CHAPTER 5

Bioactive Marine Peptides: Nutraceutical Value and Novel Approaches

Anupam Giri and **Toshiaki Ohshima**[1]

Contents

Abstract

Marine organisms represent a valuable source of nutraceuticals and functional compounds. The biodiversity of the marine environment and the associated chemical diversity constitute a practically unlimited resource of novel active substances for the development of bioactive products. Recently, a great deal of interest has been expressed in marine-derived bioactive peptides because of their numerous beneficial health effects. Moreover, several studies have reported that marine bioactive peptides can be used as

Department of Food Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, Japan
[1] Corresponding author: Toshiaki Ohshima, *E-mail address:* tohshima@kaiyodai.ac.jp

Advances in Food and Nutrition Research, Volume 65
ISSN 1043-4526, DOI: 10.1016/B978-0-12-416003-3.00005-6

antihypertensive, antioxidative, anticoagulant, and antimicrobial components in functional foods or nutraceuticals and pharmaceuticals due to their therapeutic potential in the treatment or prevention of disease. In this chapter, we provide an overview of bioactive peptides derived from marine organisms as well as information about their biological properties and mechanisms of action with potential applications in different areas.

I. INTRODUCTION

With marine species comprising ~50% of total global biodiversity, the sea represents an enormous resource for novel compounds (de Vries and Beart, 1995), and it has been classified as the largest remaining reservoir of natural molecules to be evaluated for drug activity (Gerwick, 1987). The importance of marine organisms as a source of novel bioactive substances is growing rapidly. Moreover, much attention has been paid to unraveling the structural, compositional, and sequential properties of bioactive peptides. The discovery of the bioregulatory role of different endogenous peptides in organisms as well as the characterization of the underlying molecular mechanisms of action of some novel natural bioactive peptides on specific cellular targets have contributed to the hypothesis that peptides could represent promising lead drug candidates. Recently, marine-derived peptides have opened a new area for the development of pharmaceutical compounds (Miljanich, 1997).

Marine-derived bioactive peptides have been shown to possess many physiological functions, including angiotensin I-converting enzyme (ACE) inhibition and antihypertensive (Byun and Kim, 2001; Je *et al*., 2005a), antioxidant (Kim *et al*., 2007; Mendis *et al*., 2005a), anticoagulant (Jo *et al*., 2008; Rajapakse *et al*., 2005a), and antimicrobial (Liu *et al*., 2008; Stensvag *et al*., 2008) activity. Moreover, some of these bioactive peptides may have potential benefits for the promotion of human health and the reduction of disease risk (Defelice, 1995; Shahidi and Zhong, 2008); indeed, a role for food-derived bioactive peptides in the reduction of risk for cardiovascular disease has been well demonstrated (Erdmann *et al*., 2008). Further, increasing consumer awareness about the link between diet and health has raised the demand for functional food ingredients and nutraceuticals. Bioactive peptides derived from marine organisms and the by-products from the processing of marine fish can potentially be used for the development of functional foods (Ariyoshi, 1993; Jung *et al*., 2006a; Shahidi, 2007; Yamamoto, 1997).

Marine capture fisheries contribute to more than 50% of the total global production of fish, and >70% of this output is utilized for processing (FAOSTAT, 2001). As a result, a considerable amount of the yearly total

catch is discarded as processing leftovers, which include trimmings, fins, frames, heads, skin, and viscera. In addition to fish processing, a large quantity of the by-products from marine bioprocessing plants consists of shells from crustaceans and shellfish. Therefore, there is a great potential for the marine bioprocess industry to convert and utilize more of these by-products as valuable products. Recent studies have identified a number of bioactive compounds in the discarded fish muscle proteins, collagen, gelatin, oil, bone, and internal organs and in shellfish and crustacean shells (Je *et al.*, 2005b; Jeon and Kim, 2000; Kim *et al.*, 2001). Generally, the production of foodstuffs for human consumption generates high levels of profitability; however, even higher levels of profit are expected to be derived from the production of bioactive compounds. Therefore, the development of new technology to identify and isolate novel bioactive compounds from marine processing by-products will increase the value of what is currently considered a waste product, and this represents a unique challenge and opportunity for the seafood industry.

This chapter provides an overview of the diversity of marine bioactive peptides, their biological activity, and their potential use as nutraceutical and pharmaceutical products.

II. EFFECT OF THE STRUCTURAL PROPERTIES OF PEPTIDES ON BIOACTIVITY

Bioactive peptides are inactive in the context of their parent protein, but can be released by enzymatic hydrolysis (Kim *et al.*, 1999; Lahl and Braun, 1994). Depending on their amino acid sequence, they may have roles in various biological processes such as opioid agonists or antagonists and demonstrate antihypertensive immunomodulatory, antithrombotic, antioxidant, anticancer, and antimicrobial activity, in addition to their use as nutritional compounds (Clare and Swaisgood, 2000; Elias *et al.*, 2008). On the basis of their structure and other factors, including hydrophobicity, charge, and microelement-binding properties, some bioactive peptides exhibit multifunctional activity (Cho *et al.*, 2008; Korhonen and Pihlanto-Leppala, 2003).

Bioactive peptides usually contain 3–20 amino acid residues (Pihlanto-Leppala, 2001), and the lower their molecular weight (MW), the higher their chance to cross the intestinal barrier and exert biological effects (Roberts *et al.*, 1999). Previous work on antioxidative peptides has shown that peptides with 5–16 amino acid residues can inhibit the autoxidation of linoleic acid (Chen *et al.*, 1995a). Lipid peroxidation is thought to proceed via the radical-mediated abstraction of hydrogen atoms from methylene carbons in polyunsaturated fatty acids (Rajapakse *et al.*, 2005b). Since the hydrophobicity of antioxidants is important for their

accessibility to hydrophobic targets (Chen *et al.*, 1996), it is presumed that the presence of hydrophobic amino acids in the purified peptide might contribute to their lipid peroxidation inhibitory activity by increasing the solubility of peptides in lipids, thereby facilitating their improved interaction with radical species. In addition, the presence of a hydrophobic amino acid at the N-terminus of peptide sequences is thought to be important for their antioxidative activity (Chen *et al.*, 1995b; Ranathunga *et al.*, 2006) because it is assumed that such amino acids can increase the interaction between the peptides and fatty acids.

The specific amino acid composition of a peptide is a critical factor for its ACE-inhibitory activity. Glu, Asp, Pro, Gly, and Ala are observed in many ACE-inhibitory peptides derived from food proteins, for example, Arg-Ala-Asp-His-Pro-Phe from albumin (He *et al.*, 2004), Tyr-Gly-Leu from whey (He *et al.*, 2004), Ala-Glu-Leu from alga (He *et al.*, 2004), Asp-Leu-Pro from soy (He *et al.*, 2004), Val-Pro-Pro from skimmed milk (Pan *et al.*, 2005), Leu-Ala-Ile-Pro-Val-Asn-Lys-Pro from soybean protein (Kuba *et al.*, 2005), and Gly-Pro-Pro from buckwheat (Ma *et al.*, 2006). As reported by Cushman *et al.* (1981), the active sites of two domains in ACE are structurally and functionally homologous to a dipeptidyl carboxypeptidase, and the zinc coordination geometry is critical for their hydrolytic action. However, these two catalytic sites are differentially activated by chloride ions, and the physiological substrate angiotensin-I preferentially binds to the catalytic site of the C domain. The substrate also contributes to the chloride-mediated activation of the active site. Therefore, these differences indicate that, despite the high level of primary sequence homology, there are structural and functional differences between the active sites of the C and N domains. Three subsites, S1 (antepenultimate), S0 1 (penultimate), and S0 2 (ultimate), with distinct characteristics for the binding of the carboxy-terminal amino acids of the substrate or inhibitor, are located within two homologous active sites. For the binding and interaction of the inhibitor and enzyme, these three main subsites bind with the substrate. The binding of the inhibitor or the natural substrate to the enzyme predominantly takes place via the carboxy-terminal tripeptide residues of the inhibitor and substrate. Certain C-terminus amino acid residues of the inhibitor, for example, Pro and Phe, are reported to be favorable for ACE inhibition; Ala and Val are more favorable at the antepenultimate position (S1); while Pro and Leu in the substrate sequence are most favorable at the ultimate position (S0 2) with regard to its affinity for ACE (Cushman *et al.*, 1981; Jung and Kim, 2007).

The antioxidant activity of peptides has been shown to be a result of the cooperative effect of their entire amino acid sequence; however, there is still little information concerning the structural characteristics of antioxidative peptides. At present, the main strategy has been to identify and characterize antioxidative peptides from the hydrolysates of proteins;

however, novel methods describing the relationship between the structure of the peptide and its antioxidative activity are needed to predict the antioxidant potential of food protein hydrolysates. The Muramoto research group investigated the residue–activity relationship of antioxidative peptides by combinatorial chemistry (Chen *et al*., 1996, 1998; Saito *et al*., 2003). They isolated six antioxidative peptides from soybean protein. On the basis of the smallest peptide, Leu-Leu-Pro-His-His, 28 synthetic peptides were constructed and their antioxidant activity was compared. The results indicated that the N-terminal Leu had no effect on their antioxidant activity, while His and Pro played important roles in this activity. Further analysis of 22 synthetic peptides containing His residues demonstrated that His-containing peptides can act as metal-ion chelators, active-oxygen quenchers, and hydroxyl radical scavengers (Chen *et al*., 1998). Subsequently, the Muramoto group constructed two series of tripeptide libraries (totaling 222 peptides) using combinatorial chemistry to explore the antioxidative properties of these peptides. Among the tested peptides, Tyr-(His, Lys, Arg)-Tyr was found to have the highest antioxidant activity.

According to the results from structure–activity relationship modeling by Li *et al.* (2011), the amino acid residue next to the C-terminus and the N-terminus amino acid residue are more important for antioxidant activity than the C-terminus. An amino acid residue next to the C-terminus with the ability to form hydrogen bonds and with low hydrophobicity might have higher antioxidant activity. A basic amino acid at the N-terminus with low hydrophobicity and a high hydrogen bond value was also predicted to generate high antioxidant activity. An amino acid residue next to the C-terminus with the ability to form hydrogen bonds also has an impressive effect on antioxidant activity. Saito *et al*. (2003) concluded that peptides containing Tyr-(His, Lys, Arg)-Tyr showed the highest antioxidant activity. For peptides composed of four or more amino acids, all of the four properties of the amino acid residue next to the C-terminus contribute to the antioxidant activity. In addition, the larger the hydrogen bonding and steric properties of this residue, the lower its hydrophobicity and the higher its activity. Therefore, the basic and acidic amino acids (Arg, Lys, His, Glu, and Asp) and the other hydrophilic amino acids (Thr, Ser, Asn, and Gln) have either a high value of hydrogen bonding or a high steric property with low hydrophobicity. In this position, they would have higher activity than the other amino acids. The hydrophobicity of the N-terminal amino acid residue is also very important for antioxidant activity, that is, the larger the hydrophobicity, the higher is the activity. Many other studies also speculated that hydrophobic amino acids on the N-terminus (Ala, Val, Leu, etc.) play an important role in antioxidant activity (Chen *et al*., 1995a; Tsuge *et al*., 1991).

The electronic property of the amino acid on the C-terminus also has an effect on antioxidant activity (Li *et al.*, 2011), that is, the larger the electronic property, the higher is the activity. The C-terminus is a polar position that is thus affected by its electrostatic potential, to some extent; therefore, the amino acids Trp, Glu, Leu, Ile, Met, Val, Tyr, etc. are suitable at the C-terminus. Some researchers have speculated that the identity of the amino acid on the C-terminus would play an important role in its activity. Suetsuna (2000) separated and identified a radical scavenging peptide, Tyr-Phe-Tyr-Pro-Glu-Leu, from casein hydrolysate, and it was confirmed that the Glu-Leu on the C-terminus mainly contributed to its antioxidant activity. Kim *et al.* (2009) speculated that the hydrophobic property of the amino acid on the C-terminus, for example, Val and Leu, had a distinct effect on the activity, as determined from the analysis of antioxidative peptides derived from venison hydrolysate.

Chen *et al.* (1996) investigated 28 synthetic peptides that were based on the antioxidative peptide Leu-Leu-Pro-His-His. They found that the deletion of the C-terminus His decreased the activity, whereas the deletion of the N-terminus Leu had no effect; therefore, it seemed that the C-terminus was related to the antioxidative activity of the peptide, while the N-terminus had no effect. Saito *et al.* (2003) pointed out that two Tyr-containing tripeptides (Tyr-X-Tyr) showed higher activity than did two His-containing tripeptides (His-X-His). According to the structural characteristics, the hydrophobicity of an amino acid on the N-terminus is the most important property for activity with respect to the same amino acid in the central position; in addition, Tyr on the N-terminus has stronger hydrophobicity than His. Therefore, it can be predicted that Tyr-X-Tyr has higher activity than His-X-His.

