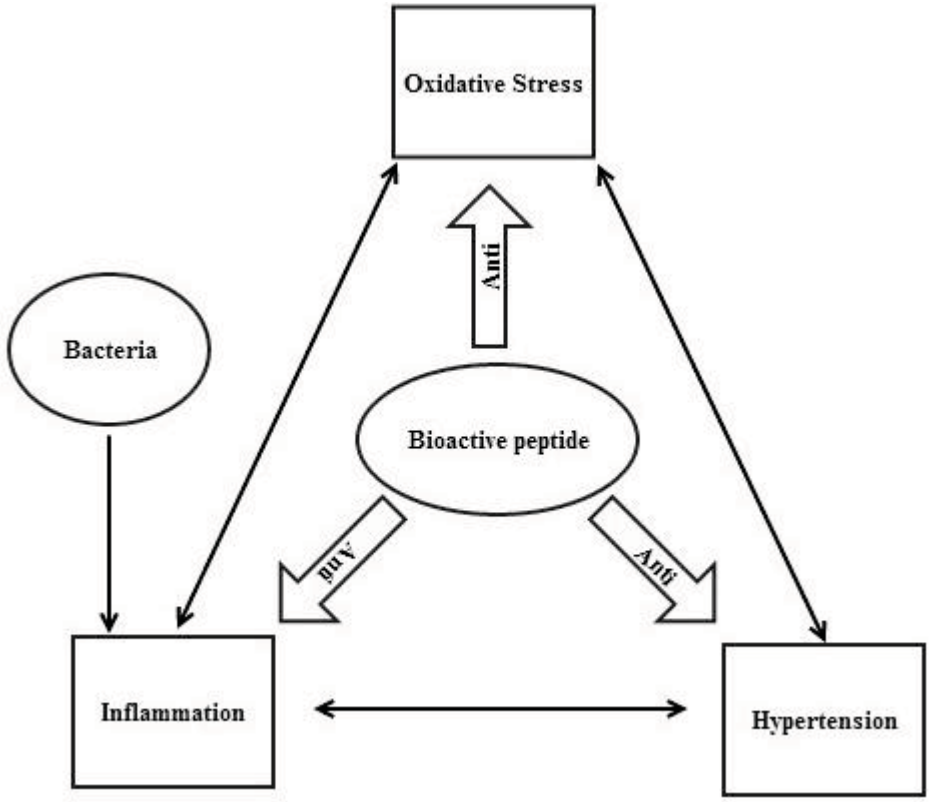

Figure1.1. Relationship between oxidative stress, hypertension and inflammation as well as the preventive roles of bioactive peptides

Sea cucumbers have been considered as a potential source for generating the bioactive peptides due to their high protein content. In this regard, bioactive peptides with ACE-inhibitory and anti-hypertensive activities have been isolated from sea cucumber (*Acaudina molpadioidea*) gelatin using bromelain and alkalase (Zhao et al., 2007; Zhao et al., 2009; Zhao et al., 2012). In another study, the giant red sea cucumber (*Parastichopus californium)* collagen proteolysates showed the ACE-inhibitory activity (Liu et al., 2011). The anti-oxidative activity of sea cucumber peptides has also been examined (Jianrong, 2010; Zhou, Wang, & Jiang, 2012).

Actinopyga lecanora commonly known as stonefish is classified among the edible species of sea cucumber (Shiell, 2004). It is considered as a by-catch of Malaysia's fishing industry and it is an unutilized species of sea cucumber and well-founded data has not yet reported on medicinal proprties of this speiceis. On the other hand, large quantities of stonefishes are discarded every year globally due to the lack of popularity. Due to its relatively high protein content, *A. lecanora* could be a potential commercial source to generate enzymatic proteolysates and bioactive peptides with multifunctional properties. Thus, this study aimed to generate multifunctional bioactive peptides with anti-hypertensive, anti-oxidative, inhibition of nitric oxide (NO) production and anti-bacterial activities from *A. lecanora* proteins. To the researcher's knowledge, this is the first time study reported on the aforementioned

multifunctional properties of *A. lecanora* proteolysates. The finding of current study can provide fundamental information for further work in this field.

The main objectives of the present study were:

1. To generate multifunctional proteolysate from *Actinopyga lecanora* protein through controlled enzymatic proteolysis
2. To fractionate and profilethe multifunctional proteolysate
3. To identify and characterize the multifunctional properties (ACE inhibitory, anti-oxidative, NO-inhibitory and anti-bacterial) of *A. lecanora* bioactive peptides

REFERENCES

Abraham, T. J., Nagarajan, J., & Shanmugam, S. A. (2002). Antimicrobial substances of potential biomedical importance from holothurian species. *Indian Journal of Marine Sciences, 31*(2), 161-164.

Addeo, F., Chianese, L., Salzano, A., Sacchi, R., Cappuccio, U., Ferranti, P., et al. (1992). Characterization of the 12% trichloroacetic acid-insoluble oligopeptides of Parmigiano-Reggiano cheese. *Journal of Dairy Research, 59*(03), 401-411.

Adler-Nissen, J. (1986). *Enzymic hydrolysis of food proteins*: Elsevier Applied Science Publishers.

Ahn, C.-B., Jeon, Y.-J., Kim, Y.-T., & Je, J.-Y. (2012). Angiotensin I converting enzyme (ACE) inhibitory peptides from salmon byproduct protein hydrolysate by Alcalase hydrolysis. *Process Biochemistry, 47*(12), 2240-2245.

Albina, J. E., & Reichner, J. S. (1998). Role of nitric oxide in mediation of macrophage cytotoxicity and apoptosis. *Cancer and Metastasis Reviews, 17*(1), 39-53.

Aleman, A., Gimnez, B., Perez-Santin, E., Gomez-Guillen, M. C., & Montero, P. (2012). Contribution of Leu and Hyp residues to antioxidant and ACE-inhibitory activities of peptide sequences isolated from squid gelatin hydrolysate. *Food Chemistry, 125*(2), 334-341.

Aleman, A., Gomez-Guillen, M. C., & Montero, P. (2013). Identification of ace-inhibitory peptides from squid skin collagen after in vitro gastrointestinal digestion. *Food Research International, 54*(1), 790-795.

Alemon, A., Gimenez, B., Perez-Santin, E., Gomez-Guillen, M. C., & Montero, P. (2011). Contribution of Leu and Hyp residues to antioxidant and ACE-inhibitory activities of peptide sequences isolated from squid gelatin hydrolysate. *Food Chemistry, 125*(2), 334-341.

Althunibat, O. Y., Hashim, R. B., Taher, M., Daud, J. M., Ikeda, M.-A., & Zali, B. I. (2009). In vitro antioxidant and antiproliferative activities of three Malaysian sea cucumber species. *European Journal of Scientific Research, 37*(3), 376-387.

Aluko, R. E., Govil, J. N., & Singh, V. K. (2008). Antihypertensive properties of plant-derived inhibitors of angiotensin I-converting enzyme activity: a review. *Phytopharmacology and Therapeutic values IV, 22*, 541-561.

Aluko, R. E., & Monu, E. (2003). Functional and bioactive properties of quinoa seed protein hydrolysates. *Journal of Food Science, 68*(4), 1254-1258.

Amado, I. R., Vazquez, J. A., Gonzalez, P., Esteban-Fernandez, D., Carrera, M., & Pineiro, C. (2014). Identification of the Major ACE-Inhibitory Peptides Produced by Enzymatic Hydrolysis of a Protein Concentrate from Cuttlefish Wastewater. *Marine Drugs, 12*(3), 1390-1405.

Ames, B. N., Shigenaga, M. K., & Hagen, T. M. (1993). Oxidants, antioxidants, and the degenerative diseases of aging. *Proceedings of the National Academy of Sciences, 90*(17), 7915-7922.

AOAC. (1995). *Official Methods of Analysis of AOAC International*. 16th ed., Vol I, Chaper 4. AOAC: Arlington, VA, USA, 1995.

Ariyoshi, Y. (1993). Angiotensin-converting enzyme inhibitors derived from food proteins. *Trends in Food Science & Technology, 4*(5), 139-144.

Ashino, T., Yamanaka, R., Yamamoto, M., Shimokawa, H., Sekikawa, K., Iwakura, Y., et al. (2008). Negative feedback regulation of lipopolysaccharide-induced inducible nitric oxide synthase gene expression by heme oxygenase-1 induction in macrophages. *Molecular Immunology, 45*(7), 2106-2115.

Asoodeh, A., Memarpoor Yazdi, M., & Chamani, J. (2012). Purification and characterisation of angiotensin I converting enzyme inhibitory peptides from lysozyme hydrolysates. *Food Chemistry, 131*(1), 291-295.

Aydin, M., Sevgili, H., Tufan, B., Emre, Y., & K0se, S. (2011). Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. *International Journal of Food Science & Technology, 46*(3), 500-508.

Balti, R., Bougatef, A., El-Hadj Ali, N., Zekri, D., Barkia, A., & Nasri, M. (2010). Influence of degree of hydrolysis on functional properties and angiotensin I-converting enzyme-inhibitory activity of protein hydrolysates from cuttlefish (*Sepia officinalis*) by-products. *Journal of the Science of Food and Agriculture, 90*(12), 2006-2014.

Battison, A. L., Summerfield, R., & Patrzykat, A. (2008). Isolation and characterisation of two antimicrobial peptides from haemocytes of the American lobster *Homarus americanus*. *Fish & Shellfish Immunology, 25*(1), 181-187.

Baumann, G., & Mueller, P. (1974). A molecular model of membrane excitability. *Journal of Supramolecular Structure, 2*(5-6), 538-557.

Baumy, J. J., Guenot, P., Sinbandhit, S., & Brule, G. (1989). Study of calcium binding to phosphoserine residues of β-casein and its phosphopeptide (1-25) by 31P NMR. *Journal of Dairy Research, 56*(03), 403-409.

Beauregard, K. A., Truong, N. T., Zhang, H., Lin, W., & Beck, G. (2001). The Detection and Isolation of a Novel Antimicrobial Peptide From the Echinoderm Cucumaria Frondosa *Phylogenetic Perspectives on the Vertebrate Immune System* (pp. 55-62): Springer.

Benzie, I. F. F. (2000). Evolution of antioxidant defence mechanisms. *European Journal of Nutrition, 39*(2), 53-61.

Blondelle, S. E., Takahashi, E., Dinh, K. T., & Houghten, R. A. (1995). The antimicrobial activity of hexapeptides derived from synthetic combinatorial libraries. *Journal of Applied Microbiology, 78*(1), 39-46.

Boman, H. G., Agerberth, B., & Boman, A. (1993). Mechanisms of action on *Escherichia coli* of cecropin P1 and PR-39, two antibacterial peptides from pig intestine. *Infection and Immunity, 61*(7), 2978-2984.

Bordbar, S., Anwar, F., & Saari, N. (2012). High-value components and bioactives from sea cucumbers for functional foods-A review. *Marine Drugs, 9*(10), 1761-1805.

Bordbar, S., Ebrahimpour, A., Abdul Hamid, A., Abdul Manap, M. Y., Anwar, F., & Saari, N. (2012). The Improvement of The Endogenous Antioxidant Property of Stone Fish (*Actinopyga lecanora)* Tissue Using Enzymatic Proteolysis. *BioMed Research International, 2013,* 1-9.

Bougatef, A., Balti, R., Haddar, A., Jellouli, K., Souissi, N., & Nasri, M. (2012). Protein hydrolysates from Bluefin Tuna *(Thunnus thynnus)* heads as influenced by the extent of enzymatic hydrolysis. *Biotechnology and Bioprocess Engineering, 17*(4), 841-852.

Bougatef, A., Hajji, M., Balti, R., Lassoued, I., Triki-Ellouz, Y., & Nasri, M. (2009). Antioxidant and free radical-scavenging activities of smooth hound (*Mustelus mustelus*) muscle protein hydrolysates obtained by gastrointestinal proteases. *Food Chemistry, 114*(4), 1198-1205.

