http://dx.doi.org/10.15433/ksmb.2015.7.1.019 ISSN 2383-5400 (Online)

# Characterization of an antioxidant peptide from katsuobushi (dried bonito) protein hydrolysates

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**Abstract** The objective of the current study was to evaluate the inhibitory and antioxidant activities of powdered katsuobushi (dried bonito) protein hydrolysates and their corresponding fractions. The powdered katsuobushi (dried bonito) hydrolysates were obtained by enzymatic hydrolysis using Alcalase, α-chymotrypsin, Neutrase, pepsin, papain, and trypsin. The antioxidant efficacy of the respective hydrolysates were evaluated using 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydroxyl, superoxide, and alkyl radical-scavenging activities. Among the hydrolysates, the peptic-derived hydrolysate exhibited the highest antioxidant activity compared to other enzymatic hydrolysates. Therefore, the peptic-derived hydrolysate was further analyzed, and was found to contain an active peptide with an amino acid sequence identified as Pro-Met-Pro-Leu-Asn-Ser-Cys (756 Da). The purified peptides from powdered katsuobushi (dried bonito) had an $EC_{50}$ value of 105.82 μM, and exhibited an inhibitory effect against DNA oxidation induced by hydroxyl radicals. Taken together, these results suggests that powdered katsuobushi (dried bonito) could be used as a natural antioxidant in functional foods and prevent oxidation reactions in food processing.

**Keywords :** Antioxidant, DPPH radical scavenging, Peptide, Electron spin resonance spectrometry, Powdered katsuobushi (dried bonito)

## Introduction

Reactive oxygen species (ROS), in particular the superoxide anion ($\bullet O2^-$), hydroxyl radical (•OH), and hydrogen peroxide ($H_2O_2$), are unwanted metabolic byproducts of normal aerobic metabolism [1]. Lipid and fatty acid oxidation generates free radicals, which lead to the development of undesirable off-flavors, odors, and potentially toxic reaction products [2,3,4]. In addition, oxidative stress can modify DNA, proteins, small cellular molecules, and is thought to play a significant role in the occurrence of diseases including cancer, arteriosclerosis, cardiovascular diseases, diabetes mellitus, neurological disorders and Alzheimer's disease [5,6]. However the body employs an array of antioxidant defenses that can scavenge for and convert ROS, such as free radicals, into harmless species. For example, the antioxidant defense system includes superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), and tripeptide glutathione (GSH). These enzymatic and non-enzymatic antioxidants work together to scavenge for and eliminate oxidative stress [7], and furthermore, a steady-state maintenance of the ROS: antioxidant ratio is essential for avoiding this increase in oxidizing species [8]. Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tbutylhydroquinone (TBHQ), and propyl gallate have been

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widely used to preserve food products by delaying the discoloration and deterioration caused by oxidation [9]. However, the use of these synthetic antioxidants has been limited in some countries due to potential health hazards [10]. Recently, enzymatic hydrolysis with proteases has garnered much attention in antioxidant research. Protein hydrolysates, which are the peptides resulting from enzymatic hydrolysis, affect health-related functions such as those in the antioxidant defense system [11]. As a result of research into this field, various antioxidant peptides produced through enzymatic hydrolysis have been isolated from marine organisms including the flounder fish (*Paralichthys olivaceus*) [12], *Sphyrna lewini* [13], blue mussel (*Mytilus edulis*) [14], marine *Chlorella ellipsoidea* [15], silver carp (*Hypophthalmichthys molitrix*) [16], sand eel [17], and rotifer [18]. These naturally-occurring marine organism-derived antioxidants are considered to be safer and have less adverse side effects compared to synthetic antioxidants.

