Prev. Nutr. Food Sci. 2015;20(1):67-72
http://dx.doi.org/10.3746/pnf.2015.20.1.67
pISSN 2287-1098 · eISSN 2287-8602

Research Note

Biofunctional Properties of Enzymatic Squid Meat Hydrolysate

Joon Hyuk Choi[1], Kyung-Tae Kim[2], and Sang Moo Kim[1]

[1]Department of Marine Food Science and Technology, Gangneung-Wonju National University, Gangwon 210-702, Korea
[2]Institute of Drug Research & Development, Chungnam National University, Daejeon 305-764, Korea

ABSTRACT: Squid is one of the most important commercial fishes in the world and is mainly utilized or consumed as sliced raw fish or as processed products. The biofunctional activities of enzymatic squid meat hydrolysate were determined to develop value-added products. Enzymatic squid hydrolysate manufactured by Alcalase effectively quenched 1,1-diphenyl-2-picrylhydrazyl radical, hydroxyl radical, and hydrogen peroxide radical with IC_{50} values of 311, 3,410, and 111.5 μg/mL, respectively. Angiotensin I-converting enzyme inhibitory activity of squid hydrolysate was strong with an IC_{50} value of 145.1 μg/mL, while tyrosinase inhibitory activity with an IC_{50} value of 4.72 mg/mL was moderately low. Overall, squid meat hydrolysate can be used in food or cosmetic industries as a bioactive ingredient and possibly be used in the manufacture of seasoning, bread, noodle, or cosmetics.

Keywords: angiotensin I-converting enzyme, antioxidant, squid meat hydrolysate, tyrosinase

INTRODUCTION

Squid is a cephalopod of the *Ommastrephes* genus which comprises around 300 species over the world and mainly utilized or produced as sliced raw fish, and dried, seasoned, smoked and fermented product. Even though squid has been used as a raw material for production of functional ingredients including chitosan and taurin, the value and biological functions are still underestimated in fact (1). During the last few decades, the major nutritional parts of animal and plant proteins have been studied as a source to produce biologically active hydrolysates including peptides and enzyme inhibitors. The bioactive hydrolysates or peptides have various bioactivities such as antioxidative (2,3), antihypertensive (4,5), and anti-whitening effects (6). The different activities of bioactive hydrolysates depend on their structural, compositional, and sequential properties of the hydrolyzing process (7).

Oxidation is one of the top-topics in food industries. Among various problems in food processing and storage, lipid peroxidation is a remarkable problem because it leads to undesirable off-flavors and potentially toxicity products (8). Nowadays, numerous synthetic antioxidants are used to retard lipid peroxidation, but the safety and the negative cognition of consumers for synthetic antioxidants restrict their applications in food products (9). Moreover, reactive radicals are implicated in age-associated chronic diseases such as cardiovascular diseases, neurodegenerative disorders, diabetes and cancer (10). Therefore, the determination of antioxidative properties in many natural sources including some dietary protein compounds has been driven by consumers. The potential value of fish protein hydrolysates from yellow fin sole frame (9), herring (11), and mackerel (12) as an antioxidant has been reported so far.

Also, protein hydrolysates are of interest as materials for medicinal purposes because of their pharmaceutical activity as angiotensin I-converting enzyme (ACE) inhibitor to reduce morbidity and mortality of patients with hypertension and other related diseases (13). ACE can increase blood pressure by converting the inactive decapeptide angiotensin-I to the potent vasoconstrictor angiotensin-II (an octapeptide) (13). Some peptides having a potential ACE-inhibitory activity are inactive within the protein sequence, but they can reveal a biological activity when they are released by hydrolysis (3). These peptides can also be generated *in vivo* by the action of gastrointestinal enzymes and specific enzymes *in vitro* or certain foods processes. There are few reports on fish protein hydrolysates with ACE inhibitory activity including Alaska pollock skin (14), oyster sauce (15), sea squirt (16), and salmon (17).