In addition, Cacciuttolo *et al.* (1993) reported that Tyr, Trp, and Phe, which have aromatic residues, can stabilize reactive oxygen species through electron transfer. Davalos *et al.* (2004) reported that Trp, Tyr, and Met have the highest antioxidant activity, followed by Cys, His, and Phe. The remaining amino acids (Arg, Asn, Gln, Asp, Pro, Ala, Val, Lys, Ile, Thr, Leu, Glu, and Gly) did not exhibit antioxidant activity. Therefore, several amino acids, for example, Tyr, Met, His, Cys, and Trp, are generally accepted as antioxidants that contribute to the activity of identified peptides (Chen *et al.*, 1995a; Hernandez-Ledesma *et al.*, 2005; Tsuge *et al.*, 1991). The antioxidative activity of peptides containing His has been attributed to the His residue because of the proton-donation ability of the His imidazole group.

In summary, the structural characteristics of peptides with high antioxidant activity are as follows: a hydrogen bonding and hydrophilic amino acid residue in the position next to the C-terminus, a hydrophobic amino acid residue at the N-terminus, and an electronic amino acid residue at the C-terminus.

III. BIOACTIVE PEPTIDES DERIVED FROM MARINE FISH

Peptides derived from fish proteins exert potent antioxidative activity in different oxidative systems (Jun *et al.*, 2004; Kim *et al.*, 2000; Rajapakse *et al.*, 2005a). There is increasing interest in identifying natural antioxidative substances that do not have side effects, and these compounds have the potential to address complications that arise from the oxidation of biomolecules. Recently, a number of studies have demonstrated that peptides derived from different marine protein hydrolysates act as potential antioxidants, for example, from marine fish such as hoki (Je *et al.*, 2005a; Kim *et al.*, 2007; Mendis *et al.*, 2005a), tuna (Je *et al.*, 2007, 2008), cod (Slizyte *et al.*, 2009), Pacific hake (Samaranayaka and Li-Chan, 2008), capelin (Amarowicz and Shahidi, 1997), scad (Thiansilakul *et al.*, 2007), Alaska pollock (Cho *et al.*, 2008; Je *et al.*, 2005b), conger eel (Ranathunga *et al.*, 2006), yellowfin sole (Jun *et al.*, 2004), and yellow stripe trevally (Klompong *et al.*, 2009; Table 5.1). The beneficial effects of marine-derived bioactive peptides include scavenging for free radicals and reactive oxygen species or preventing oxidative damage by interrupting the radical chain reaction of lipid peroxidation (Mendis *et al.*, 2005b; Qian *et al.*, 2008; Rajapakse *et al.*, 2005b; Ranathunga *et al.*, 2006). Kim *et al.* (2000) reported that some peptides derived from fish showed stronger antihypertensive activity by inhibiting the action of ACE than many other natural peptides. These peptides exhibited *in vivo* activity by lowering the blood pressure in spontaneously hypertensive rats (SHRs; Fujita and Yoshikawa, 1999; Je *et al.*, 2005a). Enzymatically hydrolyzed fish muscle peptides have also demonstrated anticoagulant and antiplatelet properties *in vitro*, and these results suggest the capability of fish peptides to inhibit the intrinsic coagulation pathway (Rajapakse *et al.*, 2005b). In addition, marine anticoagulant proteins have been purified from blood ark shell (Jung *et al.*, 2001), starfish (Koyama *et al.*, 1998), and yellowfin sole (Rajapakse *et al.*, 2005a).

Kim *et al.* (1999) and Jung *et al.* (2005b) reported that fish peptides can accelerate calcium absorption. Under many conditions, dietary calcium becomes unavailable for absorption due to the formation of insoluble compounds inside the dietary tract, and inadequate dietary calcium is associated with a number of common and chronic diseases including osteoporosis, osteoarthritis, cardiovascular disease (hypertension and stroke), diabetes, obesity, and cancer (Anderson and Garner, 1996). Further, fish protein hydrolysates contain hormone-like peptides and growth factors that accelerate calcium absorption (Fouchereau-Peron *et al.*, 1999). These peptides are capable of binding to the cell surface receptors on osteoclasts and have a role in calcium metabolism by decreasing the number of osteoclasts. Therefore, these peptides could be used in the treatment of osteoporosis and Paget's disease. Further,

TABLE 5.1 Bioactive peptides derived from marine organisms

Marine resources	Amino acid sequence/major amino acid in bioactive peptide	Activity	Reference
	Fish		
Big eye tuna (muscle)	Trp-Pro-Glu-Ala-Ala-Glu-Leu-Met-Met-Glu-Val-Asp-Pro	ACE inhibitor	Qian *et al.* (2007)
	H-Leu-Asn-Leu-Pro-Thr-Ala-Val-Tyr-Met-Val-Thr-OH	Antioxidant	Je *et al.* (2008)
	Val-Lys-Ala-Gly-Phe-Ala-Trp-Thr-Ala-Asn-Glu-Glu-Leu-Ser	Antioxidant	Je *et al.* (2007)
Big eye tuna (frame)	Gly-Asp-Leu-Gly-Lys-Thr-Thr-Thr-Val-Ser-Asn-Trp-Ser-Pro-Pro-Lys-Try-Lys-Asp-Thr-Pro	Antihypertensive	Lee *et al.* (2010)
Alaska pollock	Gly-Pro-Leu	ACE inhibitor	Byun and Kim (2001)
	Val-Leu-Ser-Gly-Gly-Thr-Thr-Met-Ala-Met-Tyr-Thr-Leu-Val	Antioxidant	Jung *et al.* (2006a)
Sea bream	Val-Ile-Tyr	ACE inhibitor	Fahmi *et al.* (2004)
Yellowfin sole	Met-Ile-Phe-Pro-Gly-Ala-Gly-Gly-Pro-Glu-Leu	ACE inhibitor	Jung *et al.* (2006a)
	Arg-Pro-Asp-Phe-Pro-Leu-Glu-Pro-Pro-Tyr	Antioxidant	Jun *et al.* (2004)
Horse mackerel (skin)	Asn-His-Arg-Tyr-Asp-Arg	Antioxidant	Sampath *et al.* (2011)
Croaker (skin)	Gly-Asn-Arg-Gly-Phe-Ala-Cys-Arg-His-Ala	Antioxidant	Sampath *et al.* (2011)
Conger eel	Leu-Gly-Leu-Asn-Gly-Asp-Asp-Val-Asn	Antioxidant	Ranathunga *et al.* (2006)
Hoki fish (skin)	His-Gly-Pro-Leu-Gly-Pro-Leu	Antioxidant	Mendis *et al.* (2005a)
Seaweed pipefish (muscle)	Thr-Phe-Pro-His-Gly-Pro	Antihypertensive	Wijesekara *et al.* (2011)
	His-Trp-Thr-Thr-Gln-Arg	Antihypertensive	Wijesekara *et al.* (2011)
Squid	Phe-Asp-Ser-Gly-Pro-Ala-Gly-Val-Leu	Antioxidant	Mendis *et al.* (2005a)
	Asn-Gly-Pro-Leu-Gln-Ala-Gly-Gln-Pro-Gly-Glu-Arg	Antioxidant	Mendis *et al.* (2005a)

TABLE 5.1 (*continued*)

Marine resources	Amino acid sequence/major amino acid in bioactive peptide	Activity	Reference
	Gly-Pro-Leu-Gly-Leu-Leu-Gly-Phe-Leu-Gly-Pro-Leu-Gly-Leu-Ser	ACE inhibitor	Alemán *et al.* (2011)
Oyster	Cys, Leu, Glu, Asp, Phe, Tyr, Ile, and Gly	Antimicrobial	Liu *et al.* (2008)
	Leu-Lys-Gln-Glu-Leu-Glu-Asp-Leu-Leu-Glu-Lys-Gln-Glu	Antioxidant	Qian *et al.* (2008)
	Leu-Leu-Glu-Tyr-Ser-Ile	Inhibitor of HIV-1 protease	Lee and Maruyama (1998)
	Leu-Leu-Glu-Tyr-Ser-Leu	Inhibitor of HIV-1 protease	Lee and Maruyama (1998)
	Arg-Arg-Trp-Trp-Cys-Arg-X	Inhibitory activity on herpes virus	Zeng *et al.* (2008)
Mussel	Glu-Ala-Asp-Ile-Asp-Gly-Asp-Gly-Gln-Val-Asn-Tyr-Glu-Glu-Phe-Val-Ala-Met-Met-Thr-Ser-Lys	Anticoagulant	Jung and Kim (2009)
	Leu-Val-Gly-Asp-Glu-Gln-Ala-Val-Pro-Ala-Val-Cys-Val-Pro	Antioxidant	Jung *et al.* (2007)
	Phe-Gly-His-Pro-Tyr	Antioxidant	Jung *et al.* (2005b)
American lobster	Gln-Tyr-Gly-Asn-Leu-Leu-Ser-Leu-Leu-Asn-Gly-Tyr-Arg	Antimicrobial	Battison *et al.* (2008)
Shrimp	Pro-Arg-Pro	Antimicrobial	Bartlett *et al.* (2002)
	Ile-Phe-Val-Pro-Ala-Phe	ACE inhibitor	Lun *et al.* (2006)
Sea urchin	Cys	Antimicrobial	Li *et al.* (2008)
Clam	Tyr-Asn	ACE inhibitor	Tsai *et al.* (2008)
	Val-Glu-Val	ACE inhibitor	Tsai *et al.* (2006)
	Val-Lys-Pro	ACE inhibitor	Tsai *et al.* (2006)
Sea cucumber	Met-Glu-Gly-Ala-Gln-Glu-Ala-Gln-Gly-Asp	ACE inhibitor	Zhao *et al.* (2009)
	Glu, Asp, Pro, Gly, and Ala	Antihypertensive	Zhao *et al.* (2007)

acidic peptide fractions from Atlantic cod hydrolysate demonstrate strong immunostimulatory effects, and treatment with these peptides generated an oxidative burst in Atlantic salmon leucocytes (Gildberg *et al*., 1996). Basically, immunomodulators enhance the production of oxygen metabolites in macrophages, which are the primary source of these oxygen metabolites, and thus determine the oxidative burst. Oxidative burst reactions are of a major importance for the bactericidal power of phagocytes.

Working on Alaska pollock (*Theragra chalcogramma*) backbones discarded from industrial processing as a source of soluble calcium, Jung *et al*. (2006a) identified a backbone peptide, Val-Leu-Ser-Gly-Gly-Thr-Thr-Met-Ala-Met-Tyr-Thr-Leu-Val (MW: 1442 Da), exhibiting an affinity for calcium ions on the surface of hydroxyapatite crystals. This *in vitro* calcium-binding assay also demonstrated that this peptide can solubilize a similar amount of calcium as casein phosphopeptide; thus, it is possible to provide a novel nutraceutical with a high bioavailability for calcium through further studies with *in vivo* assays. Fish protein hydrolysates obtained from cod backbones also enhanced product stability by preventing oxidative deterioration (Slizyte *et al*., 2009). The scavenging activity of 2,2-diphenyl-1-picrylhydrazyl (DPPH) showed that the antioxidative activity of hydrolysates could be due to their ability to scavenge lipid radicals. An increased degree of hydrolysis resulted in a slight increase of DPPH radical scavenging activity. These results also show that it is possible to obtain bioactive molecules from cod backbones by protein hydrolysis (gastrin/cholecystokinin- and calcitonin gene-related peptide-like peptides) and that the incorporation of cod hydrolysates into foods could be beneficial.

According to Lee *et al*. (2010), the peptide Gly-Asp-Leu-Gly-Lys-Thr-Thr-Thr-Val-Ser-Asn-Trp-Ser-Pro-Pro-Lys-Try-Lys-Asp-Thr-Pro, derived from big-eye tuna frame protein hydrolysate, showed a strong suppressive effect on the systolic blood pressure of SHRs, while its antihypertensive activity was similar to that of captopril, a commercially available antihypertensive drug. Further, they reported no side effects after the administration of this antihypertensive peptide to rats. In addition, these marine antihypertensive peptides exhibit stronger antihypertensive activity *in vivo* than *in vitro*. The exact mechanisms underlying this phenomenon have yet to be identified; however, it was suggested that bioactive peptides have a higher tissue affinity and are eliminated more slowly than captopril (Fujita and Yoshikawa, 1999).

To evaluate the immunomodulatory effects of marine oligopeptide preparation (MOP) from chum salmon (*Oncorhynchus keta*), Yang *et al*. (2009) demonstrated that MOP has the potential to enhance the immune function of mice, including cellular immunity, humoral immunity, and

natural killer cell function, but has no significant effect on the activity of macrophages. Moreover, this positive immunomodulation by MOP is most likely attributed to the stimulation of T helper (Th) cells, causing the secretion of Th1 and Th2 cytokines. Therefore, as a novel food source, MOP may have a beneficial effect on the immune function of consumers, and consequently help to prevent disease. Further studies have been recommended to determine and evaluate the clinical significance of these findings, particularly in those with impaired immune function and/or with cancer.

ACE-inhibitory peptides have been isolated from enzymatic hydrolysates of various sources of fish waste, for example, Alaska pollock skin (Byun and Kim, 2001), sea bream scales (Fahmi *et al.*, 2004), and yellowfin sole frame protein (Jung *et al.*, 2006b). Enzymatically hydrolyzed fish skin gelatin has higher antioxidant and antihypertensive activity than peptides derived from fish muscle protein (Byun and Kim, 2001; Kim *et al.*, 2001; Mendis *et al.*, 2005a,b). Gelatin peptides contain unique repeated Gly-Pro-Ala sequences, and it is presumed that the antioxidative and antihypertensive properties of gelatin peptides are associated with their unique amino acid composition. In addition, these peptides can accelerate the absorption of dietary calcium in animal models, thereby increasing calcium bioavailability (Kim *et al.*, 1998).