Enzymatic hydrolysates exhibited several benefits when added to foods. These benefits included improved water-binding ability, myofibrillar protein heat stability, emulsifying stability, protein solubility, and the nutritional quality of foods. Moreover, enzymatic hydrolysis has become a valuable tool for modifying protein function [19]. Generally, the bioactive properties of peptides produced through enzymatic hydrolysis remain latent within the parent protein until released by hydrolysis. Most of the bioactive peptides are 2–20 amino acids long, and their primary amino acid sequences play a critical role in their bioactivity [20]. Katsuobushi (dried bonito) has a distinct umami flavor originating from the presence of glutamic and inosinic acids. More specifically, the potent flavors come from of a mixture of components such as acids, phenols, pyridines, pyrazines, and thiazoles [21,22]. katsuobushi (dried bonito) is a traditional Japanese food additive [21], and is also used widely as a flavor enhancer. It is produced by a variety of processes including boiling, sun-drying, smoking, and in mold cultures. However, no studies to date have reported on the presence of antioxidant peptides in powdered katsuobushi (dried bonito) hydrolysates. In the present study, we investigated the radical scavenging activity of enzymatically-prepared powdered katsuobushi (dried bonito) protein hydrolysates, and further isolated a potent antioxidant peptide. Moreover, we investigated the protective effect of the purified peptide against DNA oxidation induced by the hydroxyl radicals.

## Materials and Methods

### *Materials*

katsuobushi (dried bonito) was donated by Department of a seaport and traditional fish market of Jumunjin (Gangneung, Korea), and stored at −80°C until used. Various commercial enzymes, such as α-chymotrypsin, papain, pepsin and trypsin were purchased from Sigma Chemical Co. (St. Louis, MO). Alcalase and Neutrase were purchased from Novo Co. (Novo Nordisk, Bagsvaerd, Denmark). 1,1-diphenyl-2-picryl- hydrazyl (DPPH) was also purchased from Wako Chemical Co. All other reagents used in this study were reagent grade chemicals.

### *Analysis of amino acid compositions*

Amino acids were analyzed using an automatic analyzer (Hitachi Model 835-50, Japan) with a C18column(5μm, 4.6 × 250 mm, Watchers, MA). The reaction was carried out at 38°C, with the detection wave length at 254 nm and a flow rate of 1.0 mL/min. All chemical analyses (fromeachtank) were carried out in triplicate.

### *Preparation of powdered katsuobushi (dried bonito) hydrolysates*

To prepare powdered katsuobushi (dried bonito) hydrolysates, enzymatic hydrolysis was performed using various enzymes (Alcalase, α-chymotrypsin, Neutrase, papain, pepsin, and trypsin) at their optimal conditions. The powdered katsuobushi (dried bonito) was hydrolyzed separately using various enzymes with a substrate to enzyme ratio of 1:100 for 6 hr, under optimum pH and temperature conditions (Table 1). After the reaction, reactant was conducted by glass filter and lyophilized hydrolysates were stored at −80°C until use.

**Table 1.** Optimal conditions of enzymatic hydrolysis for various enzymes.

| Enzyme | Buffer | pH | Temp.(°C) |
|---|---|---|---|
| Alcalase | 50mM *SP Buffer | 7.0 | 50 |
| α-Chymotrypsin | 50mM SP Buffer | 7.0 | 37 |
| Neutrase | 50mM SP Buffer | 7.0 | 50 |
| Papain | 50mM SP Buffer | 7.0 | 37 |
| Pepsin | 20mM HCl | 2.0 | 37 |
| Trypsin | 50mM SP Buffer | 7.0 | 37 |

*SP ; Sodium phosphate

### *Electron spin resonance (ESR) measurement DPPH radical Scavenging activity*

DPPH radical scavenging activity was measured using the method described by Nanjo et al [23]. A 30 µL sample solution (or distilled water itself as control) was added to 30 µL of DPPH (60 µM) in methanol solution. After mixing vigorously for 10 s, the solution was then transferred into a 50 µL quartz capillary tube, and the scavenging activity of hydrolysate on DPPH radical was measured using a JES-FA200 ESR spectrometer (JEOL Ltd., Tokyo, Japan). The spin adduct was measured on an ESR spectrometer exactly 2 min later. Spectrometer settings were: magnetic field, 336.5 ± 5 mT; power, 5mW; modulation frequency, 9.41 GHz; amplitude, 1 x 1000; modulation width, 0.8 mT; sweep width, 10 mT; sweep time, 30 sec. DPPH radical scavenging ability was calculated following equation in which control and sample were relative peak height of radical signals with and without sample, respectively.

