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Preparation of Antioxidative Peptides from Spanish Mackerel (*Scomberomorus niphonius*) Processing Byproducts by Enzymatic Hydrolysis

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ABSTRACT

Marine fish processing byproducts were considered as potential good protein resource for producing bioactive peptides by enzymatic hydrolysis. In this study, preparation and partial characteristics of the antioxidative peptides from Spanish mackerel (*Scomberomorus*) processing byproducts fish frame by commercial proteases were investigated. The results showed that the mackerel frame hydrolysate with alcalase had the highest degree of hydrolysis and DPPH (1,1-diphenyl-2-pycryl-hydrazyl) radical scavenging activity, the values of 31.3 and 18.5%, respectively. The alcalase hydrolysate was ultrafiltrated into four fractions and the fraction with lower molecular weight had higher DPPH radical scavenging capacity. The fraction F4 with molecular weight less than 3 kDa had the highest DPPH radical scavenging capacity of 27.7%. The molecular weight distribution of fraction F4 showed that they were mainly consisted of less than 1 kDa small peptides and free amino acids. The small peptides in fraction F4 were mainly dipeptide to nonapeptide, especially tripeptide to hexapeptide.

Key words: Spanish mackerel, *Scomberomorus niphonius*, fish frame, DPPH radical scavenging activity, enzymatic hydrolysis

INTRODUCTION

Oxidative metabolism, which produces free radicals and other reactive oxygen species, is essential for survival of cells. These free radicals play important roles in providing defense against infections and signal roles (Hancock *et al*., 2001; Johansen *et al*., 2005; Valko *et al*., 2007). However, excessive amount of free radicals can result in cell oxidative damage, which causes body ageing and initiates some diseases including atherosclerosis, arthritis, diabetes and cancer by oxidation of membrane phospholipids, cellular proteins damage, mutations in DNA, modification of low density lipoproteins and inactivation of enzymes thus shutting down cellular process (Halliwell, 1994; Jadhav *et al*., 1995). The control of oxidative stress or scavenging these excessive free radicals seems to be benefit for reducing or preventing the risk of these diseases. Recently, a lot of researches of antioxidant were reported for scavenging free radicals. Among these antioxidants, food-derived small peptides are one of the research topics, such as antioxidative peptides from milk protein (Pihlanto, 2006), bullfrog skin (Qian *et al*., 2008), porcine collagen (Lertittikul *et al*., 2007), round scad (Thiansilakul *et al*., 2007) and squid muscle (Rajapakse *et al*., 2005). These natural food derived antioxidative peptides possessed higher safety than synthetic antioxidant such as Butylated Hydroxy Anisole (BHA), Butylated Hydroxy Toluene (BHT), Tert-Butyl Hydroquinone (TBHQ) and Propyl Gallate (PG). Enzymatic hydrolysis is one of the most wildly used methods for preparing antioxidative peptides from food derived proteins. In recent years, many marine-derived processing byproducts proteins were selected to produce antioxidative peptides, such as yellowfin sole skin or frame

(Kim *et al*., 1996; Jun *et al*., 2004), Alaska pollack skin (Kim *et al*., 2001), cod frame (Jeon *et al*., 1999) and silver carp (Zhong *et al*., 2011), threadfin bream surimi (Wiriyaphan *et al*., 2013), sardinelle (Bougatef *et al*., 2010), tuna dark muscle (Hsu, 2010).

Spanish mackerel (*Scomberomorus niphonius*) is marine fish belonging to *Scombridae* (Percida) (Terada *et al*., 2007), which is extremely perishable and lower commercial value when compared to other marine fishes. Mackerel processing produces many byproducts, including frame, skin, head, viscera and others which occupy about 50% of total weight and mainly are used for animal feeds (Cho *et al*., 2014). However, these processing byproducts are enriched in protein and maybe valuable source of bioactive peptides or functional foods ingredients. A few functional components had been reported from various mackerel processing byproducts, such as antibacterial peptides (Ennaas *et al*., 2015), rhamnose-binding glycoprotein (Terada *et al*., 2007), iron-binding peptides (Wang *et al*., 2013), fish oil (Sahena *et al*., 2010), gelatin (Khiari *et al*., 2011) and polyunsaturated fatty acids (Zuta *et al*., 2003). So far, the antioxidative peptides from Spanish mackerel processing byproducts frame by enzymatic hydrolysis have not been widely studied. Investigation on antioxidative peptides from these processing byproducts will be benefit of high value utilization of waste proteins. Therefore, we investigated preparation of antioxidative peptides by enzymatic hydrolysis from Spanish mackerel processing byproducts frame and molecular weight distributions of hydrolysate.