IV. BIOACTIVE PEPTIDES DERIVED FROM LOBSTER, SHRIMP, AND CRABS

Marine-derived bioactive peptides are well described in the hemolymph of many marine invertebrates (Tincu and Taylor, 2004), including spider crab (Stensvag *et al.*, 2008), American lobster (Battison *et al.*, 2008), and shrimp (Bartlett *et al.*, 2002). Antimicrobial peptides (AMPs) in blue crab hemolymph (*Callinectes sapidus*) were highly inhibitory to Gram-negative bacteria (Edward *et al.*, 1996). Although there are several reports on the antibacterial activity of seminal plasma, few antibacterial peptides have been identified in the mud crab (*Scylla serrata*; Jayasankar and Subramonium, 1999). An AMP derived from American lobster (*Homarus americanus*) exhibited bacteriostatic activity against some Gram-negative bacteria and demonstrated protozoastatic and protozoacidal activity against two scuticociliate parasites (*Mesanophrys chesapeakensis* and *Anophryoides haemophilia*), the latter is a significant pathogen of *H. americanus* (Battison *et al.*, 2008). In addition, an AMP (arasin 1) derived from spider crab (*Hyas araneus*) inhibited the growth of *Corynebacterium glutamicum* (Stensvag *et al.*, 2008).

V. BIOACTIVE PEPTIDES DERIVED FROM SQUID, CLAMS, AND SEA URCHINS

Peptides derived from a tryptic hydrolysate of jumbo squid (*Dosidicus gigas*) skin gelatin exhibited strong inhibition of lipid peroxidation that was much higher than that of the natural antioxidant α-tocopherol (Mendis *et al.*, 2005a). Two representative peptides with comparatively higher antioxidant potency were purified and characterized as Phe-Asp-Ser-Gly-Pro-Ala-Gly-Val-Leu (880.18 Da) and Asn-Gly-Pro-Leu-Gln-Ala-Gly-Gln-Pro-Gly-Glu-Arg (1241.59 Da). The viability of radical-mediated oxidation-induced human lung fibroblasts was also enhanced following treatment with these peptides. However, they did not exhibit substantial ion chelation, and it was presumed that the observed radical scavenging potency of these peptides plays a vital role in their strong antioxidant activity. It was suggested that the hydrophobic amino acids of these peptides contributed greatly to their observed antioxidant activity.

Several ACE-inhibitory peptides were also prepared from clams, including a peptic digest of short-necked clam (Val-Glu-Val, $IC_{50}=8.7$ mM) and clam hydrolysate (Val-Lys-Pro, $IC_{50}=2.6$ mM; Tsai *et al.*, 2006). Tsai *et al.* (2008) reported that hard clam (*Meretrix lusoria*) residual meat extract can be recovered as a value-added by-product. One of the peptides isolated from hard clam residual meat hydrolysate (Tyr-Asn) showed high ACE-inhibitory activity with an IC_{50} of 51 mM (or 0.015 mg/mL).

Sea urchins possess an innate immune system and are regarded as a potential source for the discovery of new AMPs. Li *et al.* (2008) isolated two novel antibacterial peptides (strongylocin 1 and 2; 5.6 and 5.8 kDa, respectively) from coelomocyte extracts of the green sea urchin (*Strongylocentrotus droebachiensis*). These two peptides have putative isoforms (1b and 2b), similar to two putative proteins from the purple sea urchin (*Strongylocentrotus purpuratus*). The native strongylocin peptides are cationic, defensin-like peptides (cysteine-rich), but show no similarity to other known AMPs with respect to their cysteine distribution pattern. Strongylocin 1 consists of 83 amino acids that include a prepro sequence of 35 amino acids, whereas strongylocin 2a and 2b are composed of 89 and 90 amino acids, respectively, in which 38 amino acids represent a prepro sequence. The native peptides display potent activity against Gram-negative and -positive bacteria.

VI. BIOACTIVE PEPTIDES DERIVED FROM MOLLUSKS AND OYSTERS

Cytotoxic cyclic peptides have also been found in mollusks. Dolastatin peptides are a group of cyclic and linear peptides with prominent cell growth-suppressing activity that were isolated from the marine mollusk

(*Dolabella auricularia*). Another prominent family of peptides has also been isolated from mollusks; these highly compact and stable linear peptides, known as conotoxins, exhibit specific actions on the ion channels and membrane receptors of excitable cells.

D. auricularia is a shell-less mollusk that, therefore, initially appears to lack defenses against predators; however, this is only a preliminary supposition. Accumulated evidence supports the fact that Opisthobranchia mollusks have developed very powerful chemical defenses (Pettit *et al.*, 1989). Pettit *et al.* (1981) were the first to isolate some of these compounds; the pentapeptide dolastatin 10 (Fig. 5.1) was reported to be the most active natural anticancer substance at that time with an ED_{50} of 4.6×10^{-5} μg/mL against the P388 cell line (Pettit *et al.*, 2008). Dolastatin 10 was also shown to inhibit tubulin polymerization and tubulin-dependent GTP hydrolysis (Bai *et al.*, 1990).

Dolastatin 10 has been evaluated with promising results in a phase I clinical study in patients with solid tumors. Subsequently, its noticeable antitumor activity was well documented in various *in vitro* and *in vivo* tumor models (Madden *et al.*, 2000). More than a dozen dolastatin peptides have been isolated to date. Recent studies have shown, for example, that the depsipeptide dolastatin 11 arrests cells at cytokinesis by causing a rapid and massive rearrangement of the cellular actin filament network and induces the hyperpolymerization of purified actin (Bai *et al.*, 2001). The effects of dolastatin 11 were similar to those of the sponge-derived depsipeptide jasplakinolide; however, dolastatin 11 exhibited ~threefold more cytotoxicity than jasplakinolide in the cells studied.

Keenamide A is another novel cytotoxic cyclic hexapeptide isolated from a marine mollusk (*Pleurobranchus forskalii;* Fig. 5.2; Wesson and Hamann, 1996). Keenamide A exhibited significant activity against the P-388, A-549, MEL-20, and HT-29 tumor cell lines. New classes of anticancer drug candidates isolated from marine organisms have been shown to possess powerful cytotoxic activity against multiple tumor types.

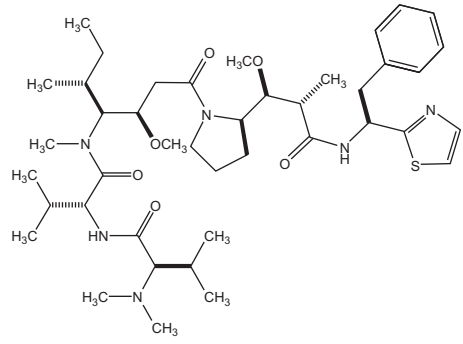

FIGURE 5.1 Structure of dolastatin 10 derived from the sea hare (mollusk) *Dolabella auricularia*.

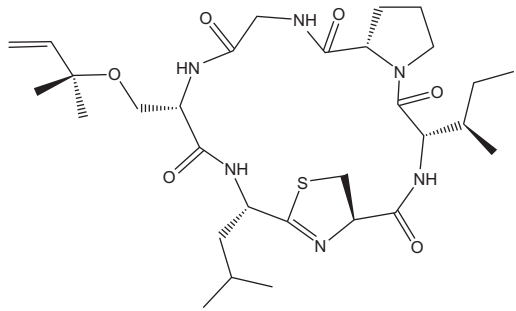

FIGURE 5.2 Structure of the cyclic peptide keenamide A derived from the notaspidean mollusk *Pleurobranchus forskalii*.

Peptides derived from marine organisms, including the oyster (Qian *et al.*, 2008) and blue mussel (Jung *et al.*, 2005b and Rajapakse *et al.*, 2005b), have been reported to exhibit functional properties. The beneficial effects of marine bioactive peptides are well known in scavenging for free radicals and reactive oxygen species or in preventing oxidative damage by interrupting the radical chain reaction of lipid peroxidation (Mendis *et al.*, 2005b; Qian *et al.*, 2008; Rajapakse *et al.*, 2005a; Ranathunga *et al.*, 2006). It has been shown that their antioxidant potency is mostly due to the presence of hydrophobic amino acids (Mendis *et al.*, 2005b). In addition, the bioactive antioxidant peptide Leu-Lys-Gln-Glu-Leu-Glu-Asp-Leu-Leu-Glu-Lys-Gln-Glu, isolated from the oyster (*Crassostrea gigas*), exhibited higher activity against polyunsaturated fatty acid peroxidation than α-tocopherol (Qian *et al.*, 2008). ACE-inhibitory peptides were also isolated from oyster protein digest (Leu-Phe; IC50=126 mM; Matsumoto *et al.*, 1994) and pearl oyster meat hydrolysate (Leu-Val-Glu; IC_{50}=14.2 mM; Suetsuna, 2002). Liu *et al.* (2008) also reported the presence of AMPs in the oyster.

Moreover, the anticoagulant peptide Glu-Ala-Asp-Ile-Asp-Gly-Asp-Gly-Gln-Val-Asn-Tyr-Glu-Glu-Phe-Val-Ala-Met-Met-Thr-Ser-Lys, derived from the blue mussel, prolonged the blood clotting time (Jung and Kim, 2009). In addition, a protein derived from the blood arch shell prolonged the clotting time more than did heparin, a commercially available anticoagulant (Jung *et al.*, 2001). However, these marine-derived anticoagulant peptides are noncytotoxic and have the potential to be used as functional ingredients in nutraceutical or pharmaceutical products.

Further, the AMP CgPep33, derived from oyster (*C. gigas*), demonstrated antimicrobial activity and growth inhibition of bacteria, for example, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*; and fungi, for example, *Botrytis cinerea* and *Penicillium expansum* (Liu *et al.*, 2008). More interestingly, the IC_{50} values of this peptide against these bacteria

and fungi ranged from 18.6 to 48.2μg/mL. In addition, antiviral bioactive peptides have also been identified. For example, Achour *et al.* (1997) found that an oyster protein extract could enhance the proliferation of immunocytes in human immunodeficiency virus 1 (HIV-1)-infected patients. In addition, Lee and Maruyama (1998) identified peptides that inhibit HIV-1 protease from the hydrolysate of oyster (*C. gigas*) proteins prepared with thermolysin. The amino acid sequences of these peptides were determined as Leu-Leu-Glu-Tyr-Ser-Ile and Leu-Leu-Glu-Tyr-Ser-Leu. These sequences are found in some proteins of various major viruses or human cytomegalovirus. Chemically synthesized Leu-Leu-Glu-Tyr-Ser-Ile and Leu-Leu-Glu-Tyr-Ser-Leu showed IC_{50} values of 20 and 15 nM, respectively and behaved as competitive inhibitors of HIV-1 protease with K_i values of 13 and 10nM, respectively. These peptides were more potent HIV-1 protease inhibitors than pepstatin A. Moreover, an active peptide, Arg-Arg-Trp-Trp-Cys-Arg-X (where X is an amino acid or an amino acid analog), against the herpes virus was isolated from the enzymatic hydrolysate of the oyster (*C. gigas*), and this peptide demonstrated high inhibitory activity against the herpes virus (Zeng *et al.*, 2008). Zeng *et al.* (2008) obtained four fractions of hydrolysates according to their MW (<1, 1–5, 5–10, and >10kDa). A peptide purified from the 5- to 10-kDa fractions had a higher biological activity than the other fractions.