Radical scavenging activity (%) =
(control-sample)/control×100%

### *Hydroxyl radical Scavenging activity*

Hydroxyl radicals were generated by iron-catalyzed Haber. Weiss reaction (Fenton driven Haber.Weiss reaction) and the generated hydroxyl radicals rapidly reacted with nitrone spin trap DMPO [24]. The resultant DMPO-OH adducts was detectable with an ESR spectrometer. The sample solution (15 µL) was mixed with DMPO (0.3 M, 15 µL), $FeSO_4$ (10 mM, 15 µL) and $H_2O_2$ (10 mM, 15 µL) in a phosphate buffer solution (pH 7.4), and then transferred into a 50 µL quartz capillary tube. After 2.5 min, the ESR spectrum was recorded using an ESR spectrometer. Spectrometer settings were: magnetic field, 336.5 ± 5 mT; power, 1 mW; modulation frequency, 9.41 GHz; amplitude, 1× 200; modulation width, 0.1 mT; sweep width, 10 mT; sweep time, 30 sec. Hydroxyl radical scavenging ability was calculated following equation in which control and sample were relative peak height of radical signals with and without sample, respectively.

Radical scavenging activity (%) =
(control-sample)/control×100%

### *Superoxide radical scavenging activity*

Superoxide radical anions were generated by UV irradiated riboflavin/EDTA system [25]. The reaction mixture containing 0.3 mM riboflavin, 1.6 mM EDTA, 800 mM DMPO and indicated concentration of sample solution was irradiated for 1 min under UV lamp at 365 nm. The reaction mixture was transferred to 50 µL quartz capillary tube of the ESR spectrometer for measurement. Spectrometer settings were: magnetic field, 336.5 ± 5 mT; power, 10 mW; modulation frequency, 9.41 GHz; amplitude, 1×1000; modulation width, 0.1 mT; sweep width, 10 mT; sweep time, 30 sec. Superoxide radical scavenging ability was calculated following equation in which control and sample were relative peak height of radical signals with and without sample, respectively.

Radical scavenging activity (%) =
(control-sample)/control×100%

### *Alkyl radical scavenging activity*

Alkyl radicals were generated by AAPH [26]. The phosphate buffered solution (pH 7.4) reaction mixtures containing 40 mM AAPH, 40 mM 4-POBN. The sample solution (15 µL) was mixed with AAPH (15 µL), POBN (15 µL). And the mixture were incubated at 37℃ for 30 min. The reaction mixture was transferred to 50 µL quartz capillary tube of the ESR spectrometer for measurement. Spectrometer settings were: magnetic field, 336.5 ± 5 mT; power, 10 mW; modulation frequency, 9.41 GHz; amplitude, 1×1000; modulation width, 0.2 mT; sweep width, 10 mT; sweep time, 30 sec. Alkyl radical scavenging ability was calculated following equation in which control and sample were relative peak height of radical signals with and without sample, respectively.

Radical scavenging activity (%) =
(control-sample)/control×100%

### *Purification and identification of antioxidant peptides*

The hydrolysate obtained from powdered katsuobushi (dried bonito) was dissolved in distilled water and loaded onto a Sephadex G-25 gel filtration column (2.5 cm × 70 cm) which had been previously equilibrated with distilled water. The column was then eluted with the distilled water at a flow rate of 1.5 ml/min (fraction volume 7.5 mL), while separated fractions showing DPPH radical scavenging activity were pooled and lyophilized. DPPH radical scavenging activity

fraction was separated by reversed-phase HPLC on a Grom-sil 120 ODS-5 ST column (5 µm, 10 mm × 250 mm) using a linear gradient of acetonitrile (0–20 %, v/v, 40 min) containing 0.1% trifluoroacetic acid (TFA) at a flow rate of 0.5 mL/min. Finally, the fraction with the DPPH radical scavenging activity was collected and lyophilized; this was followed by the identification of the amino acid sequence.