Moreover, among the various activities of protein hy-

Received 21 October 2014; Accepted 11 January 2015; Published online 31 March 2015

Correspondence to Sang Moo Kim, Tel: +82-33-640-2343, E-mail: smkim@gwnu.ac.kr

drolysates, the inhibition of tyrosinase and elastase attracted attention due to their pharmaceutical activities in the field of agriculture, food and cosmetics as well as medicine (10). Tyrosinase is a copper-containing enzyme and catalyzes two distinct reactions of melanin biosynthesis, the hydroxylation of monophenols to *O*-diphenols and the oxidation of *O*-diphenols to *O*-quinones (6). Tyrosinase is involved in hair and skin browning pigmentation, anti-hypertension, and skin trouble (18), as well as cancer and neurodegenerative diseases including Parkinson's disease (19). In this study, antioxidant activity and inhibitory activities of squid meat hydrolysate against angiotensin converting enzyme and tyrosinase were determined for the development of value-added products such as ingredients in foods or cosmetics.

MATERIALS AND METHODS

Materials

Live squid (*Todarodes pacificus*) was purchased from a local fish market (Gangneung, Korea). The sample was brought to the laboratory in ice, rapidly rinsed with tap water to eliminate contaminants at 4°C, and then stored at −40°C until use. Alcalase 2.4 L was purchased from Novozymes. (Bagsværd, Denmark). ACE (1 unit), mushroom tyrosinase, α-tocopherol, radical testing reagents including 1,1-diphenyl-2-pycryl-hydrazyl (DPPH), $FeSO_4 \cdot 7H_2O$, H_2O_2, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS) and peroxidase were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other used reagents were of analytical grade.

Preparation of enzymatic squid meat hydrolysate

The enzymatic squid hydrolysate with Alcalase was prepared under the optimal hydrolysis conditions determined in a previous study (20): a pH of 7.0, a hydrolysis time of 5.9 h, and a temperature of 55°C, and an enzyme/substrate ratio of 2.4%.

DPPH radical scavenging activity

DPPH radical scavenging activity of the enzymatic squid hydrolysate was analyzed according to the method of Heo et al. (21). One mL of enzymatic squid meat hydrolysate solution was mixed with 4 mL of 0.15 mM DPPH solution in ethanol. After 30 min at room temperature, the absorbance was determined at 516 nm with an UV-VIS spectrophotometer (V-300, JASCO Inc., Tokyo, Japan). DPPH radical scavenging activity was expressed as the degree of radical reduction by the absorbance.

Hydroxyl radical scavenging activity

Hydroxyl radical scavenging activity was determined according to the method of Heo et al. (21). Hydroxyl radical was activated by the Fenton reaction in the presence of $FeSO_4 \cdot 7H_2O$. For each tube, 0.2 mL of 10 mM $FeSO_4 \cdot 7H_2O$, 10 mM EDTA and 10 mM 2-deoxyribose were mixed with 0.2 mL of the squid meat hydrolysate. Then, 0.1 M phosphate buffer (pH 7.4) was added to obtain a 1.8 mL final volume, and 0.2 mL of 10 mM H_2O_2 was added, and the mixture incubated at 37°C for 4 h. After incubation, 1 mL of 2.8% trichloroacetic acid (TCA) and 1 mL of 1.0% thiobarbituric acid (TBA) were added to each tube. Then, the mixture was placed in a boiling water bath for 10 min. Absorbance was detected at 532 nm. Hydroxyl radical scavenging activity was expressed as the degree of radical reduction by the absorbance.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide scavenging activity was determined according to the method of Heo et al. (21). One hundred μL of 0.1 M phosphate buffer (pH 5.0) and 100 μL of sample solution were mixed in a 96 microwell plate. Twenty μL of hydrogen peroxide was added to the mixture, and then incubated at 37°C for 5 min. After the incubation, 30 μL of 1.25 mM ABTS and 30 μL of peroxidase (1 unit/ml) were added to the mixture and incubated at 37°C for 10 min. The absorbance was detected at 405 nm with an ELISA reader (EL-800, BioTek Instruments, Inc., Winooski, VT, USA). The hydrogen peroxide radical scavenging activity was expressed as the degree of radical reduction by the absorbance.