VII. FERMENTED MARINE PEPTIDES AND NOVEL APPROACHES

Fermentation, one of the oldest food preservation techniques specifically practiced in South East Asian countries such as China, Japan, and Korea, is believed to enhance the nutraceutical value of fermented foods in addition to their long-term storage. The breakdown of food proteins by microbial proteases to produce bioactive peptides may be a possible reason for the development of such properties during fermentation. Therefore, interest has developed in identifying the biological activity of fermented foods, including fish and shellfish (Ichimura *et al.*, 2003; Kim *et al.*, 2004; Wong and Mine, 2004). Health-related functional properties such as antioxidative activity and radical scavenging capacity may present as promising biological benefits of these fermented foods. Fish sauce, being a fermented food, is assumed to contain many substances, including peptides, with ACE-inhibitory activity that is produced during the fermentation of fish proteins. As they are made by the fermentation of various kinds of fish, they are assumed to be rich in degradation products, for example, small peptides and amino acids. The ACE-inhibitory activity of fermented fish sauces made from salmon, sardine, or anchovy was reported (Okamoto *et al.*, 1995a), and three fermented salmon sauce-

derived ACE-inhibitory peptides (Gly-Trp, Ile-Trp, and Val-Trp) containing Trp at the C-terminal position were further identified by Okamoto *et al*. (1995b). Earlier, Cheung *et al.* (1980) reported that Trp at the COOH terminus and Val and Ile at the NH_2 terminus of a dipeptide are the most favorable sequences for ACE-inhibitory activity. The dipeptide Gly-Trp, which was shown to be a considerable ACE inhibitor, has been isolated from sardine muscle hydrolysate by Seki *et al*. (1993). In addition, two ACE-inhibitory peptides, Gly-Pro-Pro and Val-Pro, were isolated from salted and fermented anchovy by Lee (1996). Ichimura *et al*. (2003) reported ACE-inhibitory activity in fermented fish sauce that was traditionally made from anchovy, sardine, or bonito, and purified the ACE-inhibitory peptides contained in the sauce (Table 5.2). ACE-inhibitory peptides such as Ala-Pro, Lys-Pro, Arg-Pro, Gly-Pro, Glu-Pro, Thr-Pro, Val-Pro, Gly-Ile, and Asp-Phe were isolated from fermented anchovy sauce. The ACE-inhibitory peptides Ala-Pro, Gly-Pro, Thr-Pro, Val-Pro,

TABLE 5.2 ACE-inhibitory activity of peptides derived from fermented fish sauce

Peptides	Origin	IC50[a] (μM) or % inhibition	Concentration of peptides[b] (μM)
Ala-Pro	Anchovy, sardine, bonito	29	31 (An), 29 (Sa), 1 (Bo)
Lys-Pro	Anchovy	22	28 (An)
Arg-Pro	Anchovy, bonito	21	42 (An), 2 (Bo)
Gly-Pro	Anchovy, sardine, bonito	360	1 (An), 140 (Sa), 6 (Bo)
Glu-Pro	Anchovy	1200	7 (An)
Thr-Pro	Anchovy, sardine	290	9 (An), 18 (Sa)
Val-Pro	Anchovy, sardine	570	37 (An), 8 (Sa)
Gly-Ile	Anchovy	1300	12 (An)
Asp-Phe	Anchovy	360	1 (An)
Asn-Pro	Sardine	2300	22 (Sa)
Asp-Met	Sardine	600	1 (Sa)
Asp-Leu	Sardine	2000	7 (Sa)
Ala-Val	Sardine	15% at 800 μM	8 (Sa)
Gly-Val	Sardine	17% at 800 μM	57 (Sa)
Ala-Gly-Pro	Bonito	560	4 (Bo)

Source: Ichimura *et al.* (2003). Permission has been obtained for use of copyrighted material from Elsevier B.V.
[a] IC50, concentration of the peptide required for 50% inhibition of ACE activity.
[b] Concentration of the peptide contained in the fermented fish sauce. As values were calculated from the quantity of finally purified peptide, the actual concentration of each peptide may be higher than the concentration indicated. An, anchovy; Sa, sardine; Bo, bonito.

Asn-Pro, Asp-Met, Asp-Leu, Ala-Val, and Gly-Val were isolated from fermented sardine sauce; further, the ACE-inhibitory peptides Ala-Pro, Arg-Pro, Gly-Pro, and Ala-Gly-Pro were isolated from fermented bonito sauce. Val-Pro was also identified in salted and fermented anchovy by Lee (1996). Among the peptides identified by Ichimura *et al*. (2003), Ala-Pro, Lys-Pro, and Arg-Pro showed strong and similar inhibitory activity. Ichimura *et al*. (2003) also isolated nine types of peptides containing Pro residues in their carboxy terminals. Due to the unique structure of Pro as an imino acid, peptide bonds containing Pro residues are often resistant to hydrolysis by common peptidases. This may be the reason why these Pro-containing dipeptides survived after long-term fermentation. Among these peptides, Lys-Pro was further evaluated *in vivo* in male SHRs (Charles River Japan, Yokohama) by oral administration. As shown in Fig. 5.3, orally administered Lys-Pro shows a tendency to lower the blood pressure of SHRs.

Salt-fermented anchovy sauce (FAS), a fermented fish product of Southeast and Far East Asia, made by salting anchovies, also contains anticoagulation agents (Kim *et al*., 2004). The authors reported that the degree of fibrin-clotting inhibition increased with increasing amounts of desalted FAS (Fig. 5.4), indicating the presence of a bioactive peptide that acts as an anticoagulant against fibrin clotting. It has been suggested that the active peptide has a molecular mass much smaller than nattokinase, a known fibrinolytic enzyme from fermented foods; thus, it may be more useful for oral use in the prevention of thrombotic disease. For its wider

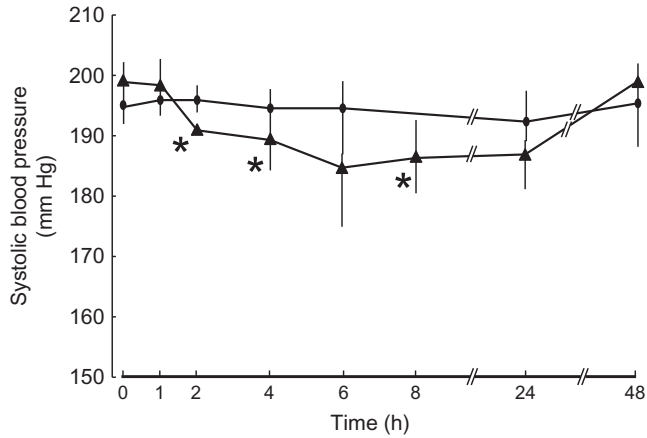

FIGURE 5.3 Change in systolic blood pressure after the oral administration of Lys-Pro. Values are the mean ± standard deviation (SD) ($n = 6$). Circles, control group (distilled water); triangles, Lys-Pro group (50 mg/kg). *$P < 0.05$ (paired t-test) compared to 0 h in the Lys-Pro group. *Source*: Ichimura *et al.* (2003). Permission has been obtained for the use of copyrighted material from Elsevier B.V.

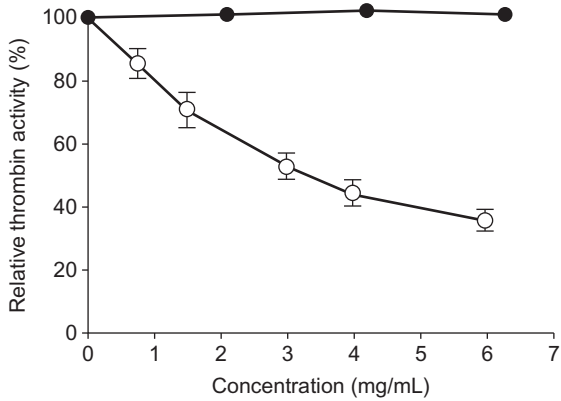

FIGURE 5.4 Relative fibrin-clotting and amidolytic activity of thrombin as a function of the concentration of desalted fermented anchovy sauce (dFAS). Anticoagulation activity was assessed by the increase in the fibrin clotting time (empty circles) and the release of *p*-nitroaniline (closed circles). Data are presented as the mean ± SD ($n = 3$). *Source*: Kim *et al.* (2004). Permission has been obtained for the use of copyrighted material from Elsevier B.V.

application, additional characteristics, for example, high thermal and pH stability and safety, are necessary for its long-term intake. The capability of this substance to control the risk of thrombosis by incorporating it into the daily diet without any adverse side effects on human health is most desirable for its application as a functional foodstuff with the intention of decreasing the medical cost of circulatory diseases.

Rajapakse *et al*. (2005b) extracted radical scavenging peptides from fermented marine blue mussel sauce. They found that the purified peptides from mussel sauce exerted strong scavenging effects on the tested radicals, although with different capacities. In addition, the mussel sauce-derived radical scavenging peptides exhibited different antioxidative mechanisms, indicating their protective action against free radicals. A cell viability assay clearly indicated the ability of these peptides to protect cells against oxidative stress-mediated injuries in a dose-dependent manner, which was similar to the observed activity of α-tocopherol (Fig. 5.5). Jung *et al*. (2005b) reported that blue mussel sauce that had been fermented for 6 months showed the highest antioxidative activity, exhibiting ~54.1% inhibition of linoleic acid peroxidation. The amino acid sequence of a purified peptide with a molecular mass of 620 Da was determined as Phe-Gly-His-Pro-Tyr. The activity of this peptide may be attributed to the chelating and lipid radical trapping ability of its imidazole ring. In addition, this peptide contained a Tyr residue, which is a potent hydrogen donor. This bioactive peptide showed antioxidative activity in lipid or fatty acid autoxidation systems and radical scavenging activity; thus, it is

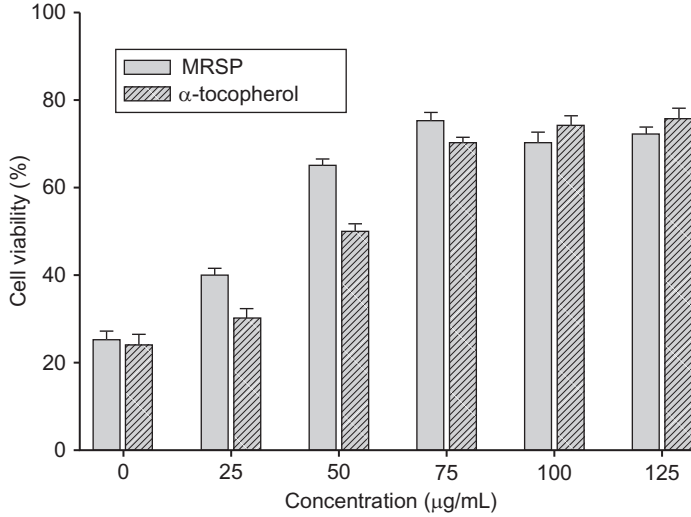

FIGURE 5.5 Effect of fermented mussel-derived radical scavenging peptides (MRSPs) on cell viability. Human lung fibroblasts were cultured in Dulbecco's modified Eagle's medium without fetal bovine serum and incubated with the purified peptide (MRSP) at various concentrations for 16 h. Oxidative stress was artificially induced in cultured cells by incubation with 200 μM *t*-BHP for 2 h. Viable cells remaining after the oxidative stress was measured as the percentage of viable cells compared to control cells using the MTT assay. Values are given as the mean ± standard error. *Source*: Rajapakse *et al.* (2005c). Permission has been obtained for the use of copyrighted material from Elsevier B.V.

possible to provide a natural antioxidant and nutraceutical food through further studies of its mechanism of action and *in vivo* assays.

The proteolytic activation of bioactive sequences by lactic acid bacteria has been debated recently due to the great advantage of using food-grade microorganisms to enrich foods with bioactive substances (Gobbetti *et al.*, 2002); however, limited applications are reported for fermented marine proteins. Wang *et al.* (2008) attempted to ferment *Acetes chinensis*, an underutilized shrimp species thriving in the Bohai Gulf of China, with lactic acid bacteria to produce a fermented shrimp sauce with high ACE-inhibitory activity. Three peptides with high ACE-inhibitory activity were isolated from the fermented shrimp sauce (Asp-Pro, Gly-Thr-Gly, and Ser-Thr). Previous studies have shown that di-, tri-, and tetra-peptides are more rapidly absorbed and reach a higher concentration in the blood than free amino acids; further, these short peptides are less susceptible to proteolytic enzymes (Chun *et al.*, 1996; Craft *et al.*, 1968; Matthews and Payne, 1980). Hence, it can be expected that Asp-Pro, Gly-Thr-Gly, and Ser-Thr will be readily absorbed by the alimentary canal, thereby reaching the peripheral target sites and producing their effect *in vivo*. The

production of *A. chinensis* fermented by *L. fermentum* SM 605 may be useful as an antihypertensive component of functional foods or nutraceuticals.

The antioxidant activity of shrimp protein hydrolysate has been demonstrated (Suetsuna, 2000). Using the water extract from Mun Goong, a paste extracted from the cephalothorax of white shrimp (*Litopenaeus vannamei*), Binsan *et al*. (2008) revealed that the water extract contained antioxidative peptides with mass ranges of m/z 400–1000. A shrimp (*A. chinensis*) hydrolysate prepared with a crude protease from *Bacillus* sp. SM98011, containing oligopeptides with a molecular mass <3 kDa, exhibited antioxidant and ACE-inhibitory activity (He *et al*., 2006).

Shrimp waste, containing 35–50% crude protein, is also an important source of bioactive molecules (Sachindra *et al*., 2005); its antioxidative property was found to be improved by ensilaging with lactic acid (Sachindra and Bhaskar, 2008). This protein-rich liquor has beneficial biological functions due to the inherent protein hydrolysis that occurs during fermentation. The potential for producing functional bioactive peptides through enzymatic hydrolysis of shrimp by-products has also been suggested by several recent reports, for example, antioxidant peptides (Guerard *et al*., 2007), ACE-inhibitory peptides (Cheung and Li-Chan, 2010; He *et al*., 2006), and AMPs (Bartlett *et al*., 2002).

A recent report by Huang *et al.* (2010) demonstrated the generation of an iron-binding peptide by the enzymatic hydrolysis of shrimp processing by-product. The MW of the hydrolysate ranged from 1 to 6 kDa, and the MW of the metal-binding peptides isolated from other proteins belong to this range, for example, a 1561-Da protein from hoki frame following pepsinolytic hydrolysis (Jung and Kim, 2007), a 3500-Da protein from hoki bone following carnivorous intestine crude proteinase hydrolysis (Jung *et al*., 2005a), and a 1442-Da protein from Alaska pollock (*T. chalcogramma*) backbone following pepsinolytic hydrolysis (Jung *et al*., 2006a). The infrared spectra of the iron-binding peptides and the iron–peptide complex shown in Fig. 5.6 suggest that the principal site of iron binding primarily corresponds to the carboxylate groups and, to a lesser extent, the peptide bonds. These attempts might facilitate the development of strong iron-binding peptides as natural functional additives in the food industry.