Bougatef, A., Nedjar-Arroume, N., Manni, L., Ravallec, R., Barkia, A., Guillochon, D., et al. (2010). Purification and identification of novel antioxidant peptides from enzymatic hydrolysates of sardinelle (*Sardinellaaurita*) by-products proteins. *Food Chemistry, 118*(3), 559-565.

Bowdish, D. M. E., Davidson, D. J., & Hancock, R. (2005). A re-evaluation of the role of host defence peptides in mammalian immunity. *Current Protein and Peptide Science, 6*(1), 35-51.

Brogden, K. A. (2005). Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? *Nature Reviews Microbiology, 3*(3), 238-250.

Brogden, K. A., Ackermann, M., McCray Jr, P. B., & Tack, B. F. (2003). Antimicrobial peptides in animals and their role in host defences. *International Journal of Antimicrobial Agents, 22*(5), 465-478.

Bruckner, A. W., Johnson, K. A., & Field, J. D. (2003). Conservation strategies for sea cucumbers: Can a CITES Appendix II listing promote sustainable international trade. *SPC Beche-de-mer information Bulletin, 18*, 24-33.

Byun, H.-G., & Kim, S.-K. (2002). Structure and activity of angiotensin I converting enzyme inhibitory peptides derived from Alaskan pollack skin. *Journal of Biochemistry and Molecular Biology, 35*(2), 239-243.

Byun, H.-G., Lee, J. K., Park, H. G., Jeon, J.-K., & Kim, S.-K. (2009). Antioxidant peptides isolated from the marine rotifer,(*Brachionus rotundiformis*). *Process Biochemistry, 44*(8), 842-846.

Cai, H., & Harrison, D. G. (2000). Endothelial dysfunction in cardiovascular diseases: the role of oxidant stress. *Circulation Research, 87*(10), 840-844.

Campagna, S., Saint, N., Molle, G. r., & Aumelas, A. (2007). Structure and mechanism of action of the antimicrobial peptide piscidin. *Biochemistry, 46*(7), 1771-1778.

Cannon, R. O. (1998). Role of nitric oxide in cardiovascular disease: focus on the endothelium. *Clinical Chemistry, 44*(8), 1809-1819.

Chan, D. I., Prenner, E. J., & Vogel, H. J. (2006). Tryptophan-and arginine-rich antimicrobial peptides: structures and mechanisms of action. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1758*(9), 1184-1202.

Chan, K. M., Decker, E. A., & Feustman, C. (1994). Endogenous skeletal muscle antioxidants. *Critical Reviews in Food Science & Nutrition, 34*(4), 403-426.

Chan, S. H. H., Wu, K. L. H., Chang, A. Y. W., Tai, M.-H., & Chan, J. Y. H. (2009). Oxidative impairment of mitochondrial electron transport chain complexes in rostral ventrolateral medulla contributes to neurogenic hypertension. *Hypertension, 53*(2), 217-227.

Cheeseman, K. H., & Slater, T. F. (1993). An introduction to free radical biochemistry. *British Medical Bulletin, 49*(3), 481-493.

Chen, H.-M., Muramoto, K., & Yamauchi, F. (1995). Structural analysis of antioxidative peptides from Soybean. beta-Conglycinin. *Journal of Agricultural and Food Chemistry, 43*(3), 574-578.

Chen, H.-M., Muramoto, K., Yamauchi, F., Fujimoto, K., & Nokihara, K. (1998). Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. *Journal of Agricultural and Food Chemistry, 46*(1), 49-53.

Chen, J. (2003). Overview of sea cucumber farming and sea ranching practices in China. *SPC beche-de-mer Information Bulletin, 18*, 18-23.

Chen, S., Xue, C., Yin, L., Tang, Q., Yu, G., & Chai, W. (2011). Comparison of structures and anticoagulant activities of fucosylated chondroitin sulfates from different sea cucumbers. *Carbohydrate Polymers, 83*(2), 688-696.

Chenghui, L., Beiwei, Z., Xiuping, D., & Liguo, C. (2007). Study on the separation and antioxidant activity of enzymatic hydrolysates from sea cucumber. *Food and Fermentation Industries, 33*, 50–53.

Cheung, H.-S., Wang, F.-l., Ondetti, M. A., Sabo, E. F., & Cushman, D. W. (1980). Binding of peptide substrates and inhibitors of angiotensin-converting enzyme. Importance of the COOH-terminal dipeptide sequence. *Journal of Biological Chemistry, 255*(2), 401-407.

Chiang, W.-D., Tsou, M.-J., Tsai, Z.-Y., & Tsai, T.-C. (2006). Angiotensin I-converting enzyme inhibitor derived from soy protein hydrolysate and produced by using membrane reactor. *Food Chemistry, 98*(4), 725-732.

Chludil, H. D., Muniain, C. C., Seldes, A. M., & Maier, M. S. (2002). Cytotoxic and Antifungal Triterpene Glycosides from the Patagonian Sea Cucumber *Hemoiedema spectabilis*. *Journal of Natural Products, 65*(6), 860-865.

Cho, Y.-S., Lee, S.-H., Kim, S.-K., Ahn, C.-B., & Je, J.-Y. (2011). Aminoethyl-chitosan inhibits LPS-induced inflammatory mediators, iNOS and COX-2 expression in RAW 264.7 mouse macrophages. *Process Biochemistry, 46*(2), 465-470.

Church, F. C., Swaisgood, H. E., Porter, D. H., & Catignani, G. L. (1983). Spectrophotometric Assay Using o-Phthaldialdehyde for Determination of Proteolysis in Milk and Isolated Milk Proteins. *Journal of Dairy Science, 66*(6), 1219-1227.

Clarkson, P. M., & Thompson, H. S. (2000). Antioxidants: what role do they play in physical activity and health? *The American Journal of Clinical Nutrition, 72*(2), 637s-646s.

Collin, P. D. (1999). Process for obtaining medicallyactive fractions from sea cucumber. United State Patent 5,876,762, 2.

Collin, P. D. (2004). Peptides having anti-cancer and anti-inflammatory activity,United State Patent,6,767,890, 27.

Conlon, J. M., Al-Ghaferi, N., Abraham, B., & Leprince, J. (2007). Strategies for transformation of naturally-occurring amphibian antimicrobial peptides into therapeutically valuable anti-infective agents. *Methods, 42*(4), 349-357.

Correa, W., Manrique-Moreno, M., Patino, E., Pelaez-Jaramillo, C., Kaconis, Y., Gutsmann, T., et al. (2014). *Galleria mellonella* Native and Analogue Peptides (Gm1 and ΔGm1. Il) Biophysical Characterization of the Interaction Mechanisms with Bacterial Model Membranes. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1838*, 2739-2744.

Coussens, L. M., & Werb, Z. (2002). Inflammation and cancer. *Nature, 420*(6917), 860-867.

Cui, F.-x., Xue, C.-h., Li, Z.-j., Zhang, Y.-q., Dong, P., Fu, X.-y., et al. (2007). Characterization and subunit composition of collagen from the body wall of sea cucumber Stichopus japonicus. *Food Chemistry, 100*(3), 1120-1125.

Cushman, D. W., & Cheung, H. S. (1971). Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochemical pharmacology, 20*(7), 1637-1648.

Daoud, R., Dubois, V., Bors-Dodita, L., Nedjar-Arroume, N., Krier, F., Chihib, N.-E., et al. (2005). New Antibacterial Peptide Derived from Bovine Hemoglobin. *Peptides, 26*(5), 713-719.

Darragh, A. J. (2002). Physiological impact of milk protein-encrypted bioactive peptides. *Bulletin-International Dairy Federation*(375), 25-31.

Dathe, M., Nikolenko, H., Meyer, J., Beyermann, M., & Bienert, M. (2001). Optimization of the antimicrobial activity of magainin peptides by modification of charge. *FEBS Letters, 501*(2), 146-150.

Davalos, A., Miguel, M., Bartolome, B., & Lopez-Fandino, R. (2004). Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. *Journal of Food Protection, 67*(9), 1939-1944.

de Cavanagh, E. M. V., Inserra, F., Ferder, L., & Fraga, C. G. (2000). Enalapril and captopril enhance glutathione-dependent antioxidant defenses in mouse tissues. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 278*(3), R572-R577.

Decker, E. A., & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and Food Chemistry, 38*(3), 674-677.

Dennison, S. R., Wallace, J., Harris, F., & Phoenix, D. A. (2005). Amphiphilic α-Helical Antimicrobial Peptides and Their Structure/Function Relationships. *Protein and Peptide Letters, 12*(1), 31-39.

Devlieghere, F., Vermeulen, A., & Debevere, J. (2004). Chitosan: antimicrobial activity, interactions with food components and applicability as a coating on fruit and vegetables. *Food Microbiology, 21*(6), 703-714.

Dia, V. P., Wang, W., Oh, V. L., De Lumen, B. O., & De Mejia, E. G. (2009). Isolation, purification and characterisation of lunasin from defatted soybean flour and in vitro evaluation of its anti-inflammatory activity. *Food Chemistry, 114*(1), 108-115.

Dinarello, C. A. (2000). Proinflammatory cytokines. *Chest Journal, 118*(2), 503-508.

Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics, 315*(1), 161-169.

Dong, S., Zeng, M., Wang, D., Liu, Z., Zhao, Y., & Yang, H. (2008). Antioxidant and biochemical properties of protein hydrolysates prepared from Silver carp (*Hypophthalmichthys molitrix*). *Food Chemistry, 107*(4), 1485-1493.

Duan, X., Zhang, M., Mujumdar, A. S., & Wang, S. (2010). Microwave freeze drying of sea cucumber (*Stichopus japonicus*). *Journal of Food Engineering, 96*(4), 491-497.

Ehlers, M. R. W., & Riordan, J. F. (1989). Angiotensin-converting enzyme: new concepts concerning its biological role. *Biochemistry, 28*(13), 5311-5318.

Eilertsen, K.-E., Mahre, H. K., Cludts, K., Olsen, J. O., & Hoylaerts, M. F. (2011). Dietary enrichment of apolipoprotein E-deficient mice with extra virgin olive oil in combination with seal oil inhibits atherogenesis. *Lipids in Health and Disease, 10*, 41.

Elavarasan, K., Naveen Kumar, V., & Shamasundar, B. A. (2013). Antioxidant and functional properties of fish protein hydrolysates from fresh water carp (*Catla catla*) as influenced by the nature of enzyme. *Journal of Food Processing and Preservation, 38*, 1207-1214.

Elias, R. J., Kellerby, S. S., & Decker, E. A. (2008). Antioxidant activity of proteins and peptides. *Critical Reviews in Food Science and Nutrition, 48*(5), 430-441.

Epstein, F. H., & Ross, R. (1999). Atherosclerosis-an inflammatory disease. *New England Journal of Medicine, 340*(2), 115-126.

Erdmann, K., Cheung, B. W. Y., & Schroder, H. (2008). The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. *The Journal of Nutritional Biochemistry, 19*(10), 643-654.

Escudero, E., Mora, L., Fraser, P. D., Aristoy, M.-C., & Toldra, F. (2013). Identification of novel antioxidant peptides generated in Spanish dry-cured ham. *Food Chemistry, 138*(2), 1282-1288.

Ezzati, M., Lopez, A. D., Rodgers, A., Vander Hoorn, S., & Murray, C. J. L. (2002). Selected major risk factors and global and regional burden of disease. *The Lancet, 360*(9343), 1347-1360.

Fang, Y.-Z., Yang, S., & Wu, G. (2002). Free radicals, antioxidants, and nutrition. *Nutrition, 18*(10), 872-879.