### *Determination of molecular weight and amino acid sequence*

Molecular weight and amino acid sequence of purified peptide from powdered katsuobushi (dried bonito) protein were determined by Q-TOF mass spectrometry (Micromass, Altrincham, UK) coupled with electrospray ionization (ESI) source. The purified peptide dissolved in methanol/water (1:1, v/v) was infused into the ESI source and molecular mass was determined by doubly charged $(M+2H)^{2+}$ statein the mass spectrum. Following molecular mass determination, the peptide was automatically selected for fragmentation and sequence information was obtained by tandem MS analysis.

### *Protection potential of induced DNA damage by hydroxyl radical*

To evaluate the protective effects of the hydrolysate against DNA damage caused by hydroxyl radicals, a reaction was induced by placing the following reagents in an Eppendorf tube: 5 µL of genomic DNA (RAW 264.7 cell line), 2 mM $FeSO_4$, and various concentrations of the purified peptide. The mixture was then incubated at 37℃ for 30 min, followed by the addition of 4 µL of 10mM $H_2O_2$ (29). Next, the mixture was subjected to 1% agarose gel electrophoresis, after which the DNA bands were stained with ethidium-bromide.

### *Statistical analysis*

All experiments were performed in triplicate. All results were expressed as means ± standard errors of measurement.

## Rusults and Discussion

### *Amino acid composition of powdered katsuobushi (dried bonito)*

The amino acid composition of powdered katsuobushi (dried bonito) is shown in Table 2. The powdered katsuobushi (dried bonito) contained high proportions of alkaline amino acids (histidine, arginine, lysine), hydrophobic amino acids (phenylalanine, valine, leucine, isoleucine, proline, alanine, glycine), and branched-chain amino acids (valine, isoleucine, leucine). The most abundant amino acids were Glu, Asp, Ala, Lys, and Leu, which accounted for 13.67%, 10.77%, 9.34%, 8.86%, and 8.31%, respectively (Table 2). Protein hydrolysates obtained after enzymatic hydrolysis are composed of free amino acids and short-chain peptides, and their amino acid profiles exhibit many advantages as nutraceuticals or functional foods. The amino acid composition of any food protein plays a significant role in various physiological activities in the human body and either directly or indirectly affects the maintenance of good health. Amino acids are the building blocks essential for the synthesis of a wide variety of proteins including nutrients carriers, enzymes, and structural proteins.

**Table 2.** Amino acid contents of powdered katsuobushi (dried bonito)

| Amino acids | Content (%) |
|---|---|
| Asp | 10.77 |
| Thr | 6.14 |
| Ser | 6.23 |
| Glu | 13.67 |
| Gly | 6.33 |
| Ala | 9.34 |
| Val | 5.00 |
| Cys | 0.32 |
| Met | 1.02 |
| Ile | 3.91 |
| Leu | 8.31 |
| Tyr | 2.37 |
| Phe | 3.37 |
| Lys | 8.86 |
| His | 5.36 |
| Arg | 4.81 |
| Pro | 0.81 |
| Hypro | 0.33 |

### *Antioxidant activity of hydrolysates*

The powdered katsuobushi (dried bonito) protein hydrolysates were prepared by enzymatic hydrolysis using the following commercially available proteases: Alcalase, α-chymotrypsin, Neutrase, papain, pepsin, and trypsin. The extent of protein degradation within the hydrolysates was estimated by evaluating the degree of hydrolysis (DH). This analysis revealed that the DH of Alcalase, Neutrase,

α-chymotrypsin, pepsin, trypsin, and papain were 86.1%, 77.8%, 68.1%, 63.6%, 38.2% and 69.8%, respectively (Table 3). The antioxidant activity of a substance can be quantified by evaluating its scavenging activities on free radicals in oxidative systems. Consequently, the protein katsuobushi (dried bonito) hydrolysates were evaluated for their free radical scavenging effects on DPPH, hydroxyl, superoxide, and alkyl radicals by using electron spin resonance technique. As shown in Table 4, scavenging of DPPH radicals was more efficient than that of hydroxyl, superoxide and alkyl radicals. Among the hydrolysates, the peptic–derived hydrolysate exhibited the highest antioxidant activity compared to the other enzymatic hydrolysates. The $EC_{50}$ (concentration of hydrolysate needed to scavenge 50% of radical activity) values of peptic hydrolysates on scavenging DPPH, hydroxyl, superoxide, and alkyl radicals were found to be 1.45, 0.74, 0.82, and 1.42 mg/mL, respectively. Recent advances in biotechnology have highlighted the ability of enzymes to produce novel food products, modified food composition, and improved waste processing [11]. Therefore, we purified peptic hydrolysates to identify the active antioxidant peptide.