MATERIALS AND METHODS

Materials: The spanish mackerel processing byproducts frame were obtained from Dongtou Fisheries Co., Ltd. (Wenzhou, China). The fish frame was transported to lab with ice and kept frozen at -20°C until used. The frame was thawed at 4°C for 24 h and then washed by running water. The washed frame was drained.

The trypsin (from bovine pancrease, type II) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Alcalase, protemax and flavourzyme were purchased from Novozymes (China) Biotechnology Co., Ltd. (Tianjin, China). The 1,1-diphenyl-2-pycryl-hydrazyl (DPPH), bovine serum albumin, cytochrome c from bovine heart, trypsin inhibitor from *Glycine max* (soybean) and vitamin B_{12} were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO). A hepta peptide with molecular weight of 804 Da was synthesized. All chemicals and reagents were of the highest grade available.

Enzymatic hydrolysis of mackerel frame: The drained frame was cut into small sections and grinded into homogenate with three folds volume of isopropanol. Then the homogenate mixture was incubated at 60°C for 4 h and the filer residue was dried under vacuum at 80°C. The dried sample was smashed, filtrated by 150 μm mesh and the defatted frame powder was achieved.

Ten grams of defatted mackerel frame powder were added to 100 mM Tris-HCl buffer (10%, w/v) at various pH (8.5, 8.5, 7.0 and 7.0) and hydrolyzed with four proteinases (alcalase, trypsin, protemax and flavourzyme) at 400 U g^{-1} powder at optimal temperature (55, 37, 50 and 50°C, respectively) for 2 h. The hydrolysis process was stopped by heating the hydrolysate at boiling water for 10 min and the hydrolysate was centrifuged at 10000 rpm for 30 min at 4°C. The supernatants were stored at -20°C until further use. The peptide content was determined by Folin-phenol method with bovine serum albumin as a standard (Lowry *et al*., 1951). Degree of hydrolysis was determined by Adler-Nissen (1979).

Ultrafiltration of hydrolysate: The hydrolysate was first filtered with a 0.22 μm membrane and then ultrafiltered successively at 4°C by three ultrafiltration with molecular weight cut-off membrane of 10, 5 and 3 kDa, respectively. And four fractions (F1-F4) were collected and the peptides content and DPPH radical scavenging activity were measured. The samples were freeze-dried and stored at -20°C until further use.

Determination of antioxidative activity: The DPPH radical scavenging activity was used to evaluate the antioxidative activity of hydrolysate. The DPPH radical scavenging activity was measured according to the method of Wu *et al.* (2003) with slight modification. All samples were diluted to solution with 1.0 mg mL^{-1} content of peptides. An aliquot of sample (0.2 mL) was mixed with 1.3 mL of distilled water and then added to 2.0 mL of 0.10 mM DPPH in alcohol. The solution was mixed vigorously and kept in dark at room temperature for 30 min. The absorbance was monitored at 517 nm. The control was determined by the same method but 0.2 mL distilled water was used instead of sample. The DPPH radical scavenging activity was calculated by following equation:

$$\text{DPPH radical scavenging activity}\,(\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

where, $A_{control}$ and A_{sample} were the absorbance of control and sample, respectively.

Measurement of molecular weight distribution: The fraction with highest DPPH radical-scavenging activity was further used for measuring the molecular weight by gel-filtration HPLC with a gel column (Waters Stragel HT5, 4.6×300 mm). The mobile phase was 40% (v/v) acetonitrile including 0.1% (v/v) trifluoroacetic acid. The flow rate was 1.0 mL min^{-1} and the elution was monitored at 214 nm. The bovine serum albumin (66.7 kDa), trypsin inhibitor (20.1 kDa), cytochrome c (12.3 kDa), vitamin B_{12} (1355 Da) and hepta peptide (804 Da) were used for molecular weight standard.