Ferreira, S. H., Bartelt, D. C., & Greene, L. J. (1970). Isolation of bradykinin-potentiating peptides from Bothrops jararaca venom. *Biochemistry, 9*(13), 2583-2593.

Ferrero-Miliani, L., Nielsen, O. H., Andersen, P. S., & Girardin, S. E. (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1β generation. *Clinical & Experimental Immunology, 147*(2), 227-235.

Fitzgerald, A. J., Rai, P. S., Marchbank, T., Taylor, G. W., Ghosh, S., Ritz, B. W., et al. (2005). Reparative properties of a commercial fish protein hydrolysate preparation. *Gut, 54*(6), 775-781.

FitzGerald, R. J., Murray, B. A., & Walsh, D. J. (2004). Hypotensive peptides from milk proteins. *The Journal of Nutrition, 134*(4), 980S-988S.

Fjell, C. D., Hiss, J. A., Hancock, R. E. W., & Schneider, G. (2011). Designing antimicrobial peptides: form follows function. *Nature Reviews Drug Discovery, 11*(1), 37-51.

Fjell, C. D., Hiss, J. A., Hancock, R. E. W., & Schneider, G. (2012). Designing antimicrobial peptides: form follows function. *Nature Reviews Drug Discovery, 11*(1), 37-51.

Fleming, I. (2006). Signaling by the angiotensin-converting enzyme. *Circulation Research, 98*(7), 887-896.

Foh, M. B. K., Qixing, J., Amadou, I., & Xia, W. S. (2010). Influence of ultrafiltration on antioxidant activity of tilapia *(Oreochromis niloticus)* protein hydrolysate. *Advance Journal of Food Science and Technology, 2*(5), 227-235.

Forghani, B., Ebrahimpour, A., Jamilah Bakar, Hamid, A. A., Hassan, Z., & Saari, N. (2012). Enzyme Hydrolysates from Stichopus horrens as a New Source for Angiotensin-Converting Enzyme Inhibitory Peptides. *Evidence-Based Complementary and Alternative Medicine, 2012*, 1-9.

Fredalina, B. D., Ridzwan, B. H., Abidin, A. A., Kaswandi, M. A., Zaiton, H., Zali, I., et al. (1999). Fatty acid compositions in local sea cucumber. *General Pharmacology: The Vascular System, 33*(4), 337-340.

Friedrich, C. L., Rozek, A., Patrzykat, A., & Hancock, R. E. W. (2001). Structure and mechanism of action of an indolicidin peptide derivative with improved activity against gram-positive bacteria. *Journal of Biological Chemistry, 276*(26), 24015-24022.

Gallo, R. L., Murakami, M., Ohtake, T., & Zaiou, M. (2002). Biology and clinical relevance of naturally occurring antimicrobial peptides. *Journal of Allergy and Clinical Immunology, 110*(6), 823-831.

Gariballa, S. E., & Sinclair, A. J. (2000). Carnosine: physiological properties and therapeutic potential. *Age and ageing, 29*(3), 207-210.

Garner, B., Waldeck, A. R., Witting, P. K., Rye, K.-A., & Stocker, R. (1998). Oxidation of high density lipoproteins II. Evidence for direct reduction of lipid hydroperoxides by methionine residues of apolipoproteins AI and AII. *Journal of Biological Chemistry, 273*(11), 6088-6095.

Ghassem, M., Arihara, K., Babji, A. S., Said, M., & Ibrahim, S. (2011). Purification and identification of ACE inhibitory peptides from Haruan (*Channa striatus*) myofibrillar protein hydrolysate using HPLC-ESI-TOF MS/MS. *Food Chemistry, 129*(4), 1770-1777.

Ghouse, F. A. H., & Ridzwan, B. H. (2007). New Bacterial Species Isolated from Malaysian Sea Cucumbers with Optimized Secreted Antibacterial Activity. *American Journal of Biochemistry and Biotechnology, 3*, 60–65.

Gilani, G. S., Xiao, C., & Lee, N. (2008). Need for accurate and standardized determination of amino acids and bioactive peptides for evaluating protein quality and potential health effects of foods and dietary supplements. *Journal of AOAC International, 91*(4), 894-900.

Goad, L. J., Garneau, F. X., Simard, J. L., ApSimon, J. W., & Girard, M. (1985). Isolation of Δ9(11)-sterols from the sea cucumber psolusfabricii. Implications for holothurin biosynthesis. *Tetrahedron letters, 26*(29), 3513-3516.

Gomez-Flores, R., & Weber, R. J. (1998). Immunomodulation of macrophage functions by opioids *Drugs of Abuse, Immunomodulation, and Aids* (pp. 13-19): Springer.

Grimble, G. K. (1994). The significance of peptides in clinical nutrition. *Annual Review of Nutrition, 14*(1), 419-447.

Guslandi, M. (1998). Nitric oxide and inflammatory bowel diseases. *European Journal of Clinical Investigation, 28*(11), 904-907.

Guzik, T., Korbut, R., & Adamek-Guzik, T. (2003). Nitric oxide and superoxide in inflammation. *Journal of Physiology and Pharmacology, 54*(4), 469-487.

Hai-Lun, H. E., Xiu-Lan, C., Cai-Yun, S., Yu-Zhong, Z., & Bai-Cheng, Z. (2006). Analysis of novel angiotensin-I-converting enzyme inhibitory peptides from protease-hydrolyzed marine shrimp Acetes chinensis. *Journal of Peptide Science, 12*(11), 726-733.

Halliwell, B. (1991). Reactive oxygen species in living systems: source, biochemistry, and role in human disease. *The American Journal of Medicine, 91*(3), S14-S22.

Hamaguchi, P., Geirsdottir, M., Vrac, A., Kristinsson, H. G., Sveinsdottir, H., Fridjonsson, O. H., et al. (2010). *In vitro antioxidant and antihypertensive properties of Icelandic sea cucumber (Cucumaria frondosa).* Paper presented at the Institute of Food Technologists Annual Meeting, Chicago, IL, Abstract.

Hancock, R. E. W. (2001). Cationic peptides: effectors in innate immunity and novel antimicrobials. *The Lancet infectious diseases, 1*(3), 156-164.

Hancock, R. E. W., & Sahl, H.-G. (2006). Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. *Nature Biotechnology, 24*(12), 1551-1557.

Harnedy, P. A., & FitzGerald, R. J. (2012). Bioactive peptides from marine processing waste and shellfish: a review. *Journal of Functional Foods, 4*(1), 6-24.

Harrison, D. G., & Gongora, M. C. (2009). Oxidative stress and hypertension. *Medical Clinics of North America, 93*(3), 621-635.

Hartmann, R., & Meisel, H. (2007). Food-derived peptides with biological activity: from research to food applications. *Current Opinion in Biotechnology, 18*(2), 163-169.

Haug, T., Kjuul, A. K., Styrvold, O. B., Sandsdalen, E., Olsen, O. M., & Stensvg, K. (2002). Antibacterial activity in *Strongylocentrotus droebachiensis* (Echinoidea),*Cucumaria frondosa* (Holothuroidea), and *Asterias rubens* (Asteroidea). *Journal of Invertebrate Pathology, 81*(2), 94-102.

He, H.-L., Chen, X.-L., Wu, H., Sun, C.-Y., Zhang, Y.-Z., & Zhou, B.-C. (2007). High throughput and rapid screening of marine protein hydrolysates enriched in peptides with angiotensin-I-converting enzyme inhibitory activity by capillary electrophoresis. *Bioresource Technology, 98*(18), 3499-3505.

He, H.-L., Liu, D., & Ma, C.-B. (2013). Review on the angiotensin-I-converting enzyme (ACE) inhibitor peptides from marine proteins. *Applied Biochemistry and Biotechnology, 169*(3), 738-749.

He, H. L., Chen, X. L., Wu, H., Sun, C. Y., Zhang, Y. Z., & Zhou, B. C. (2007). High throughput and rapid screening of marine protein hydrolysates enriched in peptides with angiotensin-I-converting enzyme inhibitory activity by capillary electrophoresis. *Bioresource Technology, 98*(18), 3499.

He, X., Cao, W., Zhao, Z., & Zhang, C. (2013). Analysis of protein composition and antioxidant activity of hydrolysates from *Paphia undulate*. *Journal of Food and Nutrition Research, 1*(3), 30-36.

Herencia, F., Ubeda, A., Ferrandiz, M. L., Terencio, M. C., Alcaraz, M. J., Garcia-Carrascosa, M., et al. (1998). Anti-inflammatory activity in mice of extracts from mediterranean marine invertebrates. *Life Sciences, 62*(9), PL115-PL120.

Hermansen, K. (2000). Diet, blood pressure and hypertension. *British Journal of Nutrition, 83*(S1), S113-S119.

Hernandez-Ledesma, B., Hsieh, C.-C., & de Lumen, B. O. (2009). Antioxidant and anti-inflammatory properties of cancer preventive peptide lunasin in RAW 264.7 macrophages. *Biochemical and Biophysical Research Communications, 390*(3), 803-808.

Hickey, M. J., Granger, D. N., & Kubes, P. (2001). Inducible nitric oxide synthase (iNOS) and regulation of leucocyte/endothelial cell interactions: studies in iNOS-deficient mice. *Acta Physiologica Scandinavica, 173*(1), 119-126.

Hing, H. L., Ambia, K. M., Azraul-Mumtazah, R., Hamidah, S. A., Sahalan, A. Z., Shamsudin, N., et al. (2007). Effect of methanol extracts from sea cucumbers Holothuria edulis and Stichopus chloronotus on Candida albicans. *Microscopy and Microanalysis, 13*(S02), 270-271.

Hirota, T., Nonaka, A., Matsushita, A., Uchida, N., Ohki, K., Asakura, M., et al. (2011). Milk casein-derived tripeptides, VPP and IPP induced NO production in cultured endothelial cells and endothelium-dependent relaxation of isolated aortic rings. *Heart and Vessels, 26*(5), 549-556.

Hsu, C.-H., Chen, C., Jou, M.-L., Lee, A. Y.-L., Lin, Y.-C., Yu, Y.-P., et al. (2005). Structural and DNA-binding studies on the bovine antimicrobial peptide, indolicidin: evidence for multiple conformations involved in binding to membranes and DNA. *Nucleic acids Research, 33*(13), 4053-4064.

Huang, D., Ou, B., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of agricultural and food chemistry, 53*(6), 1841-1856.

Humiski, L. M., & Aluko, R. E. (2007). Physicochemical and bitterness properties of enzymatic pea protein hydrolysates. *Journal of Food Science, 72*(8), S605-S611.

Hwang, J.-W., Lee, S.-J., Kim, Y.-S., Kim, E.-K., Ahn, C.-B., Jeon, Y.-J., et al. (2012). Purification and characterization of a novel peptide with inhibitory effects on colitis induced mice by dextran sulfate sodium from enzymatic hydrolysates of *Crassostrea gigas*. *Fish & Shellfish Immunology, 33*, 993-999.

Hwang, J.-Y., Shyu, Y.-S., Wang, Y.-T., & Hsu, C.-K. (2010). Antioxidative properties of protein hydrolysate from defatted peanut kernels treated with esperase. *LWT-Food Science and Technology, 43*(2), 285-290.

Hwang, J. S., Yoo, H. J., Song, H. J., Kim, K. K., Chun, Y. J., Matsui, T., et al. (2012). Inflammation-related signaling pathways implicating TGFB are revealed in the expression profiling of MCF7 cell treated with fermented soybean, Chungkookjang. *Nutrition and Cancer, 63*(4), 645-652.

Intarasirisawat, R., Benjakul, S., Wu, J., & Visessanguan, W. (2013). Isolation of antioxidative and ACE inhibitory peptides from protein hydrolysate of skipjack (*Katsuwana pelamis*) roe. *Journal of Functional Foods, 5*(4), 1854-1862.