ACE inhibitory activity

ACE inhibitory activity was determined according to the method of Miguel et al. (22). One hundred μL of squid hydrolysate was incubated with 100 μL of 0.1 M borate buffer (pH 8.3) containing 0.3 M NaCl and 5 mM hippuryl-histidyl-leucine (Sigma Chemical Co.). Twenty μL of ACE (2 mU) was added and then incubated at 37°C for 30 min. The reaction was stopped by adding 150 μL of 1 M HCl. The hippuric acid was extracted with 1,000 μL of ethyl acetate. After ethyl acetate was evaporated, hippuric acid was dissolved in 800 μL of distilled water and the absorbance at 228 nm was measured. ACE inhibitory activity was expressed as the degree of hippuric acid reduction at 228 nm by the absorbance.

Tyrosinase inhibitory activity

Tyrosinase inhibitory activity was determined according to the slightly modified method of Chang et al. (23). Briefly, 880 μL of 2 mM substrate (L-DOPA dissolved in 50 mM phosphate buffer, pH 6.8) was mixed with 100 μL of the sample in dimethyl sulfoxide (DMSO) at 25°C

for 2 min. Then, 20 μL of tyrosinase (1,000 U/mL in phosphate buffer, pH 6.8) was added to initiate the reaction. The mixture was incubated at 25°C for 10 min. The increase in absorbance at 475 nm due to the formation of dopachrome was monitored by a spectrophotometer (V-300, JASCO). The inhibition of tyrosinase activity was calculated using the following equation:

$$\% \text{ Inhibition} = \frac{A-B}{A} \times 100$$

where A was the absorbance at 475 nm with DMSO instead of sample and B was the absorbance at 475 nm with the sample.

Statistical analysis

Statistical analysis of the data was carried out using ANOVA, followed by Duncan's multiple comparison test, with a *P*-value of <0.05 set as the level of significance using the SPSS software package (version 11.0, SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Antioxidant activity

It is well known that the radical system used for antioxidant evaluation may influence the experimental results. Hence, two or more radical systems are required to investigate the radical scavenging capacities of a selected antioxidant (21).

DPPH radical scavenging activity

DPPH has been commonly used in the analysis of antioxidant activity for the primary characterization of the potential scavenging ability of natural compounds. In this study, DPPH radical scavenging ability of squid meat hydrolysate increased in a concentration-dependent manner up to 1.6 mg/mL (Fig. 1A). At 1.6 mg/mL, the squid meat hydrolysate scavenged DPPH radical by 91%. However, its IC_{50} value of 311 μg/mL against DPPH radical was higher than the IC_{50} value of 14.9 μg/mL of α-tocopherol, a positive control, but much lower than the value of 2.62 mg/mL of tetrapeptide (Tyr-pro-Pro-Ala-Lys) purified from blue mussel protein hydrolysate (24). Alemán et al. (25) reported that the glycosylated peptides that existed in squid gelatin hydrolysate contributed to its antioxidant activity. Therefore, the glycosylated peptides in squid meat hydrolysate might be involved in its DPPH radical scavenging activity.