Statistical analysis: All data presented was the Mean±Standard error of three determinations. Statistical

comparisons were performed by one-way analysis of variance (ANOVA) and the differences were calculated by Duncan's multiple range test. Statistical significance was measured at $p<0.05$.

RESULTS AND DISCUSSION

Preparation of antioxidative peptides: The four general commercial proteases, including alcalase, trypsin, protemax and flavourzyme were used for producing hydrolysates with high DPPH free radical scavenging activity. As shown in Fig. 1, among the hydrolysates resulting from four commercial proteases, the highest DPPH free radical scavenging activity was appeared in the alcalase and trypsin hydrolysates with 18.5 and 17.4%, respectively. However, the alcalase hydrolysate had higher degree of hydrolysis (31.3%) than trypsin hydrolysate (24.5%). Alcalase was one of the most effective commercial enzymes for producing antioxidative peptides from marine protein, such as yellow stripe trevally (Klompong *et al*., 2007), silver carp (Dong *et al*., 2008), shrimp processing byproduct (Zhao *et al*., 2013), Patin *Pangasius sutchi* sarcoplasmic protein round scad (*Decapterus maruadsi*) muscle protein (Jiang *et al*., 2014), skipjack (*Katsuwana pelamis*) roe (Intarasirisawat *et al*., 2013), tuna dark muscle protein (Hsu, 2010) and so on. Therefore, alcalase was selected for further producing antioxidative peptides from mackerel frame.

The enzymatic hydrolysates of mackerel frame protein are prepared by alcalase at pH 8.5 and 55°C. During enzymatic hydrolysis, the degree of hydrolysis and DPPH free radical scavenging activity were determined every 20 min and the result was shown in Fig. 2. It showed that degree of hydrolysis increased step by step during the first 100 min and then kept at a high level after 100 min. However, the DPPH free radical scavenging activity was increased during alcalase hydrolysis till to 160 min. The highest DPPH free radical scavenging activity reached to 20.2% at hydrolysis time of 160 min. Combination with degree of hydrolysis and antioxidative activity, to some extent, the DPPH free radical scavenging activity was related to degree of hydrolysis. Liu *et al.* (2010) reported that the porcine plasma protein hydrolysate by alcalase had high antioxidant activity at high degree of hydrolysis. Also, You *et al.* (2009) indicated that the antioxidative activity of loach protein hydrolysates were affected significantly by degree of hydrolysis. Therefore, the alcalase hydrolysate for 160 min was selected for further isolation and characteristic studies.

Ultrafiltration of hydrolysate: The bioactivity of protein enzymatic hydrolysate was affected significantly by molecular weight of peptides in the hydrolysate (Guo *et al*., 2009). Ultrafiltration was generally used to separate the protein hydrolysate into various molecular weight ranges fractions. In this experiment, three common molecular weight cut-off membrane of 10, 5 and 3 kDa were used for fractioning the mackerel frame alcalase hydrolysate. The hydrolysate was separated into four different molecular weight peptides

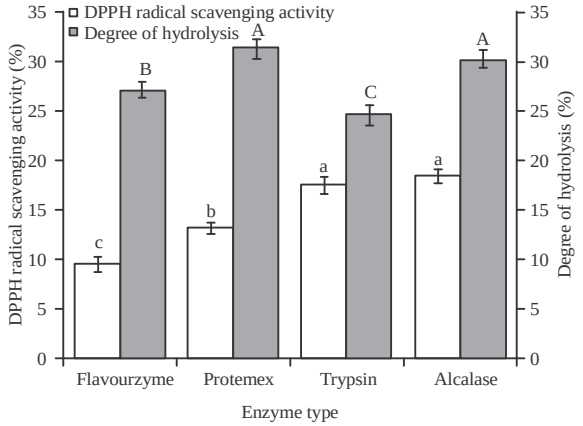

Fig. 1: Effects of protease type on degree of hydrolysis and antioxidative activity of mackerel frame protein hydrolysate. Each observation is a mean of three replicate experiments (n = 3) and the values with different letters differ significantly at $p<0.05$