Iroyuki fujita, H., Eiichi yokoyama, K., & Yoshikawa, M. (2000). Classification and Antihypertensive Activity of Angiotensin I-Converting Enzyme Inhibitory Peptides Derived from Food Proteins. *Journal of Food Science, 65*(4), 564-569.

Ismail, H., Lemriss, S., Ben Aoun, Z., Mhadhebi, L., Dellai, A., Kacem, Y., et al. (2008). Antifungal activity of aqueous and methanolic extracts from the Mediterranean sea cucumber, *Holothuria polii.Journal of Medical Mycology, 18*(1), 23-26.

Jacob, R. A., & Burri, B. J. (1996). Oxidative damage and defense. *The American Journal of Clinical Nutrition, 63*(6), 985S-990S.

Jang, A., Jo, C., Kang, K.-S., & Lee, M. (2008). Antimicrobial and human cancer cell cytotoxic effect of synthetic angiotensin-converting enzyme (ACE) inhibitory peptides. *Food Chemistry, 107*(1), 327-336.

Je, J.-Y., & Kim, S.-K. (2012). Chitosan as Potential Marine Nutraceutical. In AFNR. & S.-K. Kim (Eds.), *Marine Medicinal Foods.* (Vol. 65, pp. 121-136). UK: Academic Press.

Je, J.-Y., Lee, K.-H., Lee, M. H., & Ahn, C.-B. (2009). Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis. *Food Research International, 42*(9), 1266-1272.

Jeong, H. J., Lam, Y., & de Lumen, B. O. (2002). Barley lunasin suppresses ras-induced colony formation and inhibits core histone acetylation in mammalian cells. *Journal of Agricultural and Food Chemistry, 50*(21), 5903-5908.

Jia, J., Zhou, Y., Lu, J., Chen, A., Li, Y., & Zheng, G. (2010). Enzymatic hydrolysis of Alaska pollack (*Theragra chalcogramma*) skin and antioxidant activity of the resulting hydrolysate. *Journal of the Science of Food and Agriculture, 90*(4), 635-640.

Jianrong, C. H. Y. P. L. (2010). The Preparation of Collagen Polypeptide with Free Radical Scavenging Ability Purified from *Acaudina Molpadioides* Semper [J]. *Journal of Chinese Institute of Food Science and Technology, 10*, 19-25.

Jimsheena, V. K., & Gowda, L. R. (2009). Colorimetric, high-throughput assay for screening angiotensin I-converting enzyme inhibitors. *Analytical chemistry, 81*(22), 9388-9394.

Johansen, J. S., Harris, A. K., Rychly, D. J., & Ergul, A. (2005). Oxidative stress and the use of antioxidants in diabetes: linking basic science to clinical practice. *Cardiovascular Diabetology, 4*(1), 5.

Jung, W.-K., Rajapakse, N., & Kim, S.-K. (2005). Antioxidative activity of a low molecular weight peptide derived from the sauce of fermented blue mussel, *Mytilus edulis*. *European Food Research and Technology, 220*(5-6), 535-539.

Jung, W.-K., Mendis, E., Je, J.-Y., Park, P.-J., Son, B. W., Kim, H. C., et al. (2006). Angiotensin I-converting enzyme inhibitory peptide from yellowfin sole (*Limanda aspera*) frame protein and its antihypertensive effect in spontaneously hypertensive rats. *Food Chemistry, 94*(1), 26-32.

Kawasaki, H., & Iwamuro, S. (2008). Potential roles of histones in host defense as antimicrobial agents. *Infectious Disorders-Drug Targets, 8*(3), 195-205.

Kearney, P. M., Whelton, M., Reynolds, K., Muntner, P., Whelton, P. K., & He, J. (2005). Global burden of hypertension: analysis of worldwide data. *The Lancet, 365*(9455), 217-223.

Kehrer, J. P. (1993). Free radicals as mediators of tissue injury and disease. *CRC Critical Reviews in Toxicology, 23*(1), 21-48.

Kelly, F. J. (1999). Gluthathione: in defence of the lung. *Food and Chemical Toxicology, 37*(9), 963-966.

Kerr, R. G., & Chen, Z. (1995). In vivo and in vitro biosynthesis of saponins in sea cucumbers. *Journal of natural products, 58*(2), 172-176.

Khan, J. K., Kuo, Y.-H., Kebede, N., & Lambein, F. (1994). Determination of non-protein amino acids and toxins in *Lathyrus* by high-performance liquid chromatography with precolumn phenyl isothiocyanate derivatization. *Journal of Chromatography A, 687*(1), 113-119.

Kim, E.-K., Kim, Y.-S., Hwang, J.-W., Choi, D.-K., Lee, K.-H., Lee, J. S., et al. (2013). Purification of a novel nitric oxide inhibitory peptide derived from enzymatic hydrolysates of *Mytilus coruscus*. *Fish & Shellfish Immunology, 34*, 1416-1420.

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

Kim, S., & Ponka, P. (2002). Nitric oxide-mediated modulation of iron regulatory proteins: implication for cellular iron homeostasis. *Blood Cells, Molecules, and Diseases, 29*(3), 400-410.

Kitts, D. D., & Weiler, K. (2003). Bioactive proteins and peptides from food sources. Applications of bioprocesses used in isolation and recovery. *Current Pharmaceutical Design, 9*(16), 1309-1323.

Kleinert, H., Wallerath, T., Fritz, G., Ihrig-Biedert, I., Rodriguez-Pascual, F., Geller, D. A., et al. (1998). Cytokine induction of NO synthase II in human DLD-1 cells: roles of the JAK-STAT, AP-1 and NF-kappaB-signaling pathways. *British Journal of Pharmacology, 125*(1), 193-201.

Klompong, V., Benjakul, S., Kantachote, D., & Shahidi, F. (2007). Antioxidative activity and functional properties of protein hydrolysate of yellow stripe trevally (*Selaroides leptolepis*) as influenced by the degree of hydrolysis and enzyme type. *Food Chemistry, 102*(4), 1317-1327.

Knowles, R. G., & Moncada, S. (1994). *Nitric oxide synthases in mammals*: Biochemical Society.

Kohmura, M., Nio, N., Kubo, K., Minoshima, Y., Munekata, E., & Ariyoshi, Y. (1989). Inhibition of angiotensin-converting enzyme by synthetic peptides of human β-casein. . *Agricultural and Biological Chemistry, 53*, 2107-2114.

Korhonen, H. (2002). Technology options for new nutritional concepts. *International Journal of Dairy Technology, 55*(2), 79-88.

Korhonen, H., & Pihlanto, A. (2003). Food-derived bioactive peptides-opportunities for designing future foods. *Current Pharmaceutical Design, 9*(16), 1297-1308.

Korhonen, H., & Pihlanto, A. (2006). Bioactive peptides: production and functionality. *International Dairy Journal, 16*(9), 945-960.

Korhonen, R., Lahti, A., Kankaanranta, H., & Moilanen, E. (2005). Nitric oxide production and signaling in inflammation. *Current Drug Targets-Inflammation & Allergy, 4*(4), 471-479.

Kris-Etherton, P. M., Hecker, K. D., Bonanome, A., Coval, S. M., Binkoski, A. E., Hilpert, K. F., et al. (2002). Bioactive compounds in foods: their role in the prevention of cardiovascular disease and cancer. *The American Journal of Medicine, 113*(9), 71-88.

Ktari, N., Fakhfakh, N., Balti, R., Ben Khaled, H., Nasri, M., & Bougatef, A. (2013). Effect of degree of hydrolysis and protease type on the antioxidant activity of protein hydrolysates from cuttlefish (*Sepia officinalis*) by-products. *Journal of Aquatic Food Product Technology, 22*(5), 436-448.

Kumar, R., Chaturvedi, A. K., Shukla, P. K., & Lakshmi, V. (2007). Antifungal activity in triterpene glycosides from the sea cucumber *Actinopyga lecanora*. *Bioorganic & Medicinal Chemistry Letters, 17*(15), 4387-4391.

Kuo, P.-L., & Pu, C. (2011). The contribution of depression to mortality among elderly with self-reported hypertension: analysis using a national representative longitudinal survey. *Journal of Hypertension, 29*(11), 2084-2090.

Lakshmi, S. V., Padmaja, G., Kuppusamy, P., & Kutala, V. K. (2009). Oxidative stress in cardiovascular disease.

Lam, H.-T., Josserand, J., Lion, N., & Girault, H. H. (2007). Modeling the isoelectric focusing of peptides in an OFFGEL multicompartment cell. *Journal of Proteome Research, 6*(5), 1666-1676.

Larsen, R., Eilertsen, K.-E., & Elvevoll, E. O. (2011). Health benefits of marine foods and ingredients. *Biotechnology Advances, 29*(5), 508-518.

Lee, J. K., Hong, S., Jeon, J.-K., Kim, S.-K., & Byun, H.-G. (2009). Purification and characterization of angiotensin I converting enzyme inhibitory peptides from the rotifer, *Brachionus rotundiformis*. *Bioresource Technology, 100*(21), 5255-5259.

Lee, K.-H., Chow, Y.-L., Sharmili, V., Abas, F., Alitheen, N. B. M., Shaari, K., et al. (2012). BDMC33, a curcumin derivative suppresses inflammatory responses in macrophage-like cellular system: Role of inhibition in NF-Îºb and MAPK signaling pathways. *International Journal of Molecular Sciences, 13*(3), 2985-3008.

Lee, S.-H., Qian, Z.-J., & Kim, S.-K. (2010). A novel angiotensin I converting enzyme inhibitory peptide from tuna frame protein hydrolysate and its antihypertensive effect in spontaneously hypertensive rats. *Food Chemistry, 118*(1), 96-102.

Lee, S.-J., Kim, E.-K., Kim, Y.-S., Hwang, J.-W., Lee, K. H., Choi, D.-K., et al. (2012). Purification and characterization of a nitric oxide inhibitory peptide from *Ruditapes philippinarum*. *Food and Chemical Toxicology, 50*(5), 1660-1666.

Lee, W.-S., Jeon, J.-K., & Byun, H.-G. (2011). Characterization of a novel antioxidative peptide from the sand eel Hypoptychus dybowskii. *Process Biochemistry, 46*(5), 1207-1211.

Li, A., Lee, P. Y., Ho, B., Ding, J. L., & Lim, C. T. (2007). Atomic force microscopy study of the antimicrobial action of Sushi peptides on Gram negative bacteria. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1768*(3), 411-418.

Li, G.-H., Le, G.-W., Shi, Y.-H., & Shrestha, S. (2004). Angiotensin I-converting enzyme inhibitory peptides derived from food proteins and their physiological and pharmacological effects. *Nutrition Research, 24*(7), 469-486.

Li, X.-x., Han, L.-j., & Chen, L.-j. (2008). In vitro antioxidant activity of protein hydrolysates prepared from corn gluten meal. *Journal of the Science of Food and Agriculture, 88*(9), 1660-1666.

Li, Y., Jiang, B., Zhang, T., Mu, W., & Liu, J. (2008). Antioxidant and free radical-scavenging activities of chickpea protein hydrolysate (CPH). *Food Chemistry, 106*(2), 444-450.

Lindsay, R. C. (1996). Food additives. In O. R. Fennema (Ed.), Food chemistry (pp.778–780). New York: Marcel Dekker Inc.

Lineweaver, H., & Burk, D. (1934). The determination of enzyme dissociation constants. *Journal of the American Chemical Society, 56*(3), 658-666.

Liu, B. I. N., Gao, H.-M., Wang, J.-Y., Jeohn, G.-H., Cooper, C. L., & Hong, J.-S. (2002). Role of Nitric Oxide in Inflammation-Mediated Neurodegeneration. *Annals of the New York Academy of Sciences, 962*(1), 318-331.