Hydroxyl radical scavenging activity

The biological activity of hydroxyl radical is very strong,

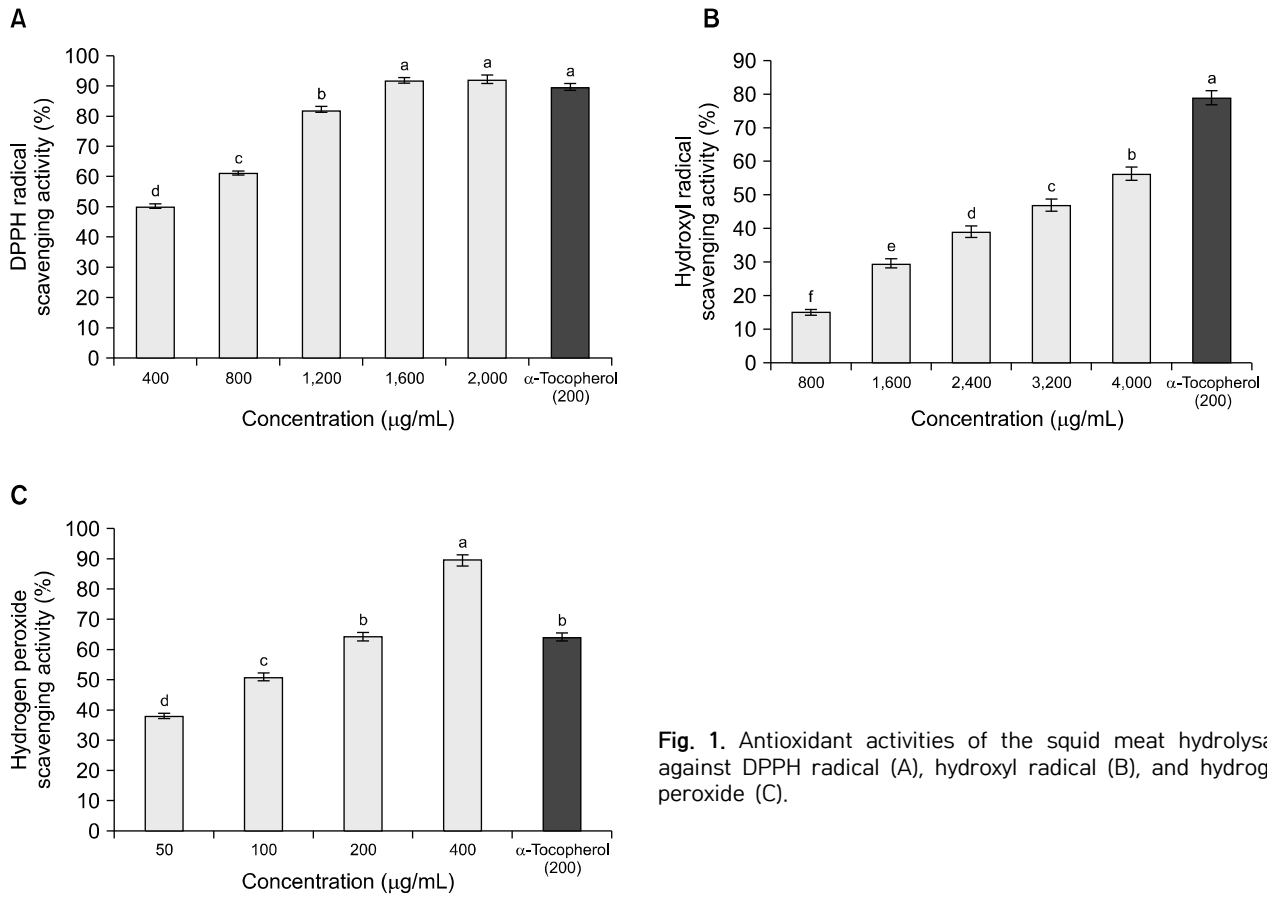

Fig. 1. Antioxidant activities of the squid meat hydrolysate against DPPH radical (A), hydroxyl radical (B), and hydrogen peroxide (C).