Meat-based products have been fermented with *Aspergillus* in an attempt to identify novel properties. Yin *et al*. (2005) reported that *Aspergillus oryzae* produces multiple enzymes and can hydrolyze minced mackerel. The present authors (Giri *et al*., 2009a,b) also developed a marine fish meat-based functional paste by utilizing the traditional Japanese koji fermentation technique with improved food functionality and aroma attributes. Several trash fish, including horse mackerel, spotted mackerel, lizard fish, and squid meat, were utilized to produce a functional paste

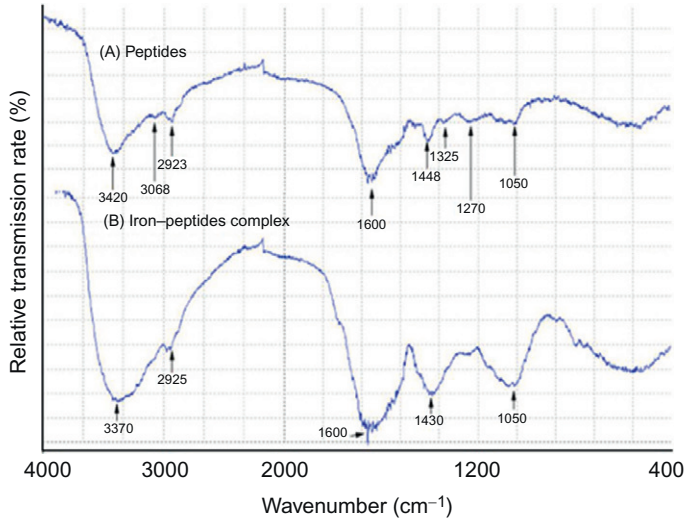

FIGURE 5.6 Infrared spectra of iron-binding peptides (A) and iron–peptide complex (B). *Source*: Huang *et al.* (2010). Permission has been obtained for the use of copyrighted material from Springer.

from *A. oryzae*-inoculated koji. Analysis of several physicochemical parameters of the finished products, including free amino acid, oligopeptide, organic acid, and mineral content, revealed the potential utility of marine fish meat for the production of miso-like fermented fish pastes (Giri *et al.*, 2009a,b, 2010a,b). There are extensive reports on the nutritional value, taste, and aroma (Giri *et al.*, 2009a,b, 2010b) as well as the antioxidative properties (Giri *et al.*, 2011a) of fish miso. A fish miso preparation of rice malt koji inoculated with *A. oryzae* provides several proteolytic, lipolytic, and amylolytic enzymes; thus, hydrolyzed protein and carbohydrate substrates were efficiently produced from marine fish meat (Giri *et al.*, 2011b). Interestingly, the scavenging activity of aqueous fish miso extracts against DPPH, hydroxyl, NO, and carbon-centered radicals, estimated through ESR, increased with the prolongation of fermentation (Fig. 5.7). These time course observations indicated that the substrate responsible for radical scavenging developed during the process of fermentation. Using the online high-performance liquid chromatography-DPPH method, the production of peptides with radical-scavenging activity and their molecular mass distributions were estimated (Fig. 5.8). As fermentation proceeded (>60 days), peptides with a low molecular mass (1.45 kDa) and radical-scavenging ability developed, indicating the involvement of those peptides in the improved antioxidative properties of fish miso. Scavenging activity increased remarkably after 270 days due to the

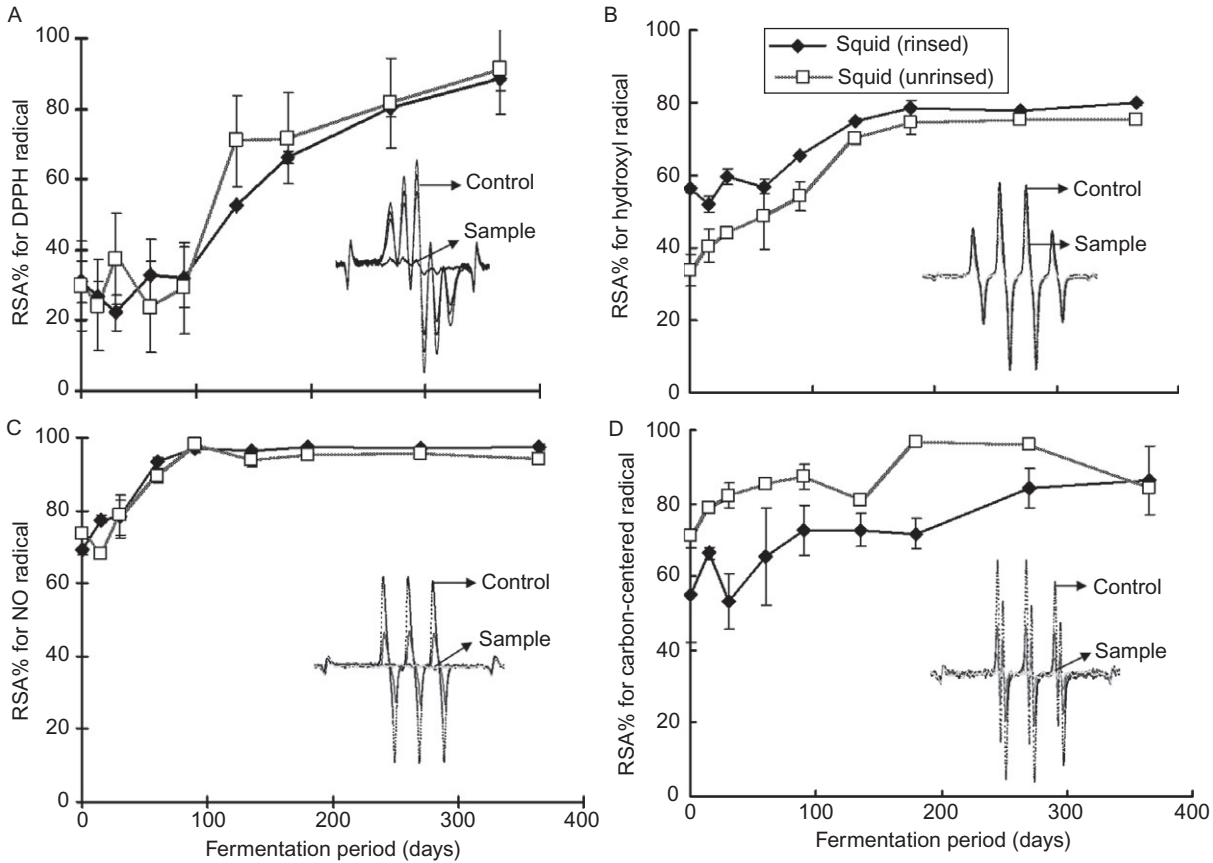

FIGURE 5.7 Changes in the levels of DPPH (A), hydroxyl (B), NO (C), and carbon-centered (D) radical-scavenging activity of miso prepared from rinsed and unrinsed squid meat at different points of the fermentation period (the ESR signal patterns for the control and sample are inset for all types of radicals). *Source*: Giri *et al.* (2011a). Permission has been obtained for the use of copyrighted material from Elsevier B.V.

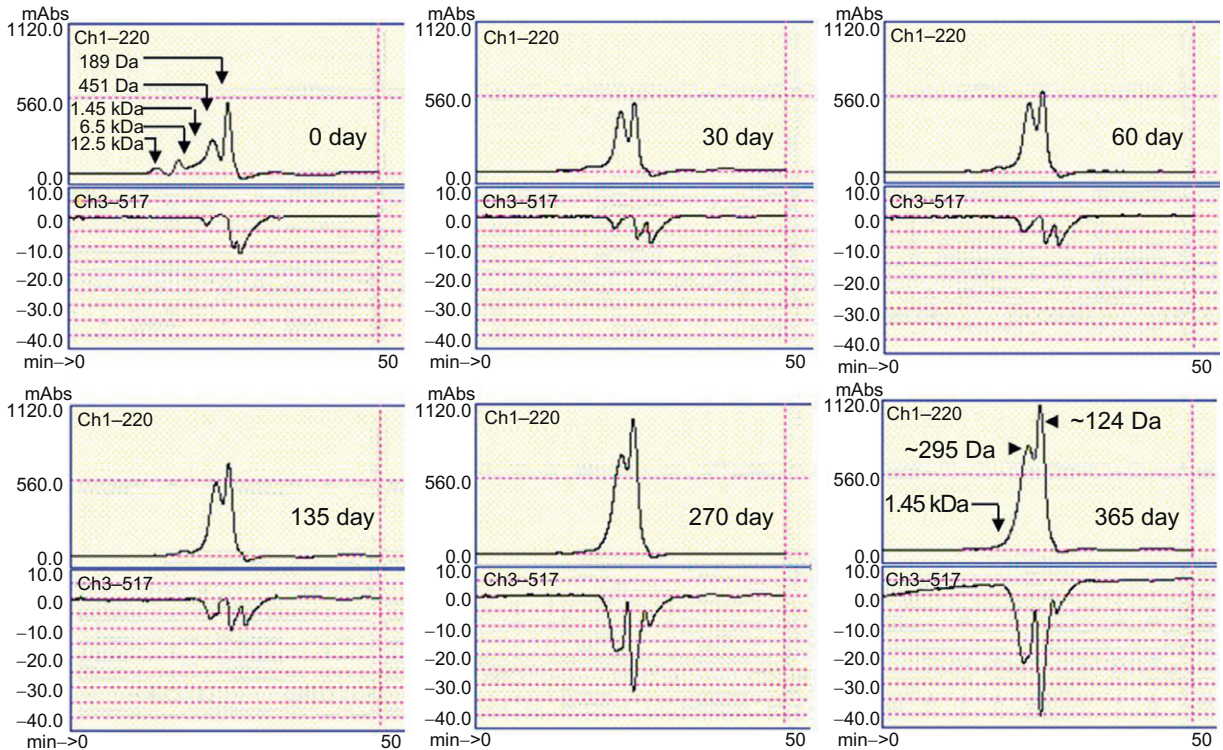

FIGURE 5.8 Changes in the molecular mass distribution of peptides and the radical-scavenging capacity of miso extracts prepared from unrinsed squid meat at different points in the fermentation period measured with an online HPLC-DPPH system. *Source*: Giri *et al.* (2011a). Permission has been obtained for the use of copyrighted material from Elsevier B.V.

development of peptides with molecular masses below ~500 Da. Changes in the free amino acid content of fish miso indicated that the levels of free amino acids, including Asp/Asn, Glu/Gln, Ala, and Leu, increased rapidly over the 270 days of fermentation (Table 5.3). The levels of oligopeptide amino acids (Table 5.4) also increased during this time period, demonstrating the development of low MW peptides as fermentation proceeded. The oligopeptides were mainly comprised of Glu/Gln, Pro, Gly, Ala, Val, Lys, and Leu. The presence of peptides with enhanced hydrophobicity (derived from many protein sources) is linked to antioxidative properties (Chen *et al.*, 1995a). An increase in hydrophobicity increases the affinity of peptides to lipids and, therefore, enhances their antioxidative properties (Rajapakse *et al.*, 2005a), suggesting that the hydrophobic amino acids, for example, Leu, Val, and Ala, in fish miso peptides might have promoted their radical-scavenging properties. These amino acids are effective as inhibitors of linoleic acid oxidation in the oil/water emulsion model system (Marcuse, 1962). In addition, the amino acid sequences of peptides strongly affect their antioxidative activity; the correct positioning of Glu/Gln, Leu, and His in antioxidative peptide sequences improves their radical-scavenging activity (Chen *et al.*, 1996). Decomposition of the imidazole ring in His is responsible for the strong radical-scavenging activity of this amino acid; thus, it was assumed that the radical-scavenging activity of fish miso resulted from the biophysical properties of some of the peptides that developed during fermentation.

VIII. CONCLUDING REMARKS

The marine-derived peptidic compounds discussed here were obtained from very different marine organisms, exhibited different chemical structures, and displayed a large variety of pharmacological effects on human health and nutrition. The possibilities of designing new functional foods and pharmaceuticals to support the reduction or regulation of diet-related chronic malfunctions are highly promising. Further, the processing of fish by-products as food proteins can be easily utilized for the production of bioactive peptides. This review suggests that due to their valuable biological functions with beneficial health effects, marine-derived bioactive peptides have the potential to be used as active ingredients for the preparation of various functional foods or nutraceutical and pharmaceutical products. Considering that the majority of the studies discussed in this chapter were conducted *in vitro* or in mouse model systems, further studies are needed to investigate the bioactivity of functional peptides in human subjects.