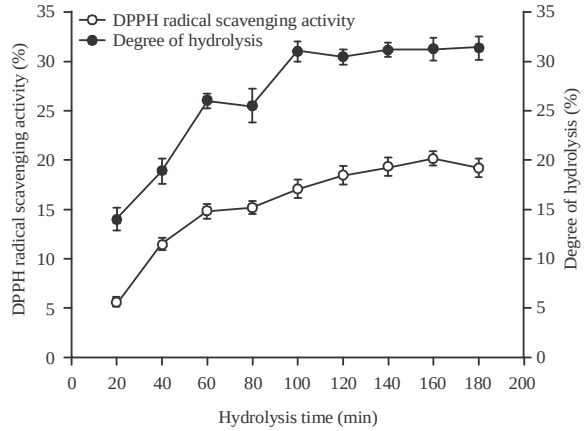

Fig. 2: Effects of hydrolysis time on degree of hydrolysis and antioxidative activity of mackerel frame protein by alcalase

fractions as F1 (>10 kDa), F2 (5~10 kDa), F3 (3~5 kDa) and F4 (<3 kDa), respectively. The peptides content and DPPH radical-scavenging activity of four fractions were determined and results were shown in Table 1. As shown in Table 1, F4 fraction occupied 29.0% of total peptides content in the mackerel frame alcalase hydrolysate and also it had the highest DPPH radical-scavenging activity of 27.7%, which was 1.82, 1.65 and 2.25 times higher than that of fraction F1, F2 and F3, respectively. A lot of researches had improved that antioxidative activity of hydrolysate was related to molecular weight of peptides in hydrolysate. Many other original protein hydrolysate with molecular weight less than 3 kDa showed higher antioxidant activity than other fractions, such as from chickpea protein (Li *et al*., 2008), wheat germ protein (Zhu *et al*., 2006), Silver carp (Dong *et al*., 2008), Patin sarcoplasmic protein grass carp muscle (Ren *et al*., 2008) and patin myofibrillar protein (Najafian *et al*., 2013).