and it easily reacts with biomolecules such as amino acids, proteins, and DNA (6,26). Therefore, the removal of the hydroxyl radical is probably one of most expected effects for the extension of food storage period and for the defense of a living body against various diseases. The hydroxyl radical is the most reactive free radical and can be formed from superoxide anion and hydrogen peroxide in the presence of metal-ions such as copper or iron, and it is involved in initiating lipid peroxidation reactions. Therefore, the determination of the hydroxyl radical scavenging activity provides useful information on antioxidant activity (27). The hydroxyl radical scavenging activity of squid meat hydrolysate increased in a concentration-dependent manner (Fig. 1B). Its IC_{50} value on the hydroxyl radical was 3,410 μg/mL, which was much higher than the 120 μg/mL of α-tocopherol (Table 1) and the 228 μg/mL of tetrapeptide (Tyr-pro-Pro-Ala-Lys) purified from blue mussel protein hydrolysate (24). It was reported that the hydroxyl radical scavenging ability of protein hydrolysates might be due to peptides derived from enzymatic hydrolysis of fish protein (3,9). Therefore, the hydroxyl radical scavenging activity of squid meat hydrolysate might be due to its peptides produced by Alcalase, a commercial protease.

Hydrogen peroxide radical scavenging activity

Hydrogen peroxide is also involved in the oxidation of foods and in the human body. Hence, hydrogen peroxide radical scavenging ability is normally combined with DPPH radical scavenging ability to determine antioxidant ability of compounds. The hydrogen peroxide scavenging activity of squid meat hydrolysate also increased in a dose-dependent manner (Fig. 1C). At 200 μg/mL, the squid meat hydrolysate scavenged hydrogen peroxide by 64.27%, which was almost the same as the 64.11% of α-tocopherol, a positive control at the same concentration. Furthermore, its IC_{50} value, 111.5 μg/mL, was much lower than the 182.4 μg/mL of t α-tocopherol, a positive control. According to the study of Chalamaiah et al. (2), the antioxidant activity of the peptides is highly dependent on the amino acid composition and their sequences. In addition, hydrophobic amino acids and one or more residues of His, Pro, Met, Cys, Tyr, Trp, and Phe are believed to enhance the activities of the antioxidant peptides (28). In a previous study (20), the hydrophobic amino acid content (45.5%) of squid meat hydrolysate was also higher than the hydrophilic amino acid content (33.0%). Therefore, hydrophobic amino acids contributed to the antioxidant activity of squid meat hydrolysate because both hydrophobic amino acids and hydrophobic amino acid-containing peptides had chelating and lipid radical trapping abilities (26). Therefore, it was concluded that the antioxidant ability of squid hydrolysate was related to its amino acid composition.

ACE inhibitory activity

ACE is related to hypertension and congestive heart failure. Hence, ACE inhibitor is very effective to help prevent these diseases. The ACE inhibitory activity of enzymatic squid meat hydrolysate increased in a concentration-dependent manner (Fig. 2). Over 90% of ACE was inhibited beyond 320 μg of squid meat hydrolysate. The IC_{50} value of squid meat hydrolysate on ACE was 145.1 μg/mL, which was lower than the 2.45 mg/mL of oyster sauce (15), the 330 μg/mL of HSSG-III with <2 kDa fractionated from peptic squid gelatin hydrolysate (5), but much higher than the 24.7 μM (14.0 μg/mL) of pentapeptide (Met-Leu-Leu-Cys-Ser) purified from a sea squirt (16), and the 9.1, 10.77, and 7.72 μM of three different octa, deca, and septa peptides purified form salmon protein hydrolysate by Alcalase, respectively (17). ACE inhibitors are known to contain one or more zinc-binding ligands, a hydrogen-bond donor and carboxyl terminal groups (29). In addition, Trp, Tyr, Phe, Pro, and hydrophobic amino acids were the preferred amino

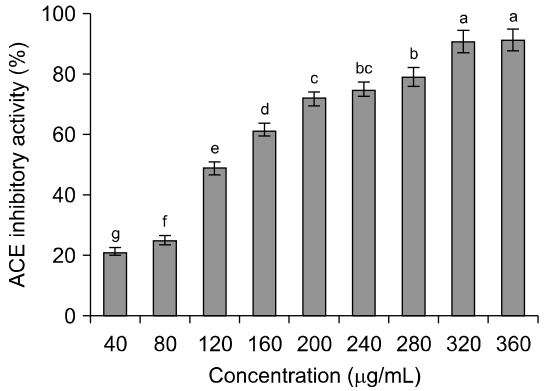

Fig. 2. ACE inhibitory activity of the squid meat hydrolysate.