TABLE 5.3 Free amino acids and related cocompounds (mg/g dry extract) and mol% (in parenthesis) in squid *miso* during fermentation period

Amino acids	0 day	30 day	60 day	135 day	270 day	365 day
Taurine	38.05^{a} (25.74)	27.56^{b} (10.42)	24.08^{b} (8.66)	22.34^{b} (7.64)	20.99^{c} (7.35)	17.49^{c} (6.23)
Aspartic acid	4.07^{d,e} (2.59)	21.70^{b} (7.71)	30.67^{a,b} (10.37)	39.80^{a} (12.80)	42.63^{a} (14.03)	45.07^{a} (15.09)
Threonine	2.25^{d,e} (1.59)	7.18^{c,d} (2.85)	7.73^{d} (2.92)	8.16^{d} (2.93)	7.93^{d} (2.91)	7.31^{d} (2.73)
Serine	3.51^{d,e} (2.83)	12.25^{c} (5.51)	13.26^{c} (5.68)	14.15^{c,d} (5.76)	12.58^{c,d} (5.24)	10.41^{c,d} (4.41)
Glutamic acid	3.52^{d,e} (2.02)	24.50^{b} (7.87)	29.58^{a,b} (9.05)	36.88^{a} (10.73)	34.91^{b} (10.39)	27.53^{b} (8.34)
Proline	14.50^{c} (10.66)	14.07^{c} (5.78)	15.57^{c} (6.09)	17.73^{c} (6.59)	18.18^{b,c} (6.92)	14.68^{c} (5.68)
Glycine	3.17^{d,e} (3.58)	6.24^{d} (3.93)	7.22^{d} (4.33)	8.11^{d} (4.62)	8.02^{d} (4.68)	7.16^{d} (4.25)
Alanine	7.84^{d} (7.45)	22.31^{b,c} (11.84)	25.21^{b} (12.74)	27.56^{b} (13.24)	27.30^{b} (13.42)	25.15^{b} (12.58)
Valine	3.49^{d,e} (2.51)	10.34^{c} (4.17)	11.17^{c,d} (4.29)	12.06^{c,d} (4.40)	13.10^{c,d} (4.90)	12.35^{c,d} (4.70)
Cysteine	4.27^{d,e} (2.98)	2.86^{d,e} (1.11)	2.72^{e} (1.01)	2.68^{e} (0.94)	3.58^{e} (1.29)	15.72^{c} (5.78)
Methionine	2.52^{d,e} (1.42)	6.53^{d} (2.07)	5.83^{e} (1.75)	5.24^{e} (1.50)	5.94^{d,e} (1.74)	9.52^{c,d} (2.84)
Isoleucine	2.28^{d,e} (1.46)	6.65^{d} (2.39)	7.02^{d,e} (2.41)	7.25^{d,e} (2.36)	7.95^{d,e} (2.65)	9.47^{c,d} (3.21)
Leucine	28.00^{b} (18.01)	40.89^{a} (14.69)	38.05^{a,b} (13.01)	34.94^{a} (11.36)	25.42^{b,c} (8.45)	24.55^{b} (8.31)
Tyrosine	3.65^{d,e} (1.70)	6.73^{d} (1.75)	7.00^{d} (1.74)	7.17^{d} (1.69)	7.64^{d} (1.84)	8.00^{d} (1.96)
Phenylalanine	3.01^{d,e} (1.54)	6.38^{d} (1.82)	6.23^{d} (1.69)	6.44^{d} (1.67)	7.58^{d} (2.01)	6.65^{d,e} (1.79)
β-Alanine	0.13^{e} (0.12)	0.13^{e} (0.07)	0.12^{f} (0.06)	0.11^{f} (0.05)	0.09^{f} (0.04)	0.09^{f} (0.04)
4-Aminobutyric acid	2.27^{d,e} (1.86)	1.74^{d,e} (0.79)	1.54^{e,f} (0.67)	1.43^{e,f} (0.59)	1.47^{e,f} (0.62)	1.21^{e} (0.52)
Histidine	1.63^{d,e} (0.88)	2.27^{d,e} (0.69)	2.11^{e,f} (0.61)	1.85^{e,f} (0.51)	1.48^{e,f} (0.41)	1.39^{e} (0.40)
Ornithine	0.33^{e} (0.21)	0.35^{e} (0.12)	0.29^{f} (0.09)	0.84^{f} (0.27)	1.98^{e,f} (0.65)	4.05^{d,e} (1.36)
Lysine	8.06^{c} (4.15)	24.39^{b} (7.03)	24.35^{b} (6.68)	21.95^{b,c} (5.72)	16.06^{c} (4.28)	12.83^{c,d} (3.48)
Arginine	13.67^{c} (6.64)	26.85^{b} (7.29)	23.53^{b} (6.08)	18.51^{c} (4.55)	24.35^{b,c} (6.12)	24.32^{b} (6.22)

Different letters (*a–f*) represent significant differences at $P<0.05$.
Source: Giri *et al.* (2011a). Permission has been obtained for use of copyrighted material from Elsevier B.V.

TABLE 5.4 Amino acids and related compounds in oligopeptides (mg/g dry extract) and mol% (in parenthesis) in squid *miso* during fermentation period

Amino acids	0 day	30 day	60 day	135 day	270 day	365 day
Taurine	7.24^{c} (3.78)	10.03^{d} (3.32)	13.33^{d} (3.97)	13.52^{d} (4.01)	$9.60^{d,e}$ (2.60)	12.66^{e} (3.17)
Aspartic acid/asparagine	13.69^{b} (6.73)	$17.08^{c,d}$ (5.33)	14.91^{d} (4.18)	$9.71^{d,e}$ (2.70)	$10.49^{d,e}$ (2.67)	10.56^{e} (2.49)
Threonine	6.26^{c} (3.43)	10.46^{d} (3.64)	12.50^{d} (3.91)	13.39^{d} (4.17)	13.45^{d} (3.83)	14.05^{e} (3.70)
Serine	$8.16^{b,c}$ (5.08)	9.23^{d} (3.65)	$10.68^{d,e}$ (3.79)	$11.23^{d,e}$ (3.96)	$11.12^{d,e}$ (3.59)	12.89^{e} (3.85)
Glutamic acid/glutamine	38.53^{a} (17.13)	64.25^{a} (18.14)	72.05^{a} (18.27)	72.70^{a} (18.34)	77.17^{a} (17.81)	88.60^{a} (18.90)
Proline	$8.63^{b,c}$ (4.90)	15.10^{c} (5.45)	12.33^{d} (3.99)	17.17^{d} (5.53)	27.82^{c} (8.20)	29.19^{c} (7.96)
Glycine	16.45^{b} (14.34)	$21.65^{b,c}$ (11.98)	22.69^{c} (11.28)	$23.19^{c,d}$ (11.46)	$20.88^{c,d}$ (9.44)	22.18^{d} (9.27)
Alanine	$10.63^{b,c}$ (7.80)	13.86^{d} (6.46)	15.10^{d} (6.32)	15.14^{d} (6.31)	14.76^{d} (5.62)	$18.44^{d,e}$ (6.49)
Valine	6.72^{c} (3.75)	12.72^{d} (4.51)	15.30^{d} (4.87)	16.86^{d} (5.34)	18.37^{d} (5.32)	$21.19^{d,e}$ (5.67)
Cysteine	5.48^{c} (2.95)	15.53^{d} (5.32)	14.78^{d} (4.55)	$4.02^{e,f}$ (1.23)	$12.85^{d,e}$ (3.60)	2.99^{f} (0.77)
Methionine	1.55^{d} (0.68)	$6.98^{e,f}$ (1.94)	$11.77^{d,e}$ (2.94)	$4.61^{e,f}$ (1.14)	1.76^{f} (0.40)	5.54^{f} (1.16)
Isoleucine	5.77^{c} (2.88)	12.18^{d} (3.85)	15.16^{d} (4.31)	17.33^{d} (4.90)	19.70^{d} (5.10)	20.31^{d} (4.86)
Leucine	16.02^{b} (7.96)	$19.24^{b,c}$ (6.06)	5.98^{e} (1.69)	$12.01^{d,e}$ (3.38)	$11.30^{d,e}$ (2.91)	12.16^{e} (2.89)
Tyrosine	$8.61^{b,c}$ (3.11)	13.58^{d} (3.11)	27.40^{c} (5.64)	29.04^{c} (5.95)	32.81^{c} (6.14)	46.34^{b} (8.02)
Phenylalanine	6.33^{c} (2.50)	1.86^{e} (0.46)	$10.27^{d,e}$ (2.32)	8.40^{e} (1.88)	$10.97^{d,e}$ (2.25)	11.50^{e} (2.18)
β-Alanine	$3.55^{c,d}$ (2.60)	$7.30^{d,e}$ (3.40)	8.47^{e} (3.55)	$9.54^{d,e}$ (3.97)	$10.86^{d,e}$ (4.13)	11.48^{e} (4.04)
4-Aminobutyric acid	0.31^{e} (0.19)	0.56^{f} (0.22)	0.99^{f} (0.35)	1.39^{f} (0.50)	2.22^{f} (0.73)	3.11^{f} (0.94)
Histidine	$3.22^{c,d}$ (1.35)	$6.73^{e,f}$ (1.80)	7.31^{e} (1.75)	7.39^{d} (1.76)	$5.93^{e,f}$ (1.29)	5.20^{f} (1.05)
Ornithine	$3.94^{c,d}$ (1.94)	$4.19^{e,f}$ (1.31)	$4.42^{e,f}$ (1.24)	$4.78^{d,e}$ (1.34)	$11.65^{d,e}$ (2.99)	$7.66^{e,f}$ (1.81)
Lysine	14.52^{b} (5.78)	31.72^{b} (8.02)	37.61^{b} (8.55)	40.71^{b} (9.20)	45.75^{b} (9.46)	47.81^{b} (9.14)
Arginine	$2.81^{c,d}$ (1.05)	8.29^{d} (1.97)	$11.50^{d,e}$ (2.46)	13.33^{d} (2.84)	$9.42^{d,e}$ (1.83)	8.61^{e} (1.55)

Different letters (*a–f*) represent significant differences at $P<0.05$.
Source: Giri *et al.* (2011a). Permission has been obtained for use of copyrighted material from Elsevier B.V.

REFERENCES

Achour, A., Lachgar, A., and Astgen, A. (1997). Potentialization of IL-2 effect on immune cells by oyster extract (JCOE) in normal and HIV-infected individuals. *Biomed. Pharmacother.* **51**, 427–429.

Alemán, A., Giménez, B., Pérez-Santin, E., Gómez-Guillén, M. C., and Monter, P. (2011). Contribution of Leu and Hyp residues to antioxidant and ACE-inhibitory activities of peptide sequences isolated from squid gelatin hydrolysate. *Food Chem.* **125**, 334–341.

Amarowicz, R. and Shahidi, F. (1997). Antioxidant activity of peptide fractions of capelin protein hydrolysates. *Food Chem.* **58**, 355–359.

Anderson, J. J. B. and Garner, S. C. (1996). Calcium and phosphorous nutrition in health and disease: Introduction. *In* ''Calcium and Phosphorous in Health and Disease'', (J. J. B. Anderson and S. C. Garner, Eds), pp. 1–5. CRC Press, New York.

Ariyoshi, Y. (1993). Angiotensin-converting enzyme inhibitors derived from food proteins. *Trends Food Sci. Technol.* **4**, 139–144.

Bai, R., Pettit, G. R., and Hamel, E. (1990). Binding of dolastatin 10 to tubulin at exchangeable nucleotide and Vinca alkaloid sites. *J. Biol. Chem.* **265**, 17141–17149.

Bai, R., Verdier-Pinard, P., Gangwar, S., Stessman, C. C., McKlure, K. J., Sausville, E. A., Pettit, G. R., Bates, R. B., and Hamel, E. (2001). Dolastatin 11, a marine depsipeptide, A arrests cells at cytokinesis and induces hyperpolymerization of purified actin. *Mol. Pharmacol.* **59**, 462–469.

Bartlett, T. C., Cuthbertson, B. J., Shepard, E. F., Chapman, R. W., Grops, P. S., and Warr, G. W. (2002). Crustins, homologues of an 11.5-kDa antibacterial peptide, from two species of penaeid shrimp, *Litopenaeus vannamei* and *Litopenaeus setiferus*. *Mar. Biotechnol.* **4**, 278–293.

Battison, A. L., Summerfield, R., and Patrzykat, A. (2008). Isolation and characterization of two antimicrobial peptides from haemocytes of the American lobster *Homarus americanus*. *Fish Shellfish Immunol.* **25**, 181–187.

Binsan, W., Benjakul, S., Visessanguan, W., Roytrakul, S., Tanaka, M., and Kishimura, H. (2008). Antioxidative activity of Mungoong, an extract paste, from the cephalothorax of white shrimp (*Litopenaeus vannamei*). *Food Chem.* **106**, 185–193.

Byun, H. G. and Kim, S. K. (2001). Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from Alaska Pollack (*Theragra chalcogramma*) skin. *Process Biochem.* **36**, 1155–1162.

Cacciuttolo, M. A., Trinh, L., Lumpkin, J. A., and Rao, G. (1993). Hyperoxia induces DNA damage in mammalian cells. *Free Radic. Biol. Med.* **14**, 267–276.

Chen, H. M., Muramoto, K., and Yamauchi, F. (1995a). Structural analysis of antioxidative peptides from soybean β-conglycinin. *J. Agric. Food Chem.* **43**, 574–578.

Chen, J., Suetsuna, K., and Yamauchi, F. (1995b). Isolation and characterization of immunostimulative peptides from soybean. *J. Nutr. Biochem.* **6**, 310–313.

Chen, H., Muramoto, K., Yamauchi, F., and Nokihara, K. (1996). Antioxidant activity of designed peptides based on the antioxidative peptide isolated from digests of a soybean protein. *J. Agric. Food Chem.* **44**, 2619–2623.