**Table 3.** The yield of powdered katsuobushi (dried bonito) hydrolysates

| Enzyme | Yield (%) |
|---|---|
| Alcalase | 86.1 |
| α-Chymotrypsin | 77.8 |
| Neutrase | 68.1 |
| Papain | 63.6 |
| Pepsin | 38.2 |
| Trypsin | 69.8 |

**Table 4.** The $EC_{50}$ values of enzymatic hydrolysates to scavenging DPPH, hydroxyl, superoxide and alkyl radicals.

| Hydrolysate | $EC_{50}$ (mg/mL) | | | |
|---|---|---|---|---|
| | DPPH | Hydroxyl | Superoxide | Alkyl |
| Alcalase | 2.89 | 3.92 | 4.63 | 0.99 |
| α-Chymotrypsin | 2.32 | 4.57 | 3.79 | 0.91 |
| Neutrase | 6.88 | 5.32 | 3.76 | 3.21 |
| Papain | 2.65 | 3.97 | 3.51 | 1.61 |
| Pepsin | 1.45 | 3.97 | 0.82 | 1.42 |
| Trypsin | 8.11 | 0.74 | 3.43 | 0.63 |

* Concentration of hydrolysate required to efficacy 50% of the respective radical ESR signal intensity.

***Purification of the katsuobushi (dried bonito) antioxidant peptide***

To identify the antioxidant peptides derived from the powdered katsuobushi (dried bonito) peptic-derived hydrolysates with the highest antioxidant activity, hydrolysates were separated by Sephadex G-25 column chromatography into four fractions (F1–F4) (Fig. 1(a) lower layer). Sephadex G-25 column chromatography separates according to molecular size, where the preliminary fractions contain larger peptides, and secondary fractions contain smaller peptides. According to a report by [27], most bioactive peptides are of small molecular size between 2 and 20 amino acids in length. Therefore, secondary fractions were assumed to have the greatest potential of containing the bioactive peptides of interest. Accordingly, we found that fraction F4 possessed strong antioxidant activity. Specifically, the DPPH radical scavenging activity of F4 had the highest antioxidant activity with an $EC_{50}$ value of 0.64 mg/mL (Fig. 1(b) upper layer). F4 was further separated by an column and subsequently separated into five fractions (A–E) (Fig. 1(b) lower layer). Fraction A showed the highest DPPH radical scavenging activity with an $EC_{50}$ value of 0.35mg/mL. This active fraction identified through revers phase-HPLC (Fraction A) was further purified using the $C_{18}$ analytical column with a linear gradient of acetonitrile (0-30%, v/v, 50 min) at a flow rate of 1.0 mL/min. As shown in Fig. 1(c), the active peaks were analyzed, and the $EC_{50}$ values of A1 were 0.14 mg/mL. The same active fraction was further purified using the same column with a different linear gradient of acetonitrile (5-10 %, v/v, 50 min) at a lower flow rate (0.5 mL/min). As shown in Fig. 1(d), the active peaks of A1-b had $EC_{50}$ values of 80.1 μg/mL. The radical scavenging activities of purified peptide is shown Table 5.

**Table 5.** The $EC_{50}$ values of purified peptide to scavenging DPPH, hydroxyl, superoxide and alkyl radicals.

| Hydrolysate | $EC_{50}$ (μg/mL) | | | |
|---|---|---|---|---|
| | DPPH | Hydroxyl | Superoxide | Alkyl |
| Purified peptide | 80.1 | 39.2 | 46.3 | 99.0 |

* Concentration of hydrolysate required to efficacy 50% of the respective radical ESR signal intensity.