Liu, J., Yu, Z., Zhao, W., Lin, S., Wang, E., Zhang, Y., et al. (2010). Isolation and identification of angiotensin-converting enzyme inhibitory peptides from egg white protein hydrolysates. *Food Chemistry, 122*(4), 1159-1163.

Liu, P., Zhao, M., Cao, Y., Lu, Y., Liang, M., Huang, Z., et al. (2013). Purification and identification of antioxidant soybean peptides by consecutive chromatography and electrospray ionization-mass spectrometry. *Rejuvenation Research, 17*(2), 209-211.

Liu, Z.-Y., Chen, D., Su, Y.-C., & Zeng, M.-Y. (2011). Optimization of hydrolysis conditions for the production of the angiotensin-I converting enzyme inhibitory peptides from sea cucumber collagen hydrolysates. *Journal of Aquatic Food Product Technology, 20*(2), 222-232.

Liu, Z., Dong, S., Xu, J., Zeng, M., Song, H., & Zhao, Y. (2008). Production of cysteine-rich antimicrobial peptide by digestion of oyster (Crassostrea gigas) with alcalase and bromelin. *Food Control, 19*(3), 231-235.

Lohner, K. (2001) The role of membrane lipid composition in cell targeting of antimicrobial peptides.Development of novel antimicrobial agents: emerging strategies.K.Lohner, ed. (Norfolk, England: Horizon Scientific Press(pp. 149-165).

Ludtke, S. J., He, K., Heller, W. T., Harroun, T. A., Yang, L., & Huang, H. W. (1996). Membrane pores induced by magainin. *Biochemistry, 35*(43), 13723-13728.

Lugnier, C., Keravis, T., & Eckly-Michel, A. (1999). Cross talk between NO and cyclic nucleotide phosphodiesterases in the modulation of signal transduction in blood vessel. *Journal of Physiology and Parmacology: An official Journal of the Polish Physiological Society, 50*(4), 639-652.

Ma, Y., Xiong, Y. L., Zhai, J., Zhu, H., & Dziubla, T. (2010). Fractionation and evaluation of radical scavenging peptides from in vitro digests of buckwheat protein. *Food Chemistry, 118*(3), 582-588.

MacCallum, J. L., Bennett, W. F., & Tieleman, D. P. (2008). Distribution of amino acids in a lipid bilayer from computer simulations. *Biophysical Journal, 94*(9), 3393-3404.

Majumder, K., Chakrabarti, S., Morton, J. S., Panahi, S., Kaufman, S., Davidge, S. T., et al. (2013). Egg-derived Tri-peptide IRW exerts antihypertensive effects in spontaneously hypertensive rats. *PloS one, 8*(11), e82829.

Makinen, S., Johannson, T., Vegarud Gerd, E., Pihlava, J. M., & Pihlanto, A. (2012). Angiotensin I-converting enzyme inhibitory and antioxidant properties of rapeseed hydrolysates. *Journal of Functional Foods, 4*(3), 575-583.

Mamelona, J., Pelletier, E., Girard-Lalancette, K., Legault, J., Karboune, S., & Kermasha, S. (2007). Quantification of phenolic contents and antioxidant capacity of Atlantic sea cucumber,*Cucumaria frondosa*. *Food Chemistry, 104*(3), 1040-1047.

Mamelona, J., Saint-Louis, R., & Pelletier, E. (2010). Nutritional composition and antioxidant properties of protein hydrolysates prepared from echinoderm byproducts. *International Journal of Food Science & Technology, 45*(1), 147-154.

Mao, X.-Y., Cheng, X., Wang, X., & Wu, S.-J. (2011). Free-radical-scavenging and anti-inflammatory effect of yak milk casein before and after enzymatic hydrolysis. *Food Chemistry, 126*(2), 484-490.

Marchand, C., Krajewski, K., Lee, H.-F., Antony, S., Johnson, A. A., Amin, R., et al. (2006). Covalent binding of the natural antimicrobial peptide indolicidin to DNA abasic sites. *Nucleic acids Research, 34*(18), 5157-5165.

Matsufuji, H., Matsui, T., Seki, E., Osajima, K., Nakashima, M., & Osajima, Y. (1994). Angiotensin I-converting enzyme inhibitory peptides in an alkaline protease hydrolyzate derived from sardine muscle. *Bioscience, Biotechnology, and Biochemistry, 58*(12), 2244-2245.

Matsuzaki, K. (1998). Magainins as paradigm for the mode of action of pore forming polypeptides. *Biochimica et Biophysica Acta (BBA)-Reviews on Biomembranes, 1376*(3), 391-400.

Megias, C., Pedroche, J., Yust, M. M., Giron-Calle, J., Alaiz, M., Millan, F., et al. (2008). Production of copper-chelating peptides after hydrolysis of sunflower proteins with pepsin and pancreatin. *LWT-Food Science and Technology, 41*(10), 1973-1977.

Meisel, H. (1997). Biochemical properties of regulatory peptides derived from mil proteins. *Peptide Science, 43*(2), 119-128.

Meisel, H. (2004). Multifunctional peptides encrypted in milk proteins. *Biofactors, 21*(1), 55-61.

Meisel, H. (2005). Biochemical properties of peptides encrypted in bovine milk proteins. *Current Medicinal Chemistry, 12*(16), 1905-1919.

Meisel, H., & FitzGerald, R. J. (2003). Biofunctional peptides from milk proteins: mineral binding and cytomodulatory effects. *Current Pharmaceutical Design, 9*(16), 1289-1296.

Memarpoor-Yazdi, M., Asoodeh, A., & Chamani, J. (2012). A novel antioxidant and antimicrobial peptide from hen egg white lysozyme hydrolysates. *Journal of Functional Foods, 4*(1), 278-286.

Mendis, E., Rajapakse, N., & Kim, S.-K. (2005). Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. *Journal of Agricultural and Food Chemistry, 53*(3), 581-587.

Migliore-Samour, D., & Jolles, P. (1988). Casein, a prohormone with an immunomodulating role for the newborn? *Experientia, 44*(3), 188-193.

Ming, S. (2001). Investigation on Component and Pharmacology of Sea Cucumber [J]. *Chinese Traditional Patent Medicine, 10*, 021.

Mitchell, B. M., Dorrance, A. M., Ergul, A., & Webb, R. C. (2004). Sepiapterin decreases vasorelaxation in nitric oxide synthase inhibition-induced hypertension. *Journal of Cardiovascular Pharmacology, 43*(1), 93-98.

Moller, N. P., Scholz-Ahrens, K. E., Roos, N., & Schrezenmeir, J. (2008). Bioactive peptides and proteins from foods: indication for health effects. *European Journal of Nutrition, 47*(4), 171-182.

Monera, O. D., Sereda, T. J., Zhou, N. E., Kay, C. M., & Hodges, R. S. (1995). Relationship of sidechain hydrophobicity and α-helical propensity on the stability of the single-stranded amphipathic α-helix. *Journal of Peptide Science, 1*(5), 319-329.

Morgan, A., & Archer, J. (1999). Overview: Aspects of sea cucumber industry research and development in the South Pacific. *SPC Beche-de-mer Inf. Bull, 1*, 15-17.

Mourao, P. A. S., & Pereira, M. S. (1999). Searching for alternatives to heparin: sulfated fucans from marine invertebrates. *Trends in Cardiovascular Medicine, 9*(8), 225-232.

Mourao, P. A. S., Pereira, M. S., Pavao, M. S. G., Mulloy, B., Tollefsen, D. M., Mowinckel, M.-C., et al. (1996). Structure and anticoagulant activity of a fucosylated chondroitin sulfate from echinoderm Sulfated fucose branches on the polysaccharide account for its high anticoagulant action. *Journal of Biological Chemistry, 271*(39), 23973-23984.

Moure, A., Dominguez, H., & Parajo, J. C. (2006). Antioxidant properties of ultrafiltration-recovered soy protein fractions from industrial effluents and their hydrolysates. *Process Biochemistry, 41*(2), 447-456.

Mueller, M. M. (2006). Inflammation in epithelial skin tumours: old stories and new ideas. *European Journal of Cancer, 42*(6), 735-744.

Murase, H., Nagao, A., & Terao, J. (1993). Antioxidant and emulsifying activity of N-(long-chain-acyl) histidine and N-(long-chain-acyl) carnosine. *Journal of Agricultural and Food Chemistry, 41*(10), 1601-1604.

Murray, A. P., Muniain, C., Seldes, A. M., & Maier, M. S. (2001). Patagonicoside A: a novel antifungal disulfated triterpene glycoside from the sea cucumber Psolus patagonicus. *Tetrahedron, 57*(47), 9563-9568.

Murray, B. A., & FitzGerald, R. J. (2007). Angiotensin converting enzyme inhibitory peptides derived from food proteins: biochemistry, bioactivity and production. *Current Pharmaceutical Design, 13*(8), 773-791.

Nagase, H., Enjyoji, K.-i., Minamiguchi, K., Kitazato, K. T., Kitazato, K., Saito, H., et al. (1995). Depolymerized holothurian glycosaminoglycan with novel anticoagulant actions: antithrombin III-and heparin cofactor II-independent inhibition of factor X activation by factor IXa-factor VIIIa complex and heparin cofactor II-dependent inhibition of thrombin. *Blood, 85*(6), 1527-1534.

Nakagomi, K., Fujimura, A., Ebisu, H., Sakai, T., Sadakane, Y., Fujii, N., et al. (1998). Acein-1, a novel angiotensin-I-converting enzyme inhibitory peptide isolated from tryptic hydrolysate of human plasma. *FEBS Letters, 438*(3), 255-257.

Nakajima, Y., Ishibashi, J., Yukuhiro, F., Asaoka, A., Taylor, D., & Yamakawa, M. (2003). Antibacterial activity and mechanism of action of tick defensin against Gram-positive bacteria. *Biochimica et Biophysica Acta (BBA)-General Subjects, 1624*(1), 125-130.

Nasri, R., Bougatef, A., Ben Khaled, H., Nedjar-Arroume, N., Karra Chaabouni, M., Dhulster, P., et al. (2012). Antioxidant and Free Radical-Scavenging Activities of Goby (*Zosterisessor ophiocephalus*) Muscle Protein Hydrolysates Obtained by Enzymatic Treatment. *Food Biotechnology, 26*(3), 266-279.

Natesh, R., Schwager, S. L. U., Sturrock, E. D., & Acharya, K. R. (2003). Crystal structure of the human angiotensin-converting enzyme-lisinopril complex. *Nature, 421*(6922), 551-554.

Nathan, C. (2002). Points of control in inflammation. *Nature, 420*(6917), 846-852.

Nejati, F., Rizzello, C. G., Di Cagno, R., Sheikh-Zeinoddin, M., Diviccaro, A., Minervini, F., et al. (2013). Manufacture of a functional fermented milk enriched of Angiotensin-I Converting Enzyme (ACE)-inhibitory peptides and α-amino butyric acid (GABA). *LWT-Food Science and Technology, 51*(1), 183-189.

Nielsen, S. S. (2010). Food analysis: Springer Verlag

Ngo, D.-H., Wijesekara, I., Vo, T.-S., Van Ta, Q., & Kim, S.-K. (2011). Marine food-derived functional ingredients as potential antioxidants in the food industry: An overview. *Food Research International, 44*(2), 523-529.

Nokihara, K., Yamamoto, R., Hazama, M., Wakizawa, O., & Nakamura, S. (1992). Design and applications of a novel simultaneous multiple solid-phase peptide synthesizer, In R. Epton (Ed.) (pp. 445–448).Andover,UK: Intercept Limited.

O'Loughlin, I. B., Murray, B. A., Brodkorb, A., FitzGerald, R. J., & Kelly, P. M. (2014). Production of whey protein isolate hydrolysate fractions with enriched ACE-inhibitory activity. *International Dairy Journal*, *38*(2), 101-103.