Chen, H., Muramoto, K., Yamauchi, F., Fujimoto, K., and Nokihara, K. (1998). Antioxidative properties of histidine-containing peptides designed from peptide fragments found in the digests of a soybean protein. *J. Agric. Food Chem.* **46**, 49–53.

Cheung, I. W. Y. and Li-Chan, E. C. Y. (2010). Angiotensin-I-converting enzyme inhibitory activity and bitterness of enzymatically-produced hydrolysates of shrimp (*Pandalopsis dispar*) processing byproducts investigated by Taguchi design. *Food Chem.* **122**, 1003–1012.

Cheung, H. S., Wang, F. L., Ondetti, M. A., Sabo, E. F., and Cushman, D. W. (1980). Binding of peptide substrates and inhibitors of angiotensin-converting enzyme. *J. Biol. Chem.* **255**, 401–407.

Cho, S. S., Lee, H. K., Yu, C. Y., Kim, M. J., Seong, E. S., Ghimire, B. K., Son, E. H., Choung, M. G., and Lim, J. D. (2008). Isolation and characterization of bioactive peptides from Hwangtae (yellowish dried Alaska pollack) protein hydrolysate. *J. Food Sci. Nutr.* **13**, 196–203.

Chun, H., Sasaki, M., Fujiyama, Y., and Bamba, T. (1996). Effect of peptide chain length on absorption and intact transport of hydrolyzed soybean peptide in rat intestinal everted sac. *J. Clin. Biochem. Nutr.* **21**, 131–140.

Clare, D. A. and Swaisgood, H. E. (2000). Bioactive milk peptides: A prospectus. *J. Dairy Sci.* **83**, 1187–1195.

Craft, I. L., Geddes, D., Hyde, C. W., Wise, I. J., and Matthews, D. M. (1968). Absorption and malabsorption of glycine and glycine peptides in man. *Gut* **9**, 425–437.

Cushman, D. W., Cheung, H. S., Sabo, E. F., and Ondetti, M. A. (1981). Angiotensin-converting enzyme inhibitors: Evaluation of a new class of antihypertensive drugs. *In* ''Angiotensin-Converting Enzyme Inhibitors: Mechanism of Action and Clinical Implications'', (Z. P. Horovitz, Ed.), pp. 1–25. Urban and Schwarzenberg, Baltimore and Munich.

Davalos, A., Miguel, M., Bartolomé, B., and López-Fandiño, R. (2004). Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. *J. Food Prot.* **67**, 1939–1944.

de Vries, D. J. and Beart, P. M. (1995). Fishing for drugs from the sea: Status and strategies. *Trends Pharmacol. Sci.* **16**, 275–279.

Defelice, S. L. (1995). The nutritional revolution: Its impact on food industry R&D. *Trends Food Sci. Technol.* **6**, 59–61.

Edward, N. J., Arroll, T. A., and Fan, Z. (1996). Specificity and some physicochemical characteristics of the antibacterial activity from blue crab *Callinectus sapidus*. *Fish Shellfish Immunol.* **6**, 403–413.

Elias, R. J., Kellerby, S. S., and Decker, E. A. (2008). Antioxidant activity of proteins and peptides. *Crit. Rev. Food Sci. Nutr.* **48**, 430–441.

Erdmann, K., Cheung, B. W. Y., and Schroder, H. (2008). The possible roles of food-derived bioactive peptides in reducing the risk of cardiovascular disease. *J. Nutr. Biochem.* **19**, 643–654.

Fahmi, A., Morimura, S., Guo, H. C., Shigematsu, T., Kida, K., and Uemura, Y. (2004). Production of angiotensin I converting enzyme inhibitory peptides from sea bream scales. *Process Biochem.* **39**, 1195–1200.

FAOSTAT, FAO Statistical Databases, Fisheries Data (2001). Food and Agriculture Organization of the United Nations, Rome, Italy. http://www.fao.org. Available from Internet, URL.

Fouchereau-Peron, M., Duvail, L., Michel, C., Gildberg, A., Batista, I., and Gal, Y. I. (1999). Isolation of an acid fraction from a fish protein hydrolysate with a calcitonin–gene-related-peptide-like biological activity. *Biotechnol. Appl. Biochem.* **29**, 87–92.

Fujita, H. and Yoshikawa, M. (1999). LKPNM: A prodrug-type ACE-inhibitory peptide derived from fish protein. *Immunopharmacology* **44**, 123–127.

Gerwick, W. H. (1987). Drugs from the Sea—The Search Continues. *J. Pharm. Tech.* (July/August), 136–141.

Gildberg, A., Bogwald, J., Johansen, A., and Stenberg, E. (1996). Isolation of acid peptide fractions from a fish protein hydrolysate with strong stimulatory effect on Atlantic salmon (*Salmo salar*) head kidney leucocytes. *Comp. Biochem. Physiol.* **11**, 97–101.

Giri, A., Osako, K., and Ohshima, T. (2009a). Effect of raw materials on the extractive component sand taste aspects of fermented fish paste: Sakana miso. *Fish. Sci.* **75**, 785–796.

Giri, A., Osako, K., and Ohshima, T. (2009b). Extractive components and taste aspects of fermented fish pastes and bean pastes prepared using different koji molds as starters. *Fish. Sci.* **75**, 481–489.

Giri, A., Osako, K., and Ohshima, T. (2010a). Identification and characterization of headspace volatiles of fish miso, a Japanese fish meat based fermented paste, with special emphasis on effect of fish species and meat washing. *Food Chem.* **120**, 621–631.

Giri, A., Osako, K., Okamoto, A., and Ohshima, T. (2010b). Olfactometric characterization of aroma active compounds in fermented fish paste in comparison with fish sauce, fermented soy paste and sauce products. *Food Res. Int.* **43**, 1027–1040.

Giri, A., Osako, K., Okamoto, A., Okazaki, E., and Ohshima, T. (2011a). Antioxidative properties of aqueous and aroma extracts of squid miso prepared with *Aspergillus oryzae*-inoculated koji. *Food Res. Int.* **44**, 317–325.

Giri, A., Osako, K., Okamoto, A., Okazaki, E., and Ohshima, T. (2011b). Effect of meat washing on the development of impact odorants in fish miso prepared from spotted mackerel. *J. Sci. Food Agric.* **91**, 850–859.

Gobbetti, M., Stepaniak, L., Angelis, M. D., Corsetti, A., and Cagno, R. D. (2002). Latent bioactive peptides in milk proteins: Proteolytic activation and significance in dairy processing. *Crit. Rev. Food Sci. Nutr.* **42**, 223–239.

Guerard, F., Sumaya-Martinez, M. T., Laroque, D., Chabeaud, A., and Dufosse, L. (2007). Optimization of free radical scavenging activity by response surface methodology in the hydrolysis of shrimp processing discards. *Process Biochem.* **42**, 1486–1491.

He, H., Chen, X., Sun, C., and Zhang, Y. (2004). Research progress in inhibitory peptides of angiotensin-converting enzyme. *China Biotechnol.* **24**, 7–11.

He, H., Chen, X., Sun, C., Zhang, Y., and Gao, P. (2006). Preparation and functional evaluation of oligopeptide-enriched hydrolysate from shrimp (*Acetes chinensis*) treated with crude protease from *Bacillus* sp SM98011. *Bioresour. Technol.* **97**, 385–390.

Hernandez-Ledesma, B., D'avalos, A., Bartolom'e, B., and Amigo, L. (2005). Preparation of antioxidant enzymatic hydrolysates from α-lactalbumin and β-lactoglobulin. Identification of active peptides by HPLC-MSMS. *J. Agric. Food Chem.* **53**, 588–593.

Huang, G., Ren, Z., and Jiang, J. (2010). Separation of iron-binding peptides from shrimp processing by-products hydrolysates. *Food Bioprocess Technol.* **4**, 1527–1532.

Ichimura, T., Hu, J., Aita, D. Q., and Maruyama, S. (2003). Angiotensin I-converting inhibitory activity and insulin secretion stimulative activity of fermented fish sauce. *J. Biosci. Bioeng.* **5**, 496–499.

Jayasankar, V. and Subramonium, T. (1999). Antibacterial activity of seminal plasma of the mud crab *Scylla serrata* (forskal). *J. Exp. Mar. Biol. Ecol.* **236**, 253–259.

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

Je, J. Y., Park, P. J., Byun, H. K., Jung, W. K., and Kim, S. K. (2005b). Angiotensin I converting enzyme (ACE) inhibitory peptide derived from the sauce of fermented blue mussel, *Mytilus edulis*. *Bioresour. Technol.* **96**, 1624–1629.

Je, J. Y., Qian, Z. J., Byun, H. G., and Kim, S. K. (2007). Purification and characterization of an antioxidant peptide obtained from tuna backbone protein by enzymatic hydrolysis. *Process Biochem.* **42**, 840–846.

Je, J. Y., Qian, Z. J., Lee, S. H., Byun, H. G., and Kim, S. K. (2008). Purification and antioxidant properties of bigeye tuna (*Thunnus obesus*) dark muscle peptide on free radical-mediated oxidation systems. *J. Med. Food* **11**, 629–637.

Jeon, Y. J. and Kim, S. K. (2000). Continuous production of chitooligosaccharides using a dual reactor system. *Process Biochem.* **35**, 623–632.

Jo, H. Y., Jung, W. K., and Kim, S. K. (2008). Purification and characterization of a novel anticoagulant peptide from marine echiuroid worm, *Urechis unicinctus*. *Process Biochem.* **43**, 179–184.

Jun, S. Y., Park, P. J., Jung, W. K., and Kim, S. K. (2004). Purification and characterization of an antioxidative peptide from enzymatic hydrolysates of yellowfin sole (*Limanda aspera*) frame protein. *Eur. Food Res. Technol.* **219**, 20–26.

Jung, W. K. and Kim, S. K. (2007). Calcium-binding peptide derived from pepsinolytic hydrolysates of hoki (*Johnius belengerii*) frame. *Eur. Food Res. Technol.* **224**, 763–767.

Jung, W. K. and Kim, S. K. (2009). Isolation and characterization of an anticoagulant oligopeptide from blue mussel, *Mytilus edulis*. *Food Chem.* **117**, 687–692.

Jung, W. K., Je, J. Y., and Kim, S. K. (2001). A novel anticoagulant protein from *Scapharca broughtonii*. *J. Biochem. Mol. Biol.* **35**, 199–205.

Jung, W. K., Park, P. J., Byun, H. G., Moon, S. H., and Kim, S. K. (2005a). Preparation of hoki (*Johnius belengerii*) bone oligophosphopeptide with a high affinity to calcium by carnivorous intestine crude proteinase. *Food Chem.* **91**, 333–340.

Jung, W. K., Rajapakse, N., and Kim, S. K. (2005b). Antioxidative activity of a low molecular weight peptide derived from the sauce of fermented blue mussel, *Mytillus edulis*. *Eur. Food Res. Technol.* **220**, 535–539.

Jung, W. K., Karawita, R., Heo, S. J., Lee, B. J., Kim, S. K., and Jeon, Y. J. (2006a). Recovery of a novel Ca-binding peptide from Alaska pollack (*Theragra chalcogramma*) backbone by pepsinolytic hydrolysis. *Process Biochem.* **41**, 2097–2100.

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

Jung, W. K., Qian, Z. J., Lee, S. H., Choi, S. Y., Sung, N. J., Byun, H. G., and Kim, S. K. (2007). Free radical scavenging activity of a novel antioxidative peptide isolated from in vitro gastrointestinal digests of *Mytilus coruscus*. *J. Med. Food* **10**, 197–202.

Kim, G. H., Jeon, Y. J., Byun, H. G., Lee, Y. S., and Kim, S. K. (1998). Effect of calcium compounds from oyster shell bound fish skin gelatine peptide in calcium deficient rats. *J. Korean Fish. Soc.* **31**, 149–159.

Kim, S. K., Jeon, Y. J., Byun, H. G., and Park, P. J. (1999). Calcium absorption acceleration effect on phosphorylated and nonphosphorylated peptides from hoki (*Johnius belengeri*) frame. *J. Korean Fish. Soc.* **32**, 713–717.

Kim, S. K., Choi, Y. R., Park, P. J., Choi, J. H., and Moon, S. H. (2000). Screening of biofunctional peptides from cod processing wastes. *J. Korean Soc. Agric. Chem. Biotechnol.* **43**, 225–227.

Kim, S. K., Kim, Y. T., Byun, H. G., Nam, K. S., Joo, D. S., and Shahidi, F. (2001). Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Allaska Pollack skin. *J. Agric. Food Chem.* **49**, 1984–1989.

Kim, D. C., Chae, H. J., and In, M. J. (2004). Existence of stable fibrin-clotting inhibitor in salt fermented anchovy sauce. *J. Food Compost. Anal.* **17**, 113–118.

Kim, S. Y., Je, J. Y., and Kim, S. K. (2007). Purification and characterization of antioxidant peptide from hoki (*Johnius balengerii*) frame protein by gastrointestinal digestion. *J. Nutr. Biochem.* **18**, 31–38.

Kim, E., Lee, S., Jeon, B., Moon, S., Kim, B., Park, T., Han, J., and Park, P. (2009). Purification and characterisation of antioxidative peptides from enzymatic hydrolysates of venison protein. *Food Chem.* **114**, 1365–1370.