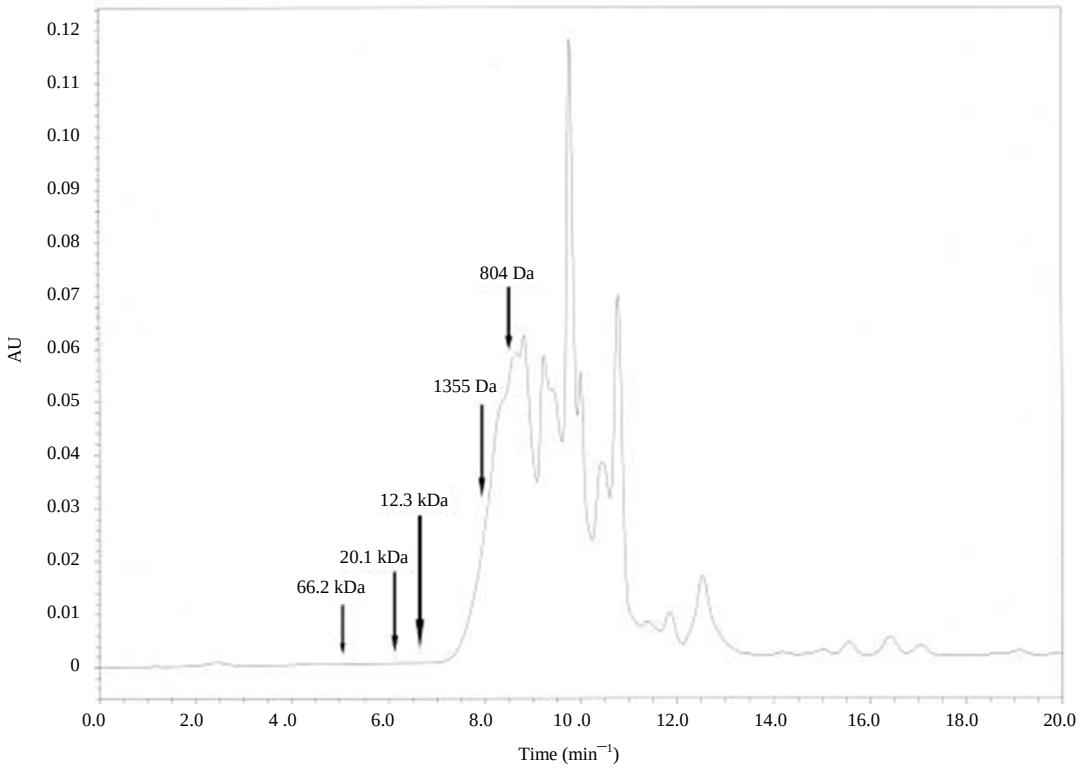

Fig. 3: Molecular weight distribution of mackerel frame antioxidative peptides. The bovine serum albumin (66.7 kDa), trypsin inhibitor (20.1 kDa), cytochrome c (12.3 kDa), vitamin B_{12} (0.1355 Da) and hepta peptide (804 Da) were used for molecular weight standard.

Table 1: Effects of ultrafiltration on antioxidative activity of mackerel frame alcalase hydrolysate

	Fractions			
Items	F1	F2	F3	F4
Molecular weight range (kDa)	>10	5~10	3~5	<3
Peptide contents (mg mL^{-1})	6.68±0.16^{a}	5.32±0.24^{b}	3.12±0.19^{c}	0.56±0.11^{d}
Total volume (mL)	23	34	61	382
Total peptides ratio (%)	20.8	24.5	25.8	29.0
DPPH radical scavenging activity (%)	15.2±1.2^{b}	16.8±0.7^{b}	12.3±0.8^{c}	27.7±1.3^{a}

Each observation is a mean of three replicate experiments (n = 3) and the values with different lowercase differ significantly by the same row at $p<0.05$

Molecular weight distribution of antioxidative peptides: The molecular weight distribution of the fraction F4 with the highest DPPH radical-scavenging activity was determined using size exclusion chromatography, shown in Fig. 3. The result showed that the molecular weight of most peptides in fraction F4 were smaller than 1.0 kDa. The average weight of twenty amino acids commonly found in protein was 110 kDa. Therefore, the fraction F4 with the highest DPPH radical-scavenging activity was consisted of free amino acids and small peptides (dipeptide to nonapeptide, especially tripeptide to hexapeptide). This result was similar that other original marine protein hydrolysate with small molecular weight had higher antioxidative activity. Byun *et al.* (2009) reported that the molecular weight of highest antioxidative activity two peptides from marine rotifer protein hydrolysate was 1033 and 1076 Da. Ko *et al.* (2012) reported that a peptide of molecular weight 702 Da had high antioxidative activity, which was purified from marine *Chlorella ellipsoidea* protein hydrolysate. Other small peptides from marine protein hydrolysate, such as round scad (*Decapterus maruadsi*) muscle protein (Jiang *et al*., 2014), Indian squid protein (Sudhakar and Nazeer, 2015), silver carp processing by-product protein (Zhong *et al*., 2011), threadfin bream surimi byproduct protein (Wiriyaphan *et al*., 2013) and sardinelle (*Sardinella aurita*) by-products proteins (Bougatef *et al*., 2010) and tuna liver protein (Je *et al*., 2009), had been reported to possess high antioxidative activity.

CONCLUSION

Spanish mackerel processing byproduct, fish frame, had high protein content and usual was used for animal feeds. In this study, the mackerel frame was defatted and hydrolyzed with several commercial proteases and their DPPH radical scavenging activities investigated. The results suggested that alcalase was the best commercial enzyme for producing antioxidative peptides from mackerel frame protein. The small

molecular weight fraction less than 3 kDa possessed the highest DPPH radical scavenging activity. This fraction was consisted of free amino acids and small peptides (dipeptide to nonapeptide). However, further detailed information, such as *in vivo* antioxidative activities, further purification and amino acids sequence, are needed.

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REFERENCES

Adler-Nissen, J., 1979. Determination of the degree of hydrolysis of food protein hydrolysates by trinitrobenzenesulfonic acid. J. Agric. Food. Chem., 27: 1256-1262.