Ondetti, M. A., & Cushman, D. W. (1982). Enzymes of the renin-angiotensin system and their inhibitors. *Annual Review of Biochemistry, 51*(1), 283-308.

Ondetti, M. A., Rubin, B., & Cushman, D. W. (1977). Design of specific inhibitors of angiotensin-converting enzyme: New class of orally active antihypertensive agents. *Sience, 196*(10), 441-444.

Ono, S., Hosokawa, M., Miyashita, K., & Takahashi, K. (2006). Inhibition properties of dipeptides from salmon muscle hydrolysate on angiotensin I-converting enzyme. *International Journal of Food Science & Technology, 41*(4), 383-386.

Oren, Z., & Shai, Y. (1997). Selective lysis of bacteria but not mammalian cells by diastereomers of melittin: structure-function study. *Biochemistry, 36*(7), 1826-1835.

Oren, Z., & Shai, Y. (1998). Mode of action of linear amphipathic α- helical antimicrobial peptides *Peptide Science, 47*(6), 451-463.

Ozer, N. P., Mol, S., & Varlik, C. (2004). Effect of the handling procedures on the chemical composition of sea cucumber. *Turkish Journal of Fisheries and Aquatic Sciences, 4*, 71-74.

Pacheco, R. G., Vicente, C. P., Zancan, P., & Mourao, P. A. S. (2000). Different antithrombotic mechanisms among glycosaminoglycans revealed with a new fucosylated chondroitin sulfate from an echinoderm. *Blood Coagulation &Fibrinolysis, 11*(6), 563-573.

Pacher, P. l., Beckman, J. S., & Liaudet, L. (2007). Nitric oxide and peroxynitrite in health and disease. *Physiological reviews, 87*(1), 315-424.

Pan, S.-K., Yao, D.-R., Zhou, M.-Q., & Wu, S.-J. (2014). Hydroxyl radical scavenging activity of peptide from sea cucumber using enzyme complex isolated from the digestive tract of sea cucumber. *African Journal of Biotechnology, 11*(5), 1214-1219.

Park, C. B., Kim, H. S., & Kim, S. C. (1998). Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and inhibiting cellular functions. *Biochemical and Biophysical Research Communications, 244*(1), 253-257.

Park, C. B., Yi, K.-S., Matsuzaki, K., Kim, M. S., & Kim, S. C. (2000). Structure-activity analysis of buforin II, a histone H2A-derived antimicrobial peptide: the proline hinge is responsible for the cell-penetrating ability of buforin II. *Proceedings of the National Academy of Sciences, 97*(15), 8245-8250.

Park, E. Y., Imazu, H., Matsumura, Y., Nakamura, Y., & Sato, K. (2012). Effects of peptide fractions with different isoelectric points from wheat gluten

hydrolysates on lipid oxidation in pork meat patties. *Journal of Agricultural and Food Chemistry, 60*(30), 7483-7488.

Park, E. Y., Morimae, M., Matsumura, Y., Nakamura, Y., & Sato, K. (2008). Antioxidant activity of some protein hydrolysates and their fractions with different isoelectric points. *Journal of Agricultural and Food Chemistry, 56*(19), 9246-9251.

Park, K. H., Nan, Y. H., Park, Y., Kim, J. I., Park, I.-S., Hahm, K.-S., et al. (2009). Cell specificity, anti-inflammatory activity, and plausible bactericidal mechanism of designed Trp-rich model antimicrobial peptides. *Biochimica et Biophysica Acta (BBA)-Biomembranes, 1788*(5), 1193-1203.

Patrzykat, A., Friedrich, C. L., Zhang, L., Mendoza, V., & Hancock, R. E. W. (2002). Sublethal concentrations of pleurocidin-derived antimicrobial peptides inhibit macromolecular synthesis in *Escherichia coli*. *Antimicrobial Agents and Chemotherapy, 46*(3), 605-614.

Pedroche, J., Yust, M. M., Giron-Calle, J., Alaiz, M., Millan, F., & Vioque, J. (2002). Utilisation of chickpea protein isolates for production of peptides with angiotensin I-converting enzyme (ACE)-inhibitory activity. *Journal of the Science of Food and Agriculture, 82*(9), 960–965.

Pena-Ramos, E. A., Xiong, Y. L., & Arteaga, G. E. (2004). Fractionation and characterisation for antioxidant activity of hydrolysed whey protein. *Journal of the Science of Food and Agriculture, 84*(14), 1908-1918.

Peng, X., Xiong, Y. L., & Kong, B. (2009). Antioxidant activity of peptide fractions from whey protein hydrolysates as measured by electron spin resonance. *Food Chemistry, 113*(1), 196-201.

Perez-Vega, J. A., Olivera-Castillo, L., Gomez-Ruiz, J. A., & Hernandez-Ledesma, B. (2013). Release of multifunctional peptides by gastrointestinal digestion of sea cucumber (*Isostichopus badionotus*). *Journal of Functional Foods, 5*(2), 869-877.

Pfeffer, M. A., & Frohlich, E. D. (2006). Improvements in clinical outcomes with the use of angiotensin-converting enzyme inhibitors: cross-fertilization between clinical and basic investigation. *American Journal of Physiology-Heart and Circulatory Physiology, 291*(5), H2021-H2025.

Pham-Huy, L. A., He, H., & Pham-Huy, C. (2008). Free radicals, antioxidants in disease and health. *International Journal of Biomedical Science: IJBS, 4*(2), 89.

Pihlanto, A., Akkanen, S., & Korhonen, H. J. (2008). ACE-inhibitory and antioxidant properties of potato *(Solanum tuberosum)*. *Food Chemistry, 109*(1), 104-112.

Pober, J. S., & Sessa, W. C. (2007). Evolving functions of endothelial cells in inflammation. *Nature Reviews Immunology, 7*(10), 803-815.

Poli, G., Leonarduzzi, G., Biasi, F., & Chiarpotto, E. (2004). Oxidative stress and cell signalling. *Current Medicinal Chemistry, 11*(9), 1163-1182.

Pou, S., Pou, W. S., Bredt, D. S., Snyder, S. H., & Rosen, G. M. (1992). Generation of superoxide by purified brain nitric oxide synthase. *Journal of Biological Chemistry, 267*(34), 24173-24176.

Power, O., Jakeman, P., & FitzGerald, R. J. (2013). Antioxidative peptides: enzymatic production, in vitro and in vivo antioxidant activity and potential applications of milk-derived antioxidative peptides. *Amino Acids, 44*(3), 797-820.

Pownall, T. L., Udenigwe, C. C., & Aluko, R. E. (2010). Amino acid composition and antioxidant properties of pea seed *(Pisum sativum L.)* enzymatic protein hydrolysate fractions. *Journal of Agricultural and Food Chemistry, 58*(8), 4712-4718.

Pripp, A. H. (2008). Effect of peptides derived from food proteins on blood pressure: a metaanalysis of randomized controlled trials. *Food & Nutrition Research, 52*(10), 1-9.

Qian, Z.-J., Je, J.-Y., & Kim, S.-K. (2007). Antihypertensive effect of angiotensin I converting enzyme-inhibitory peptide from hydrolysates of bigeye tuna dark muscle, Thunnus obesus. *Journal of Agricultural and Food Chemistry, 55*(21), 8398-8403.

Qian, Z.-J., Jung, W.-K., & Kim, S.-K. (2008). Free radical scavenging activity of a novel antioxidative peptide purified from hydrolysate of bullfrog skin, Rana catesbeiana Shaw. *Bioresource Technology, 99*(6), 1690-1698.

Quist, E. E., Phillips, R. D., & Saalia, F. K. (2009). Angiotensin converting enzyme inhibitory activity of proteolytic digests of peanut (*Arachis hypogaea L.*) flour. *LWT-Food Science and Technology, 42*(3), 694-699.

Rafiuddin Ahmed, M., Venkateshwarlu, U., & Jayakumar, R. (2004). Multilayered peptide incorporated collagen tubules for peripheral nerve repair. *Biomaterials, 25*(13), 2585-2594.

Rajapakse, N., Mendis, E., Byun, H.-G., & Kim, S.-K. (2005). Purification and in vitro antioxidative effects of giant squid muscle peptides on free radical-mediated oxidative systems. *The Journal of Nutritional Biochemistry, 16*(9), 562-569.

Rajapakse, N., Mendis, E., Jung, W.-K., Je, J.-Y., & Kim, S.-K. (2005). Purification of a radical scavenging peptide from fermented mussel sauce and its antioxidant properties. *Food Research International, 38*(2), 175-182.

Ren, J., Zhao, M., Shi, J., Wang, J., Jiang, Y., Cui, C., et al. (2008). Purification and identification of antioxidant peptides from grass carp muscle hydrolysates by consecutive chromatography and electrospray ionization-mass spectrometry. *Food Chemistry, 108*(2), 727-736.

Ridzwan, B. H., Kaswandi, M. A., Azman, Y., & Fuad, M. (1995). Screening for antibacterial agents in three species of sea cucumbers from coastal areas of Sabah. *General Pharmacology: The Vascular System, 26*(7), 1539-1543.

Ritchlin, C. T., Haas-Smith, S. A., Li, P., Hicks, D. G., & Schwarz, E. M. (2003). Mechanisms of TNF-α and RANKL-mediated osteoclastogenesis and bone resorption in psoriatic arthritis. *Journal of Clinical Investigation, 111*(6), 821-831.

Roginsky, A., Singh, B., Ding, X. Z., Collin, P., Woodward, C., Talamonti, M. S., et al. (2004). Frondanol(R)-A5p From the Sea Cucumber, Cucu-Maria Frondosa Induces Cell Cycle Arrest and Apoptosis in Pancreatic Cancer Cells. *Pancreas, 29*(4), 335.

Rui, X., Boye, J. I., Simpson, B. K., & Prasher, S. O. (2013). Purification and characterization of angiotensin I-converting enzyme inhibitory peptides of small red bean (*Phaseolus vulgaris)* hydrolysates. *Journal of Functional Foods, 5*(3), 1116-1124.

Ryan, J. T., Ross, R. P., Bolton, D., Fitzgerald, G. F., & Stanton, C. (2011). Bioactive peptides from muscle sources: meat and fish. *Nutrients, 3*(9), 765-791.

Saiga, A. I., Tanabe, S., & Nishimura, T. (2003). Antioxidant activity of peptides obtained from porcine myofibrillar proteins by protease treatment. *Journal of agricultural and food chemistry, 51*(12), 3661-3667.

Saito, K., Jin, D.-H., Ogawa, T., Muramoto, K., Hatakeyama, E., Yasuhara, T., et al. (2003). Antioxidative properties of tripeptide libraries prepared by the combinatorial chemistry. *Journal of agricultural and food chemistry, 51*(12), 3668-3674.

Saito, M., Kunisaki, N., Urano, N., & Kimura, S. (2002). Collagen as the major edible component of sea cucumber *(Stichopus japonicus)*. *Journal of food science, 67*(4), 1319-1322.

Salampessy, J., Phillips, M., Seneweera, S., & Kailasapathy, K. (2010). Release of antimicrobial peptides through bromelain hydrolysis of leatherjacket (Meuchenia sp.) insoluble proteins. *Food Chemistry, 120*(2), 556-560.

Samaranayaka, A. G. P., & Li-Chan, E. C. Y. (2011). Food-derived peptidic antioxidants: A review of their production, assessment, and potential applications. *Journal of Functional Foods, 3*(4), 229-254.

Sampath Kumar, N. S., Nazeer, R. A., & Jaiganesh, R. (2011). Purification and biochemical characterization of antioxidant peptide from horse mackerel (*Magalaspis cordyla*) viscera protein. *Peptides, 32*(7), 1496-1501.

San Miguel-Ruiz, J. E., & Garcia-Arraras, J. E. (2007). Common cellular events occur during wound healing and organ regeneration in the sea cucumber Holothuria glaberrima. *BMC Developmental Biology, 7*(1), 115.