Klompong, V., Benjakul, S., Yachai, M., Visessanguan, W., Shahidi, F., and Hayes, K. D. (2009). Amino acid composition and antioxidative peptides from protein hydrolysates of yellow stripe trevally (*Selaroides leptolepsis*). *J. Food Sci.* **74**, 126–133.

Korhonen, H. and Pihlanto-Leppala, A. (2003). Food-derived bioactive peptides: Opportunities for designing future foods. *Curr. Pharm. Des.* **9**, 1297–1308.

Koyama, T., Noguchi, K., Aniya, Y., and Sakanashi, M. (1998). Analysis for sites of anticoagulant action of plancinin, a new anticoagulant peptide isolated from the starfish *Acanthaster planci*, in the blood coagulation cascade. *Gen. Pharmacol.* **31**, 277–282.

Kuba, M., Tana, C., Tawata, S., and Yasuda, M. (2005). Production of angiotensin-I converting enzyme inhibitory peptides from soybean protein with *Monascus purpureus* acid proteinase. *Process Biochem.* **40**, 2191–2196.

Lahl, W. J. and Braun, S. D. (1994). Enzymatic production of protein by hydrolysates for food use. *Food Technol.* **48**, 68–71.

Lee, T. G. (1996). Functional peptides from hydrolysate of marine oyster proteins. Ph.D. thesis, Pukyong National University, Korea.

Lee, T. G. and Maruyama, S. (1998). Isolation of HIV-1 protease-inhibiting peptides from thermolysin hydrolysate of oyster proteins. *Biochem. Biophys. Res. Commun.* **253**, 604–608.

Lee, S. H., Qian, Z. J., and 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 Chem.* **118**, 96–102.

Li, C., Haug, T., Styrvold, O. B., Jørgensen, T.Ø., and Stensvag, K. (2008). Strongylocins, novel antimicrobial peptides from the green sea urchin, *Strongylocentrotus droebachiensis*. *Dev. Comp. Immunol.* **32**, 1430–1440.

Li, Y. W., Li, B., He, J., and Qian, P. (2011). Structure–activity relationship study of antioxidative peptides by QSAR modeling: The amino acid next to C-terminus affects the activity. *J. Pept. Sci.* **17**, 454–462.

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

Lun, H. H., Lan, C. X., Yun, S. C., Zhong, Z. Y., and Cheng, Z. B. (2006). Analysis of novel angiotensin-I-converting enzyme inhibitory peptides from protease-hydrolyzed marine shrimp *Acetes chinensis*. *J. Pept. Sci.* **12**, 726–733.

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

Madden, T., Tran, H. T., Beck, D., Huie, R., Newman, R. A., Pusztai, L., Wright, J. J., and Abbruzzese, J. L. (2000). Novel marine-derived anticancer agents: A phase I clinical, pharmacological, and pharmacodynamic study of dolastatin 10 (NSC 376128) in patients with advanced solid tumors. *Clin. Cancer Res.* **6**, 1293–1301.

Marcuse, R. (1962). The effect of some amino acids on the oxidation of linoleic acid and its methyl ester. *J. Am. Oil Chem. Soc.* **39**, 97–107.

Matsumoto, K., Ogikubo, A., Yoshino, T., Matsui, T., and Osajima, Y. (1994). Separation and purification of angiotensin I converting enzyme inhibitory peptide in peptic hydrolysate of oyster. *Nippon Shokuhin Kogyo Gakkaishi* **41**, 589–594. (in Japanese).

Matthews, D. M. and Payne, J. W. (1980). Transmembrane transport of small peptides. *J. Curr. Top. Membr. Transp.* **14**, 331–425.

Mendis, E., Rajapakse, N., and Kim, S. K. (2005a). Antioxidant properties of a radical-scavenging peptide purified from enzymatically prepared fish skin gelatin hydrolysate. *J. Agric. Food Chem.* **53**, 581–587.

Mendis, E., Rajapakse, N., Byun, H. G., and Kim, S. K. (2005b). Investigation of jumbo squid (*Dosidicus gigas*) skin gelatin peptides for their in vitro antioxidant effects. *Life Sci.* **77**, 2166–2178.

Miljanich, G. P. (1997). Venom peptides as human pharmaceuticals. *Sci. Med.* (Sept/Oct), 6–15.

Okamoto, A., Hanagata, H., Matsumoto, E., Kawamura, Y., Koizumi, Y., and Yanagida, F. (1995a). Angiotensin I converting enzyme inhibitory activities of various fermented foods. *Biosci. Biotech. Biochem.* **59**, 1147–1149.

Okamoto, A., Matsumoto, E., Iwashita, A., Yasuhara, T., Kawamura, Y., Koizumi, Y., and Yanagida, F. (1995b). Angiotensin I-converting enzyme inhibitory action of fish sauce. *Food Sci. Technol. Int.* **1**, 101–106.

Pan, D. D., Luo, Y. K., and Tanokura, M. (2005). Antihypertensive peptides from skimmed milk hydrolysate digested by cell-free extract of *Lactobacillus helveticus*. *Food Chem.* **91**, 123–129.

Pettit, G. R., Kamano, Y., Fuji, Y., Herald, C. L., Inoue, M., Brown, P., Gust, D., Kitahara, K., Shmidt, J. M., Doubek, D. L., and Michel, C. (1981). Marine animal biosynthetic constituents for cancer chemotherapy. *J. Nat. Prod.* **44**, 482–485.

Pettit, G. R., Kamano, Y., Herald, C. L., Dufresne, C., Cerny, R. L., Herald, D. L., Schmidt, J. M., and Kizu, H. (1989). Isolation and structure of the cytostatic depsipeptide dolastatin 13 from the sea hare *Dolabella auricularia*. *J. Am. Chem. Soc.* **111**, 5015–5017.

Petitt, R., Hogan, F., Xu, J. P., Tan, R., Nogawa, T., Cichacz, Z., Pettit, R. K., Du, J., Ye, Q. H., Cragg, G. M., Herald, C. L., *et al*. (2008). Antineoplastic Agents. 536. New Sources of Naturally Occurring Cancer Cell Growth Inhibitors from Marine Organisms, Terrestrial Plants, and Microorganisms. *J. Nat. Prod.* **71**(3), 438–444.

Pihlanto-Leppala, A. (2001). Bioactive peptides derived from bovine proteins: Opioid and ACE-inhibitory peptides. *Trends Food Sci. Technol.* **11**, 347–356.

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

Qian, Z. J., Jung, W. K., Byun, H. G., and Kim, S. K. (2008). Protective effect of an antioxidative peptide purified from gastrointestinal digests of oyster, *Crassostrea gigas* against free radical induced DNA damage. *Bioresour. Technol.* **99**, 3365–3371.

Rajapakse, N., Jung, W. K., Mendis, E., Moon, S. H., and Kim, S. K. (2005a). A novel anticoagulant purified from fish protein hydrolysate inhibits factor XIIa and platelet aggregation. *Life Sci.* **76**, 2607–2619.

Rajapakse, N., Mendis, E., Byun, H. G., and Kim, S. K. (2005b). Purification and *in vitro* antioxidative effects of giant squid muscle peptides on free radical-mediated oxidative systems. *J. Nutr. Biochem.* **16**, 562–569.

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

Ranathunga, S., Rajapakse, N., and Kim, S. K. (2006). Purification and characterization of antioxidantative peptide derived from muscle of conger eel (*Conger myriaster*). *Eur. Food Res. Technol.* **222**, 310–315.

Roberts, P. R., Burney, J. D., Black, K. W., and Zaloga, G. P. (1999). Effect of chain length on absorption of biologically active peptides from the gastrointestinal tract. *Digestion* **60**, 332–337.

Sachindra, N. M. and Bhaskar, N. (2008). *In vitro* antioxidant activity of liquor from fermented shrimp biowaste. *Bioresour. Technol.* **99**, 9013–9016.

Sachindra, N. M., Bhaskar, N., and Mahendrakar, N. S. (2005). Carotenoids in different body components of Indian shrimps. *J. Sci. Food Agric.* **85**, 167–172.

Saito, K., Jin, D., Ogawa, T., Muramoto, K., Hatakeyama, E., Yasuhara, T., and Nokihara, K. (2003). Antioxidative properties of tripeptide libraries prepared by the combinatorial chemistry. *J. Agric. Food Chem.* **51**, 3668–3674.

Samaranayaka, A. G. P. and Li-Chan, E. C. Y. (2008). Autolysis-assisted production of fish protein hydrolysates with antioxidant properties from Pacific hake (*Merluccius productus*). *Food Chem.* **107**, 768–776.

Sampath, K. N. S., Nazeer, R. A., and Jaiganesh, R. (2011). Purification and identification of antioxidant peptides from the skin protein hydrolysate of two marine fishes, horse mackerel (*Magalaspis cordyla*) and croaker (*Otolithes ruber*). *Amino Acids*. doi: 10.1007/s00726-011-0858-6.

Seki, E., Osajima, K., Matsui, T., and Osajima, Y. (1993). Separation and purification of angiotensin I converting enzyme inhibitory peptides from heated sardine meat by treatment with alkaline protease. *Nippon Shokuhin Kogyo Gakkaishi* **40**, 783–791. (in Japanese).

Shahidi, F. (2007). Antioxidants from marine by-products. *In* ''Maximising the Value of Marine By-products'', (F. Shahidi, Ed.), pp. 397–412. CRC Press, USA.

Shahidi, F. and Zhong, Y. (2008). Bioactive peptides. *J. AOAC Int.* **91**, 914–931.

Slizyte, R., Mozuraityte, R., Martı'nez-Alvarez, O., Falch, E., Fouchereau-Peron, M., and Rustad, T. (2009). Functional, bioactive and antioxidative properties of hydrolysates obtained from cod (*Gadus morhua*) backbones. *Process Biochem.* **44**, 668–677.

Stensvag, K., Haug, T., Sperstad, S. V., Rekdal, O., Indrevoll, B., and Styrvold, O. B. (2008). Arasin 1, a proline-arginine-rich antimicrobial peptide isolated from the spider crab, *Hyas araneus*. *Dev. Comp. Immunol.* **32**, 275–285.

Suetsuna, K. (2000). Antioxidant peptides from the protease digest of prawn (*Penaeus Japonicus*) muscle. *Mar. Biotechnol.* **2**, 5–10.

Suetsuna, K. (2002). Identification of antihypertensive peptides from peptic digest of the short-necked clam tapes philippinarum and the pearl oyster pinctada fucata martensii. *Fish Sci.* **68**, 233–235.

Thiansilakul, Y., Benjakul, S., and Shahidi, F. (2007). Antioxidative activity of protein hydrolysate from round scad muscle using alcalase and flavourzyme. *J. Food Biochem.* **31**, 266–287.

Tincu, J. A. and Taylor, S. W. (2004). Antimicrobial peptides from marine invertebrates. *Antimicrob. Agents Chemother.* **48**, 3645–3654.

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

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

Tsuge, N., Eikawa, Y., Nomura, Y., Yamamoto, M., and Sugisawa, K. (1991). Antioxidative activity of peptides prepared by enzymatic hydrolysis of egg-white albumin. *Nippon Nogeikagaku Kaishi* **65**, 1635–1641.

Wang, Y. K., He, H. L., Chen, X. L., Sun, C. Y., Zhang, Y. Z., and Zhou, B. C. (2008). Production of novel angiotensin I-converting enzyme inhibitory peptides by fermentation of marine shrimp *Acetes chinensis* with *Lactobacillus fermentum* SM 605. *Appl. Microbiol. Biotechnol.* **79**, 785–791.

Wesson, K. J. and Hamann, M. T. (1996). Keenamide A, a bioactive cyclic peptide from the marine mollusk *Pleurobranchus forskalii*. *J. Nat. Prod.* **59**, 629–631.

Wijesekara, I., Qian, Z. J., Ryu, B. M., Ngo, D. H., and Kim, S. K. (2011). Purification and identification of antihypertensive peptides from seaweed pipefish (Syngnathus schlegeli) muscle protein hydrolysate. *Food Res. Int.* **44**(3), 703–707.

Wong, A. H. K. and Mine, Y. (2004). Novel fibrinolytic enzyme in fermented shrimp paste, a traditional Asian fermented seasoning. *J. Agric. Food Chem.* **52**, 980–986.

Yamamoto, N. (1997). Antihypertensive peptides derived from food proteins. *Biopolymers* **43**, 129–134.

Yang, R., Zhang, Z., Pei, X., Han, X., Wang, J., Wang, L., Long, Z., Shen, X., and Li, Y. (2009). Immunomodulatory effects of marine oligopeptide preparation from chum salmon (*Oncorhynchus keta*) in mice. *Food Chem.* **113**, 464–470.

Yin, L. J., Tong, Y. L., and Jiang, S. T. (2005). Effect of combining proteolysis and lactic acid bacterial fermentation on the characteristics of minced mackerel. *J. Food Sci.* **70**, 186–192.

Zeng, M., Cui, W., Zhao, Y., Liu, Z., Dong, S., and Guo, Y. (2008). Antiviral active peptide from oyster. *Chin. J. Oceanol. Limnol.* **26**, 307–312.

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

Zhao, Y., Bafang, L., Dong, S., Liu, Z., Zhao, X., Wang, J., and Zeng, M. (2009). A novel ACE inhibitory peptide isolated from *Acaudina molpadioidea* hydrolysate. *Peptides* **30**, 1028–1033.