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

Byun, H.G., J.K. Lee, H.G. Park, J.K. Jeon and S.K. Kim, 2009. Antioxidant peptides isolated from the marine rotifer, *Brachionus rotundiformis*. Process Biochem., 44: 842-846.

Cho, S., S.Y. Kim, M. Yoon and S.B. Kim, 2014. Physicochemical profiles of chub mackerel *Scomber japonicas* bones as a food resource. Fish Aquat. Sci., 17: 175-180.

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

Ennaas, N., R. Hammami, L. Beaulieu and I. Fliss, 2015. Purification and characterization of four antibacterial peptides from protamex hydrolysate of Atlantic mackerel (*Scomber scombrus*) by-products. Biochem. Biophys. Res. Commun., 462: 195-200.

Guo, H., Y. Kouzuma and M. Yonekura, 2009. Structures and properties of antioxidative peptides derived from royal jelly protein. Food Chem., 113: 238-245.

Halliwell, B., 1994. Free radicals, antioxidants and human disease: Curiosity, cause, or consequence? Lancet, 344: 721-724.

Hancock, J.T., R. Desikan and S.J. Neill, 2001. Role of reactive oxygen species in cell signalling pathways. Biochem. Soc. Trans., 29: 345-350.

Hsu, K.C., 2010. Purification of antioxidative peptides prepared from enzymatic hydrolysates of tuna dark muscle by-product. Food Chem., 122: 42-48.

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

Jadhav, S.J., S.S. Nimbalkar, A.D. Kulkarni and D.L. Madhavi, 1995. Lipid Oxidation in Biological and Food Systems. In: Food Antioxidants: Technological, Toxicological and Health Perspectives, Madhavi, D.L., S.S. Deshpande and D.K. Salunkhe (Eds). Marcel Dekker, New York, USA., pp: 5-63.

Je, J.Y., K.H. Lee, M.H. Lee and C.B. Ahn, 2009. Antioxidant and antihypertensive protein hydrolysates produced from tuna liver by enzymatic hydrolysis. Food Res. Int., 42: 1266-1272.

Jeon, Y.J., H.G. Byun and S.K. Kim, 1999. Improvement of functional properties of cod frame protein hydrolysates using ultrafiltration membranes. Process Biochem., 35: 471-478.

Jiang, H., T. Tong, J. Sun, Y. Xu, Z. Zhao and D. Liao, 2014. Purification and characterization of antioxidative peptides from round scad (*Decapterus maruadsi*) muscle protein hydrolysate. Food Chem., 154: 158-163.

Johansen, J.S., A.K. Harris, D.J. Rychly and A. Ergul, 2005. Oxidative stress and the use of antioxidants in diabetes: Linking basic science to clinical practice. Cardiovascular Diabetol., Vol. 4. 10.1186/1475-2840-4-5

Jun, S.Y., P.J. Park, W.K. Jung and S.K. Kim, 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.

Khiari, Z., D. Rico, A.B. Martin-Diana and C. Barry-Ryan, 2011. The extraction of gelatine from mackerel (*Scomber scombrus*) heads with the use of different organic acids. J. Fish. Sci., 5: 52-63.

Kim, S.K., H.C. Lee, H.G. Byun and Y.J. Jeon, 1996. Isolation and characterization of antioxidants peptides from enzymatic hydrolysis of yellowfin sole skin gelatin. Korean J. Fish. Aquat. Sci., 29: 246-255.

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

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

Ko, S.C., D. Kim and Y.J. Jeon, 2012. Protective effect of a novel antioxidative peptide purified from a marine *Chlorella ellipsoidea* protein against free radical-induced oxidative stress. Food Chem. Toxicol., 50: 2294-2302.

Lertittikul, W., S. Benjakul and M. Tanaka, 2007. Characteristics and antioxidative activity of Maillard reaction products from a porcine plasma protein-glucose model system as influenced by pH. Food Chem., 100: 669-677.

Li, Y.H., B. Jiang, T. Zhang, W.M. Mu and J. Liu, 2008. Antioxidant and free radical-scavenging activities of Chickpea Protein Hydrolysate (CPH). Food Chem., 106: 444-450.