Santos, S. D. A., Martins, V. G., Salas-Mellado, M., & Prentice, C. (2011). Evaluation of Functional Properties in Protein Hydrolysates from Bluewing Searobin (*Prionotus punctatus*) Obtained with Different Microbial Enzymes. *Food and Bioprocess Technology, 4*(8), 1399-1406.

Sarmadi, B., Ismail, A., & Hamid, M. (2011). Antioxidant and angiotensin converting enzyme (ACE) inhibitory activities of cocoa (*Theobroma cacao L.*) autolysates. *Food Research International, 44*(1), 290-296.

Sarmadi, B. H., & Ismail, A. (2010). Antioxidative peptides from food proteins: a review. *Peptides, 31*(10), 1949-1956.

Sato, M., Hosokawa, T., Yamaguchi, T., Nakano, T., Muramoto, K., Kahara, T., et al. (2002). Angiotensin I-converting enzyme inhibitory peptides derived from wakame (*Undaria pinnatifida*) and their antihypertensive effect in spontaneously hypertensive rats. *Journal of Agricultural and Food Chemistry, 50*(21), 6245-6252.

Schiffrin, E. L., & Touyz, R. M. (2004). From bedside to bench to bedside: role of renin-angiotensin-aldosterone system in remodeling of resistance arteries in hypertension. *American Journal of Physiology-Heart and Circulatory Physiology, 287*(2), H435-H446.

Shahidi, F., & Zhong, Y. (2008). Bioactive peptides. *Journal of AOAC International, 91*(4), 914-931.

Shamloo, M., Bakar, J., Mat Hashim, D., & Khatib, A. (2012). Biochemical properties of red tilapia *(Oreochromis niloticus)* protein hydrolysates. *International Food Research Journal, 19*(1), 183-188.

Sheih, I. C., Fang, T. J., & Wu, T.-K. (2009). Isolation and characterisation of a novel angiotensin I-converting enzyme (ACE) inhibitory peptide from the algae protein waste. *Food Chemistry, 115*(1), 279-284.

Sheriff, S. A., Sundaram, B., Ramamoorthy, B., & Ponnusamy, P. (2014). Synthesis and in vitro antioxidant functions of protein hydrolysate from backbones of Rastrelliger kanagurta by proteolytic enzymes. *Saudi Journal of Biological Sciences, 21*(1), 19-26.

Shiell, G. (2004). Field observations of juvenile sea cucumbers. *SPC Beche-de-mer Inf. Bull, 20*, 6-11.

Shimokawa, H., Flavahan, N. A., & Vanhoutte, P. M. (1991). Loss of endothelial pertussis toxin-sensitive G protein function in atherosclerotic porcine coronary arteries. *Circulation, 83*(2), 652-660.

Sila, A., Nedjar-Arroume, N., Hedhili, K., Chataigne, G., Balti, R., Nasri, M., et al. (2014). Antibacterial peptides from barbel muscle protein hydrolysates: Activity against some pathogenic bacteria. *LWT-Food Science and Technology, 55*(1), 183-188.

Silhavy, T. J., Kahne, D., & Walker, S. (2010). The bacterial cell envelope. *Cold Spring Harbor Perspectives in Biology, 2*, 414.

Smith, A. C. (1978). A proposed phylogenetic relationship between sea cucumber Polian vesicles and the vertebrate lymphoreticular system. *Journal of Invertebrate Pathology, 31*(3), 353-357.

Song, R., Wei, R., Zhang, B., & Wang, D. (2010). Optimization of the antibacterial activity of half-fin anchovy (*Setipinna taty*) hydrolysates. *Food and Bioprocess Technology, 5*(5), 1979-1989.

Sowmya, R., Rathinaraj, K., & Sachindra, N. M. (2011). An autolytic process for recovery of antioxidant activity rich carotenoprotein from shrimp heads. *Marine Biotechnology, 13*(5), 918-927.

Sreejayan, N., Rao, M. N. A., Priyadarsini, K. I., & Devasagayam, T. P. A. (1997). Inhibition of radiation-induced lipid peroxidation by curcumin. *International Journal of Pharmaceutics, 151*(1), 127-130.

Srinivas, S., & Prakash, V. (2010). Bioactive peptides from bovine milk α-casein: isolation, characterization and multifunctional properties. *International Journal of Peptide Research and Therapeutics, 16*(1), 7-15.

Stohs, S. J., & Bagchi, D. (1995). Oxidative mechanisms in the toxicity of metal ions. *Free Radical Biology and Medicine, 18*(2), 321-336.

Strom, M. B., Haug, B. E., Rekdal, O., Skar, M. L., Stensen, W., & Svendsen, J. S. (2002). Important structural features of 15-residue lactoferricin derivatives and methods for improvement of antimicrobial activity. *Biochemistry and Cell Biology, 80*(1), 65-74.

Stryer, L. (1995). *Biochemistry, Chapter 2,* (4th ed.): WH Freeman & Company:pp. 732.

Su, Y.-c., Liu, S.-j., & Wu, C.-y. (2009). Optimization of the preparation procedure and the antioxidant activity of polypeptide from sea cucumber. *Journal of Fujian Fisheries, 2*.

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

Suetsuna, K. (2002). Identification of antihypertensive peptides from peptic digest of the short-necked clam Tapes philippinarum and the pearl oyster Pinctada fucata martensii. *Fisheries Science, 68*(1), 233-235.

Suetsuna, K., Ukeda, H., & Ochi, H. (2000). Isolation and characterization of free radical scavenging activities peptides derived from casein. *The Journal of Nutritional Biochemistry, 11*(3), 128-131.

Sugawara, T., Zaima, N., Yamamoto, A., Sakai, S., Noguchi, R., & Hirata, T. (2006). Isolation of sphingoid bases of sea cucumber cerebrosides and their cytotoxicity against human colon cancer cells. *Bioscience Biotechnology and Biochemistry, 70*(12), 2906.

Suh, J.-Y., Lee, Y.-T., Park, C.-B., Lee, K.-H., Kim, S.-C., & Choi, B.-S. (1999). Structural and functional implications of a proline residue in the antimicrobial peptide gaegurin. *European Journal of Biochemistry, 266*(2), 665-674.

Sun, J., He, H., & Xie, B. J. (2004). Novel antioxidant peptides from fermented mushroom Ganoderma lucidum. *Journal of Agricultural and Food Chemistry, 52*(21), 6646-6652.

Szabo, C., & Ohshima, H. (1997). DNA damage induced by peroxynitrite: subsequent biological effects. *Nitric Oxide, 1*(5), 373-385.

Szeto, H. H. (2008). Mitochondria-targeted cytoprotective peptides for ischemia-reperfusion injury. *Antioxidants & Redox Signaling, 10*(3), 601-620.

Tabacova, S. A., & Kimmel, C. A. (2001). Enalapril: pharmacokinetic/dynamic inferences for comparativedevelopmental toxicity: A review. *Reproductive Toxicology, 15*(5), 467-478.

Tain, Y.-L., & Baylis, C. (2006). Dissecting the causes of oxidative stress in an in vivo model of hypertension. *Hypertension, 48*(5), 828-829.

Takahashi, H., & Hara, K. (2003). Cardiovascular diseases and oxidative stress. *Rinsho byori. The Japanese Journal of Clinical Pathology, 51*(2), 133-139.

Tang, C.-H., Wang, X.-S., & Yang, X.-Q. (2009). Enzymatic hydrolysis of hemp (*Cannabis sativa L.*) protein isolate by various proteases and antioxidant properties of the resulting hydrolysates. *Food Chemistry, 114*(4), 1484-1490.

Tang, L., Sun, J., Zhang, H. C., Zhang, C. S., Yu, L. N., Bi, J., et al. (2012). Evaluation of Physicochemical and Antioxidant Properties of Peanut Protein Hydrolysate. *PloS One, 7*(5), e37863.

Tang, W., Zhang, H., Wang, L., & Qian, H. (2013). Antimicrobial peptide isolated from ovalbumin hydrolysate by immobilized liposome-binding extraction. *European Food Research and Technology*, 1-10.

Tewtrakul, S., & Itharat, A. (2007). Nitric oxide inhibitory substances from the rhizomes of Dioscorea membranacea. *Journal of ethnopharmacology, 109*(3), 412-416.

Tian, F., Zhang, X., Tong, Y., Yi, Y., Zhang, S., Li, L., et al. (2005). a new sulfated saponin from sea cucumber, exhibits anti-angiogenic and anti-tumor activitiesin vitro and in vivo. *Cancer Biol & Therapy, 4*, 874–882.

Tong, Y., Zhang, X., Tian, F., Yi, Y., Xu, Q., Li, L., et al. (2005). Philinopside a, a novel marine-derived compound possessing dual anti-angiogenic and anti-tumor effects. *International Journal of Cancer, 114*(6).

Torre, D., Pugliese, A., & Speranza, F. (2002). Role of nitric oxide in HIV-1 infection: friend or foe? *The Lancet Infectious Diseases, 2*(5), 273-280.

Torres-Fuentes, C., Alaiz, M., & Vioque, J. (2012). Iron-chelating activity of chickpea protein hydrolysate peptides. *Food Chemistry, 134*(3), 1585-1588.

Tsai, J.-S., Chen, J.-L., & Pan, B. S. (2008). ACE-inhibitory peptides identified from the muscle protein hydrolysate of hard clam (*Meretrix lusoria*). *Process Biochemistry, 43*(7), 743-747.

Tsai, J. S., Lin, T. C., Chen, J. L., & Pan, B. S. (2006). The inhibitory effects of freshwater clam (*Corbicula fluminea, Muller*) muscle protein hydrolysates on angiotensin I converting enzyme. *Process Biochemistry, 41*(11), 2276-2281.

Tschudi, M. R., Mesaros, S., Luscher, T. F., & Malinski, T. (1996). Direct in situ measurement of nitric oxide in mesenteric resistance arteries Increased decomposition by superoxide in hypertension. *Hypertension, 27*(1), 32-35.

Turko, I. V., Marcondes, S., & Murad, F. (2001). Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA: 3-oxoacid CoA-transferase. *American Journal of Physiology-Heart and Circulatory Physiology, 281*(6), H2289-H2294.

Turrens, J. F. (2003). Mitochondrial formation of reactive oxygen species. *The Journal of Physiology, 552*(2), 335-344.

Ulvatne, H., Samuelsen, A., Haukland, H. H., Kromer, M., & Vorland, L. H. (2004). Lactoferricin B inhibits bacterial macromolecular synthesis in Escherichia coli and Bacillus subtilis. *FEMS Microbiology Ietters, 237*(2), 377-384.

Vastag, Z., Popovic, L., Popovic, S., Krimer, V., & Pericin, D. (2011). Production of enzymatic hydrolysates with antioxidant and angiotensin-I converting enzyme inhibitory activity from pumpkin oil cake protein isolate. *Food Chemistry, 124*(4), 1316-1321.

Venugopal, D., Klapper, D., Srouji, A. H., Bhonsle, J. B., Borschel, R., Mueller, A., et al. (2010). Novel antimicrobial peptides that exhibit activity against select agents and other drug resistant bacteria. *Bioorganic & Medicinal Chemistry, 18*(14), 5137-5147.

Vermeirssen, V., Van Camp, J., & Verstraete, W. (2002). Optimisation and validation of an angiotensin-converting enzyme inhibition assay for the screening of bioactive peptides. *Journal of Biochemical and Biophysical Methods, 51*(1), 75-87.

Vernaza, M. G., Dia, V. P., Gonzalez de Mejia, E., & Chang, Y. K. (2012). Antioxidant and antiinflammatory properties of germinated and hydrolysed Brazilian soybean flours. *Food Chemistry, 134*(4), 2217-2225.