Table 1. Inhibitory activities of the squid meat hydrolysate against oxidative radicals, ACE, and tyrosinase

Sample	IC_{50} value (μg/mL)				
	DPPH radical	Hydroxyl radical	Hydrogen peroxide	ACE	Tyrosinase
Squid hydrolysate	311.0[a]	3,410[a]	111.5[b]	145.1	>4,000[a]
α–Tocopherol	14.9[b]	120[b]	182.4[a]	–	–
Kojic acid	–	–	–	–	221[b]

[a,b]Means in the same column with different superscripts are significantly different ($P<0.05$).

acids at the C-terminal residue and thus contributed to the ACE inhibitory activity via the interaction between three subsites at the active site of ACE (30). The ACE inhibitory activities of hydrolysates might be different depending on the type or composition of proteins existing in raw materials, the type of hydrolyzing enzymes, and hydrolysis conditions. Hence, the variety of peptides with various amino acid sequences might result in different ACE inhibitory activities. However, the ACE inhibitory activity in this study compared to the results of other studies could not be completely explained due to different protein sources. Therefore, the relationship between chain length and bioactive properties of peptides together with the type of peptides should be determined to investigate the mechanism of ACE inhibition by protein hydrolysate because the antihypertensive peptides are usually in the range of 2-10 amino acid units (31). It is also reported that the presence of Leu and His residues in the peptide sequence plays an important role in antioxidant and ACE inhibitory activity (25).

Tyrosinase inhibitory activity

Tyrosinase, a copper-containing mixed-function oxidase, is widely distributed in microorganisms, plants and animals including humans, and it is involved in enzymatic browning. Recently, tyrosinase inhibitors have received attention because skin whitening can be achieved by inhibiting this enzyme. The tyrosinase inhibitory mechanism is related to the chelating action with the copper atom in the active site of tyrosinase (6). Among amino acids, cysteine was established as the best tyrosinase inhibitor (6). In a previous study (20), the cysteine concentration of free and compositional amino acid contents of squid meat hydrolysate were 2.2% and 0.1%, respectively. Therefore, cysteine might be an important tyrosinase inhibitor in squid meat hydrolysate. The tyrosinase inhibitory activity of enzymatic squid meat hydrolysate increased in a dose-dependent manner (Fig. 3). However, its IC_{50} value, 4,720 μg/mL, was much higher than the 221 μg/mL of kojic acid, a positive control (Table 1). However, in this study, the tyrosinase inhibitory activity (48.4%) of squid meat hydrolysate at 4 mg/mL was much higher than the 21.7%, 53.9% and 47.2% at 5 mg/mL of three fractions derived from jellyfish collagen hydrolysate by trypsin (32). A moderately low tyrosinase inhibition of squid meat hydrolysate might be an advantage because too strong inhibition of this enzyme can cause rickets or malignant melanoma by over-expression under sunlight (18). The potential of squid meat hydrolysate as a cosmetic ingredient can be considerable to control the skin whitening effects without side-effects and to properly maintain the quality of whitening cosmetics. In order to evaluate the potential of squid meat hydrolysate as an ingredient for whitening cosmetics, more study is needed to consider actual processes and quality control of cosmetic products.

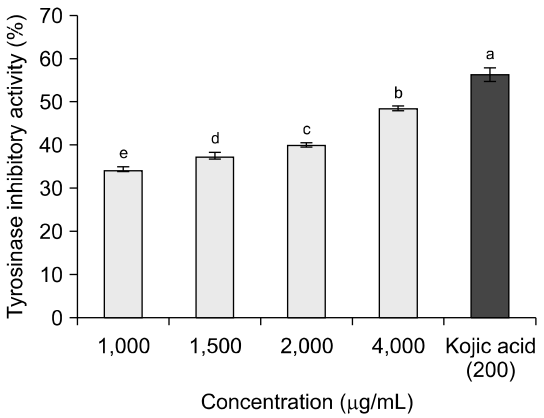

Fig. 3. Tyrosinase inhibitory activity of the squid meat hydrolysate.