Liu, Q., B.H. Kong, Y.L. Xiong and X.F. Xia, 2010. Antioxidant activity and functional properties of porcine plasma protein hydrolysate as influenced by the degree of hydrolysis. Food Chem., 118: 403-410.

Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193: 265-275.

Najafian, L., M. Jafarzade, M. Said and A.S. Babji, 2013. Biochemical properties and antioxidant activity of myofibrillar protein hydrolysates obtained from patin (*Pangasius sutchi*). Int. J. Food Sci. Technol., 48: 2014-2022.

Pihlanto, A., 2006. Antioxidative peptides derived from milk proteins. Int. Dairy J., 16: 1306-1314.

Qian, Z.J., W.K. Jung and S.K. Kim, 2008. Free radical scavenging activity of a novel antioxidative peptide purified from hydrolysate of bullfrog skin, *Rana catesbeiana* Shaw. Bioresour. Technol., 99: 1690-1698.

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

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

Sahena, F., I.S.M. Zaidul, S. Jinap, M.H.A. Jahurul, A. Khatib and N.A.N. Norulaini, 2010. Extraction of fish oil from the skin of Indian mackerel using supercritical fluids. J. Food Eng., 99: 63-69.

Sudhakar, S. and R.A. Nazeer, 2015. Structural characterization of an Indian squid antioxidant peptide and its protective effect against cellular reactive oxygen species. J. Funct. Foods, 14: 502-512.

Terada, T., Y. Watanabe, H. Tateno, T. Naganuma, T. Ogawa, K. Muramoto and H. Kamiya, 2007. Structural characterization of a rhamnose-binding glycoprotein (lectin) from Spanish mackerel (*Scomberomorous niphonius*) eggs. Biochimica Biophysica Acta (BBA)-Gen. Sub., 1770: 617-629.

Thiansilakul, Y., S. Benjakul and F. Shahidi, 2007. Compositions, functional properties and antioxidative activity of protein hydrolysates prepared from round scad (*Decapterus maruadsi*). Food Chem., 103: 1385-1394.

Valko, M., D. Leibfritz, J. Moncol, M.T.D. Cronin, M. Mazur and J. Telser, 2007. Free radicals and antioxidants in normal physiological functions and human disease. Int. J. Biochem. Cell Biol., 39: 44-84.

Wang, P.F., G.R. Huang and J.X. Jiang, 2013. Optimization of hydrolysis conditions for the production of iron-binding peptides from mackerel processing byproducts. Adv. J. Food Sci. Technol., 5: 921-925.

Wiriyaphan, C., B. Chitsomboon, S. Roytrakul and J. Yongsawadigul, 2013. Isolation and identification of antioxidative peptides from hydrolysate of threadfin bream surimi processing byproduct. J. Funct. Foods, 5: 1654-1664.

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

You, L., M. Zhao, C. Cui, H. Zhao and B. Yang, 2009. Effect of degree of hydrolysis on the antioxidant activity of loach (*Misgurnus anguillicaudatus*) protein hydrolysates. Innovative Food Sci. Emerging Technol., 10: 235-240.

Zhao, J., G. Huang and J. Jiang, 2013. Purification and characterization of a new DPPH radical scavenging peptide from shrimp processing by-products hydrolysate. J. Aquat. Food Prod. Technol., 22: 281-289.

Zhong, S., C. Ma, Y.C. Lin and Y. Luo, 2011. Antioxidant properties of peptide fractions from silver carp (*Hypophthalmichthys molitrix*) processing by-product protein hydrolysates evaluated by electron spin resonance spectrometry. Food Chem., 126: 1636-1642.

Zhu, K., H. Zhou and H. Qian, 2006. Antioxidant and free radical-scavenging activities of Wheat Germ Protein Hydrolysates (WGPH) prepared with alcalase. Process Biochem., 41: 1296-1302.

Zuta, C.P., B.K. Simpson, H.M. Chan and L. Phillips, 2003. Concentrating PUFA from mackerel processing waste. J. Am. Oil Chem. Soc., 80: 933-936.