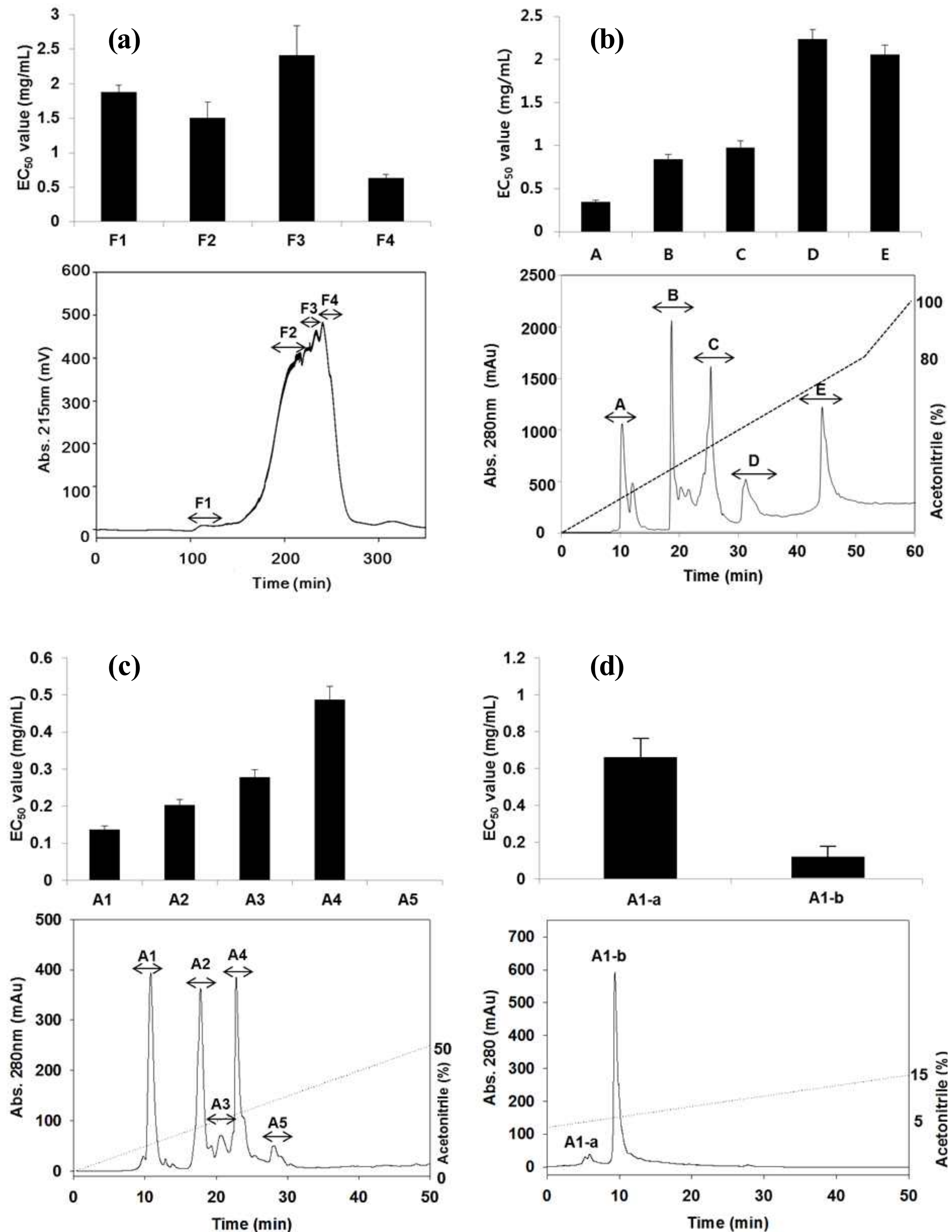


**Figure 1.** Separation scheme for the radical-scavenging peptide obtained from dried bonito hydrolysate. (A) Gel filtration chromatography on Sephadex G-25 column (2.5 × 70 cm). The column was eluted with distilled water and eluted at a flow rate of 1.5 mL/min. Protein elution was monitored at 215 nm. The fractions were collected and tested for DPPH radical scavenging activity as illustrated in the upper panel. (B) RP-HPLC on an ODS pre C18 column. The fractions with the desire peaks were pooled and tested for DPPH radical scavenging activity. (C,D) RP-HPLC on an analytical C18 column. The fractions with the desire peaks were pooled and tested for DPPH radical scavenging activity.