CONCLUSIONS

Squid meat hydrolysate was evaluated as a value-added ingredient due to its antioxidants properties, ACE and tyrosinase inhibition. Squid meat hydrolysate exhibited strong antioxidant activity on DPPH radical and hydrogen peroxide. Especially, the hydrogen peroxide scavenging ability of squid meat hydrolysate was much higher than that of α-tocopherol. Furthermore, ACE inhibitory activity of squid meat hydrolysate was very strong compared to other hydrolysates. Tyrosinase inhibitory activity was also moderate. Therefore, squid hydrolysate can be used as a functional ingredient in foods including seasonings, breads, noodles, and beverages or in cosmetics.

ACKNOWLEDGEMENTS

This reserach was supported by the 2014 Subsidy for Scientific Research of Gangneung-Wonju National University, Korea.

AUTHOR DISCLOSURE STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Kim KT, Kang OJ. 2005. Effects of carboxymethyl chitosan on yield and whey protein loss in cottage cheese. *J Food Sci Nutr* 10: 231-238.
2. Chalamaiah M, Dinesh Kumar B, Hemalatha R, Jyothirmayi T. 2012. Fish protein hydrolysates: proximate composition, amino acid composition, antioxidant activities and applica-

tions: a review. *Food Chem* 135: 3020-3038.
3. Jun SY, Park PJ, Jung WK, Kim SK. 2004. Purification and characterization of an antioxidative peptide from enzymatic hydrolysate of yellowfin sole (*Limanda aspera*) frame protein. *Eur Food Res Technol* 219: 20-26.
4. Matsui T, Yukiyoshi A, Doi S, Sugimoto H, Yamada H, Matsumoto K. 2002. Gastrointestinal enzyme production of bioactive peptides from royal jelly protein and their antihypertensive ability in SHR. *J Nutr Biochem* 13: 80-86.
5. Lin L, Lv S, Li B. 2012. Angiotensin-I-converting enzyme (ACE)-inhibitory and antihypertensive properties of squid skin gelatin hydrolysates. *Food Chem* 131: 225-230.
6. Chang TS. 2009. An updated review of tyrosinase inhibitors. *Int J Mol Sci* 10: 2440-2475.
7. Shahidi F, Han XQ, Synowiecki J. 1995. Production and characteristics of protein hydrolysates from capelin (*Mallotus villosus*). *Food Chem* 53: 285-293.
8. Maillard MN, Soum MH, Boivin P, Berset C. 1996. Antioxidant activity of barley and malt: relationship with phenolic content. *LWT-Food Sci Technol* 29: 238-244.
9. Park PJ, Jung WK, Nam KS, Shahidi F, Kim SK. 2001. Purification and characterization of antioxidative peptides from protein hydrolysate of lecithin-free egg yolk. *J Am Oil Chem Soc* 78: 651-656.
10. Ames BN, Shigenaga MK, Hagen TM. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. *Proc Natl Acad Sci USA* 90: 7915-7922.
11. Sathivel S, Bechtel PJ, Babbitt J, Smiley S, Crapo C, Reppond KD, Prinyawiwatkul W. 2003. Biochemical and functional properties of herring (*Clupea harengus*) byproduct hydrolysates. *J Food Sci* 68: 2196-2200.
12. Wu HC, Chen HM, Shiau CY. 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Res Int* 36: 949-957.
13. Erdös EG. 1975. Angiotensin-I converting enzyme. *Circ Res* 36: 247-255.
14. Byun HG, Kim SK. 2001. Purification and characterization of angiotensin I converting enzyme (ACE) inhibitory peptides from Alaska pollack (*Theragra chalcogramma*) skin. *Process Biochem* 36: 1155-1162.