Verschuren, L., Wielinga, P. Y., van Duyvenvoorde, W., Tijani, S., Toet, K., van Ommen, B., et al. (2011). A dietary mixture containing fish oil, resveratrol, lycopene, catechins, and vitamins E and C reduces atherosclerosis in transgenic mice. *The Journal of Nutrition, 141*(5), 863-869.

Vieira, R. P., Mulloy, B., & Mourao, P. A. (1991). Structure of a fucose-branched chondroitin sulphate from sea cucumber. Evidence for the presence of 3-O-sulfo-β-D-glucuronosyl residues. *Journal of Biological Chemistry, 266*, 13530–13536.

Vogel, H. J., Schibli, D. J., Jing, W., Lohmeier-Vogel, E. M., Epand, R. F., & Epand, R. M. (2002). Towards a structure-function analysis of bovine lactoferricin and related tryptophan-and arginine-containing peptides. *Biochemistry and Cell Biology, 80*(1), 49-63.

Wako, Y., Ishikawa, S., & Muramoto, K. (1996). Angiotensin I-converting enzyme inhibitors in autolysates of squid liver and mantle muscle. *Bioscience, Biotechnology, and Biochemistry, 60*(8), 1353-1355.

Wang, J.-s., Zhao, M.-m., Zhao, Q.-z., & Jiang, Y.-m. (2007). Antioxidant properties of papain hydrolysates of wheat gluten in different oxidation systems. *Food Chemistry, 101*(4), 1658-1663.

Wang, J., Hu, J., Cui, J., Bai, X., Du, Y., Miyaguchi, Y., et al. (2008). Purification and identification of a ACE inhibitory peptide from oyster proteins hydrolysate and the antihypertensive effect of hydrolysate in spontaneously hypertensive rats. *Food Chemistry, 111*(2), 302-308.

Wang, J., Wang, Y., Tang, Q., Wang, Y., Chang, Y., Zhao, Q., et al. (2010). Antioxidation activities of low-molecular-weight gelatin hydrolysate isolated from the sea cucumber *Stichopus japonicus*. *Journal of Ocean University of China, 9*(1), 94-98.

Wang, W., Mejia, D., & Gonzalez, E. (2005). A New Frontier in Soy Bioactive Peptides that May Prevent Age-related Chronic Diseases. *Comprehensive Reviews in Food Science and Food Safety, 4*(4), 63-78.

Wen, J., Hu, C., & Fan, S. (2010). Chemical composition and nutritional quality of sea cucumbers. *Journal of the Science of Food and Agriculture, 90*(14), 2469-2474.

Whitehouse, M. W., & Fairlie, D. P. (1994). Anti-Inflammatory activity of a holothurian (sea cucumber) food supplement in rats. *Inflammopharmacology, 2*(4), 411-417.

Wijesekara, I., & Kim, S.-K. (2010). Angiotensin-I-converting enzyme (ACE) inhibitors from marine resources: prospects in the pharmaceutical industry. *Marine Drugs, 8*(4), 1080-1093.

Wilcox, J. N., Subramanian, R. R., Sundell, C. L., Tracey, W. R., Pollock, J. S., Harrison, D. G., et al. (1997). Expression of multiple isoforms of nitric oxide synthase in normal and atherosclerotic vessels. *Arteriosclerosis, Thrombosis, and Vascular Biology, 17*(11), 2479-2488.

Wiriyaphan, C., Chitsomboon, B., & Yongsawadigul, J. (2012). Antioxidant activity of protein hydrolysates derived from threadfin bream surimi byproducts. *Food Chemistry, 132*(1), 104-111.

Wiseman, H., & Halliwell, B. (1996). Damage to DNA by reactive oxygen and nitrogen species: role in inflammatory disease and progression to cancer. *Biochemical Journal, 313*, 17-29.

Wolz, M., Cutler, J., Roccella, E. J., Rohde, F., Thom, T., & Burt, V. (2000). Statement from the national high blood pressure education program: prevalence of hypertension. *American Journal of Hypertension, 13*(1), 103-104.

Wu, H.-C., Chen, H.-M., & Shiau, C.-Y. (2003). Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Research International, 36*(9), 949-957.

Wu, H., He, H.-L., Chen, X.-L., Sun, C.-Y., Zhang, Y.-Z., & Zhou, B.-C. (2008). Purification and identification of novel angiotensin-I-converting enzyme inhibitory peptides from shark meat hydrolysate. *Process Biochemistry, 43*(4), 457-461.

Wu, H., Pan, B. S., Chang, C. l., & Shiau, C. (2005). Low-molecular-weight peptides as related to antioxidant properties of chicken essence. *Journal of Food and Drug Analysis, 13*(2), 176.

Wu, J., Aluko, R. E., & Muir, A. D. (2008). Purification of angiotensin I-converting enzyme-inhibitory peptides from the enzymatic hydrolysate of defatted canola meal. *Food Chemistry, 111*(4), 942-950.

Wu, J., Aluko, R. E., & Nakai, S. (2006). Structural requirements of angiotensin I-converting enzyme inhibitory peptides: quantitative structure-activity relationship study of di-and tripeptides. *Journal of Agricultural and Food Chemistry, 54*(3), 732-738.

Xie, Z., Huang, J., Xu, X., & Jin, Z. (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chemistry, 111*(2), 370-376.

Yamamoto, N., Ejiri, M., & Mizuno, S. (2003). Biogenic peptides and their potential use. *Current Pharmaceutical Design, 9*(16), 1345-1355.

Yamamoto, N., Mine, Y., Li-Chan, E., & Jiang, B. (2010). Functional Food Products with Antihypertensive Effects. *Bioactive proteins and peptides as functional foods and nutraceuticals, 29*, 169.

Yang, D., Biragyn, A., Hoover, D. M., Lubkowski, J., & Oppenheim, J. J. (2004). Multiple Roles of Antimicrobial Defensins, Cathelicidins, and Eosinophil-Derived Neurotoxin in Host Defense. *Annual Review of Immunology, 22*, 181-215.

Yang, Y., Tao, G., Liu, P., & Liu, J. (2007). Peptide with angiotensin I-converting enzyme inhibitory activity from hydrolyzed corn gluten meal. *Journal of Agricultural and Food Chemistry, 55*(19), 7891-7895.

Yau, W.-M., Wimley, W. C., Gawrisch, K., & White, S. H. (1998). The preference of tryptophan for membrane interfaces. *Biochemistry, 37*(42), 14713-14718.

Yea, C. S., Ebrahimpour, A., Hamid, A. A., Bakar, J., Muhammad, K., & Saari, N. (2014). Winged bean [Psophorcarpus tetragonolobus (L.) DC] seeds as an underutilised plant source of bifunctional proteolysate and biopeptides. *Food & function, 5*(5), 1007-1016.

Yokoyama, K., Chiba, H., & Yoshikawa, M. (1992). Peptide inhibitors for angiotensin I-converting enzyme from thermolysin digest of dried bonito. *Bioscience, Biotechnology, and Biochemistry, 56*(10), 1541.

Yoshikawa, M., Fujita, H., Matoba, N., Takenaka, Y., Yamamoto, T., Yamauchi, R., et al. (2000). Bioactive peptides derived from food proteins preventing lifestyle-related diseases. *Biofactors, 12*(1), 143-146.

You, L., Zhao, M., Regenstein, J. M., & Ren, J. (2010). Purification and identification of antioxidative peptides from loach (Misgurnus anguillicaudatus) protein hydrolysate by consecutive chromatography and electrospray ionization-mass spectrometry. *Food Research International, 43*(4), 1167-1173.

You, L., Zhao, M., Regenstein, J. M., & Ren, J. (2011). In vitro antioxidant activity and in vivo anti-fatigue effect of loach (*Misgurnus anguillicaudatus*) peptides prepared by papain digestion. *Food Chemistry, 124*(1), 188-194.

You, S.-J., Udenigwe, C. C., Aluko, R. E., & Wu, J. (2010). Multifunctional peptides from egg white lysozyme. *Food Research International, 43*(3), 848-855.

Yuan, T., Weljie, A. M., & Vogel, H. J. (1998). Tryptophan fluorescence quenching by methionine and selenomethionine residues of calmodulin: orientation of peptide and protein binding. *Biochemistry, 37*(9), 3187-3195.

Zarei, M., Ebrahimpour, A., Abdul-Hamid, A., Anwar, F., Bakar, F. A., Philip, R., et al. (2014). Identification and characterization of papain-generated antioxidant peptides from palm kernel cake proteins. *Food Research International, 62*, 726-734.

Zarei, M., Ebrahimpour, A., Abdul-Hamid, A., Anwar, F., & Saari, N. (2012). Production of defatted palm kernel cake protein hydrolysate as a valuable source of natural antioxidants. *International Journal of Molecular Sciences, 13*(7), 8097-8111.

Zeng, M., Xiao, F., Zhao, Y., Liu, Z., Li, B., & Dong, S. (2007). Study on the free radical scavenging activity of sea cucumber (*Paracaudina chinens var*.) gelatin hydrolysate. *Journal of Ocean University of China, 6*(3), 255-258.

Zhang, Y.-x., Zou, A.-h., Manchu, R.-g., Zhou, Y.-c., & Wang, S.-f. (2008). Purification and antimicrobial activity of antimicrobial protein from Brown-spotted Grouper, Epinephelus fario. *Zool Research, 6*, 627-632.

Zhang, Y., Olsen, K., Grossi, A., & Otte, J. (2013). Effect of pretreatment on enzymatic hydrolysis of bovine collagen and formation of ACE-inhibitory peptides. *Food Chemistry, 141*(3), 2343-2354.

Zhao, K., Zhao, G.-M., Wu, D., Soong, Y., Birk, A. V., Schiller, P. W., et al. (2004). Cell-permeable peptide antioxidants targeted to inner mitochondrial membrane inhibit mitochondrial swelling, oxidative cell death, and reperfusion injury. *Journal of Biological Chemistry, 279*(33), 34682-34690.

Zhao, Y., Li, B., Dong, S., Liu, Z., Zhao, X., Wang, J., et al. (2009). A novel ACE inhibitory peptide isolated from *Acaudina molpadioidea* hydrolysate. *Peptides, 30*(6), 1028-1033.

Zhao, Y., Li, B., Liu, Z., Dong, S., Zhao, X., & Zeng, M. (2007). Antihypertensive effect and purification of an ACE inhibitory peptide from sea cucumber gelatin hydrolysate. *Process Biochemistry, 42*(12), 1586-1591.

Zhao , Y. H., Li, B. F., Ma, J. J., Dong, S. Y., Liu, Z. Y., & Zeng, M. Y. (2012). Purification and synthesis of ACE-inhibitory peptide from *Acaudina molpadioidea* protein hydrolysate. *Chemical Journal of Chinese Universities-Chinese 33*, 308–312.

Zhong, Y., Khan, M. A., & Shahidi, F. (2007). Compositional characteristics and antioxidant properties of fresh and processed sea cucumber (*Cucumaria frondosa*). *Journal of Agricultural and Food Chemistry, 55*(4), 1188-1192.

Zhou, D.-Y., Zhu, B.-W., Qiao, L., Wu, H.-T., Li, D.-M., Yang, J.-F., et al. (2012). In vitro antioxidant activity of enzymatic hydrolysates prepared from abalone (*Haliotis discus hannaiIno*) viscera. *Food and Bioproducts Processing, 90*(2), 148-154.

Zhou, X., Wang, C., & Jiang, A. (2012). Antioxidant peptides isolated from sea cucumber *Stichopus Japonicus*. *European Food Research and Technology, 234*(3), 441-447.

Zhu, C.-Z., Zhang, W.-G., Zhou, G.-H., Xu, X.-L., Kang, Z.-L., & Yin, Y. (2013). Isolation and Identification of Antioxidant Peptides from Jinhua Ham. *Journal of Agricultural and Food Chemistry, 61*(6), 1265-1271.