***Characterization of the katsuobushi (dried bonito) antioxidant peptide***

The purified A-1-b fraction was analyzed by ESI.MS for molecular mass determination and ESI.MS/MS for peptide characterization. The amino acid sequence of the bioactive purified peptide from this fraction was identified to be Pro-Met-Pro-Leu-Asn-Ser-Cys (M.W.: 756 Da), and the $EC_{50}$value of this purified antioxidant peptide was 105.82µM (Fig. 2) [29]. Furthermore, we demonstrated that the purified DPPH radical scavenging peptide has a sequence similar reported, including the Tuna DPPH radical scavenging peptide (Pro-Met-Asp-Tyr-Met-Val-Thr, RSA = 85.2% at 100 µg/mL) [30] and sardinelle (*Sardinella aurita*) DPPH radical scavenging peptide (Pro-His-Tyr-Leu, RSA = Not data) [4] It is also reported that antioxidant peptides possess some metal chelation and/or proton/electron donating activities, which could allow them to interact with free radicals and terminate their chain reactions or prevent their formation [31]. Therefore, the amino acid constituents and sequence are critical for their antioxidant activity. Furthermore, it has been shown that hydrophobic amino acids in addition to one or more residues of histidine, proline, methionine, cysteine, tyrosine, tryptophan, and phenylalanine can enhance the activities of antioxidant peptides. [32,33]. Moreover, evidence suggests that functional peptides are dependent on amino acid sequence and structure [33]. Li et al. reported that the antioxidant activity of histidine-containing peptides was attributed to the proton-donating ability of the histidine imidazole group [28]. Specifically, histidine and proline participate in the antioxidant activity of designed peptides, among which Pro−His−His exhibited the greatest antioxidant activity [28]. Moreover, Dávalos et al. reported that among the amino acids, tyrosine, tryptophan, and methionine showed the highest antioxidant activity, followed by histidine, cysteine, and phenylalanine [32]. In agreement, the amino acid composition of the purified antioxidant peptide from powdered katsuobushi (dried bonito) consisted of proline, methionine and cysteine.