15. Je JY, Park JY, Jung WK, Park PJ, Kim SK. 2005. Isolation of angiotensin I converting enzyme (ACE) inhibitor from fermented oyster sauce, *Crassostrea gigas*. *Food Chem* 90: 809-814.
16. Ko SC, Kang MC, Lee JK, Byun HG, Kim SK, Lee SC, Jeon BT, Park PJ, Jung WK, Jeon YJ. 2011. Effect of angiotensin I-converting enzyme (ACE) inhibitory peptide purified from enzymatic hydrolysates of *Styela plicata*. *Eur Food Res Technol* 233: 915-922.
17. Ahn CB, Jeon YJ, Kim YT, Je JY. 2012. Angiotensin I converting enzyme (ACE) inhibitory peptides from salmon byproduct protein hydrolysate by Alcalase hydrolysis. *Process Biochem* 47: 2240-2245.
18. Schroeder HA, Adames MH. 1941. The effect of tyrosinase on experimental hypertension. *J Exp Med* 73: 531-550.
19. Hasegawa T. 2010. Tyrosinase-expressing neuronal cell line as *in vitro* model of Parkinson's disease. *Int J Mol Sci* 11: 1082-1089.
20. Choi JH, Kim KT, Kim SM. 2014. Optimization and biochemical characteristics of an enzymatic squid hydrolysate for manufacture of a squid complex seasoning. *Food Sci Biotechnol* 23: 417-423.
21. Heo SJ, Cha SH, Lee KW, Cho SK, Jeon YJ. 2005. Antioxidant activities of chlorophyta and phaeophyta from Jeju island. *Algae* 20: 251-260.
22. Miguel M, Contreras MM, Recio I, Aleixandre A. 2009. ACE-inhibitory and antihypertensive properties of a bovine casein hydrolysate. *Food Chem* 112: 211-214.
23. Chang TS, Ding HY, Lin HC. 2005. Identifying 6,7,4'-trihydroxyisoflavone as a potent tyrosinase inhibitor. *Biosci Biotech Biochem* 69: 1999-2001.
24. Wang B, Li L, Chi CF, Ma JH, Luo HY, Xu YF. 2013. Purification and characterisation of a novel antioxidant peptide derived from blue mussel (*Mytilus edulis*) protein hydrolysate. *Food Chem* 138: 1713-1719.
25. Alemán A, Giménez B, Pérez-Santin E, Gómez-Guillén MC, Montero 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.
26. Peña-Ramos EA, Xiong YL. 2002. Antioxidant activity of soy protein hydrolysates in a liposomal system. *J Food Sci* 67: 2952-2956.
27. Iyengar R, McEvily AJ. 1992. Anti-browning agents: alternatives to the use of sulfites in foods. *Trends Food Sci Technol* 3: 60-64.
28. Udenigwe CC, Aluko RE. 2011. Chemometric analysis of the amino acid requirements of antioxidant food protein hydrolysates. *Int J Mol Sci* 12: 3418-3161.
29. Andrews PR, Carson JM, Caselli A, Spark MJ, Woods R. 1985. Conformational analysis and active site modelling of angiotensin-converting enzyme inhibitors. *J Med Chem* 28: 393-399.
30. Cushman DW, Cheung HS. 1971. Spectrophotometric assay and properties of the angiotensin-converting enzyme of rabbit lung. *Biochem Pharmacol* 20: 1637-1648.
31. Barba de la Rosa AP, Montoya AB, Martínez-Cuevas P, Hernández-Ledesma B, León-Galván MF, De León-Rodríguez A, González C. 2010. Tryptic amaranth glutelin digests induce endothelial nitric oxide production through inhibition of ACE: Antihypertensive role of amaranth peptides. *Nitric Oxide* 23: 106-111.
32. Zhuang YL, Zhao X, Li BF. 2009. Optimization of antioxidant activity by response surface methodology in hydrolysates of jellyfish (*Rhopilema esculentum*) umbrella collagen. *J Zhejiang Univ Sci B* 10: 572-579.