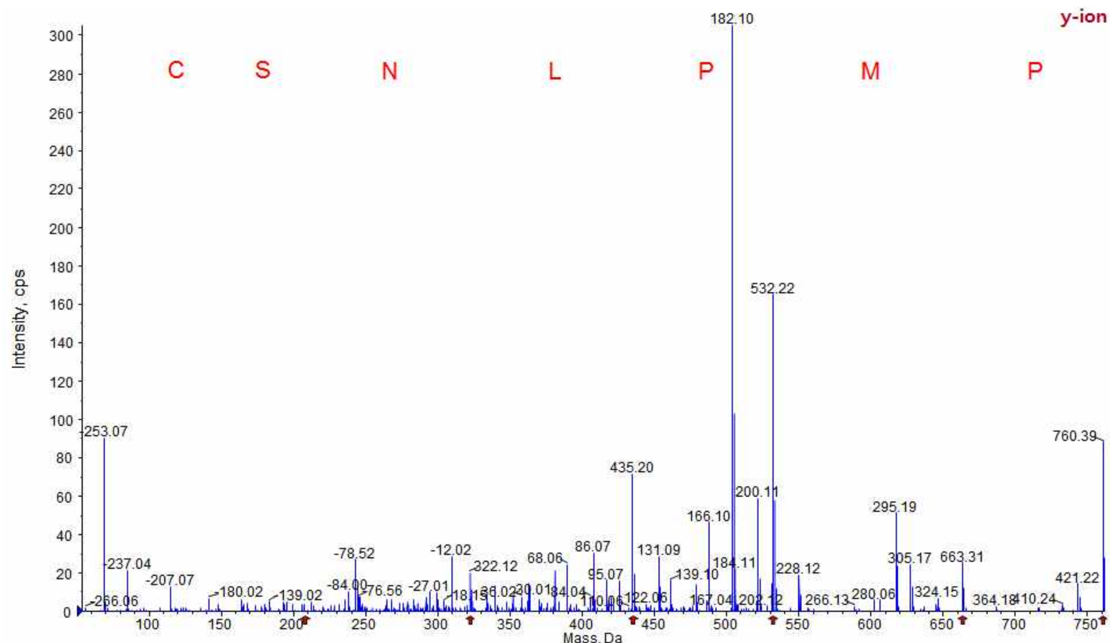


**Figure 2**. Identification of molecular mass and amino acid sequence of the purified peptides from powdered katsuobushi (dried bonito) peptic hydrolysate by HPLC. MS/MS experiments were performed on a Q-TOF tandem mass spectrometer equipped with a nano-ESI source.

***Prevention of oxidation-induced DNA damage by a katsuobushi (dried bonito) peptide***

As shown in Fig. 3, the purified katsuobushi (dried bonito) peptide had a protective effect against DNA oxidation induced by hydroxyl radicals, and this result was dose dependent with increasing peptide concentrations ranging from 0.5 to 0.125 mg/mL. These results indicate this katsuobushi (dried bonito) peptide exerted adequate protective effects on radical-mediated DNA damage. Furthermore, our results show that the katsuobushi (dried

bonito) purified peptide can prevent oxidative damage to DNA when exposed to OH radicals generated by Fe(II)/$H_2O_2$.$Fe^{2+}$-catalyzed conversion of $H_2O_2$ is a major route to the synthesis of OH radicals in physiologic systems. OH radicals are highly reactive with all components of the DNA molecule, leading to damage of both purine and pyrimidine, and also the deoxyribose backbone [34]. DNA is also a sensitive bio-target for ROS-mediated oxidative damage [35], and DNA damage by ROS is known to initiate carcinogenesis, and affect the pathogenesis for neurodegenerative diseases such as Parkinson’s disease and Alzheimer’s disease.

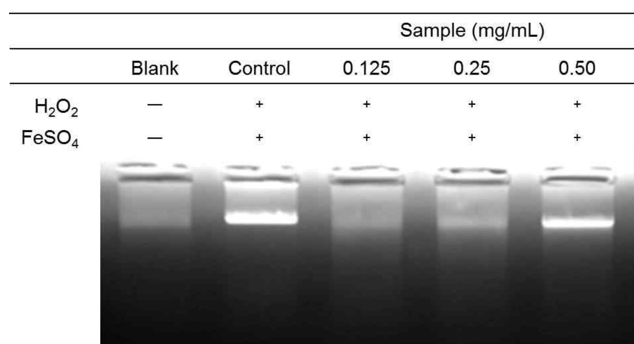


**Figure 3.** Protective effect of purified powdered katsuobushi (dried bonito) extract on hydroxyl radicals induced oxidation of genomic DNA. Blank: untreated sample and $H_2O_2$, $FeSO_4$, Control: distilled water instead of sample, Sample: Treated sample, $H_2O_2$ and $FeSO_4$ (+ : treatment, - : not treatment).

## Conclusion

In the current study, powdered katsuobushi (dried bonito) proteins were hydrolyzed with various enzymes, and the antioxidant activities of the resultant purified peptides were determined,. As a result, an antioxidant peptide with seven-amino acids (Pro-Met-Pro-Leu-Asn-Ser-Cys) from the peptic hydrolysate of powdered katsuobushi (dried bonito) protein was identified. This purified peptide exhibited an inhibitory effect against DNA oxidation induced by hydroxyl radicals. These results suggest that powdered katsuobushi (dried bonito) could be used to enhance the antioxidant effects of functional foods and prevent oxidation reactions in food processing.

## Acknowledgement

This research was the project titled '(Marine bio-regional specialization leading technology development) Functional bi omaterial development of the East coastal marine bioresource ', funded by the Ministry of Oceans and Fisheries, Korea.

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