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Reactive Oxygen Scavenging Effect of Enzymatic Extracts from *Sargassum thunbergii*

PYO-JAM PARK,[†] SOO-JIN HEO,[‡] EUN-JU PARK,[§] SE-KWON KIM,[||] HEE-GUK BYUN,[||] BYONG-TAE JEON,[⊥] AND YOU-JIN JEON*,[‡]

Department of Biotechnology and Nokyong Research Center, Konkuk University, Chungju 380-701, Korea, Faculty of Applied Marine Science, Cheju National University, Jeju 690-756, Korea, Division of Life Sciences, Kyungnam University, Masan 631-701, Korea, and Department of Chemistry, Pukyong National University, Busan 608-737, Korea

The free radical scavenging activity of water soluble natural antioxidants from *Sargassum thunbergii*, which is a brown marine alga, was evaluated by examining the radical scavenging activities of the extracts of hydrolyzates from *S. thunbergii* on hydroxyl, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and alkyl radicals. A spin-trapping electron spin resonance (ESR) spectrometer was employed, and the results were compared for their ESR signal intensity. *S. thunbergii* was enzymatically hydrolyzed to prepare water soluble extracts by five carbohydrases (AMG, Celluclast, Termamyl, Ultraflo, and Viscozyme) and proteases (Alcalase, Flavorzyme, Kojizyme, Neutrase, and Protamex). The scavenging activity of the radicals increased with increased concentrations of the extracts. The scavenging results were higher for hydroxyl and alkyl radicals and lower for DPPH radical as compared with vitamin C as a reference. The hydrogen peroxide scavenging activity of the extracts was also investigated; the Alcalase extract showed the highest scavenging activity among the extracts prepared with the five proteases and five carbohydrates. In addition, the DNA damage was determined by using the comet assay with alkaline electrophoresis and was quantified by measuring the tail length. The preventive effect of Alcalase extract from *S. thunbergii* against DNA damage increased with increments of concentration of the enzymatic extracts.

KEYWORDS: Enzymatic extract; *Sargassum thunbergii*; marine alga; electron spin resonance; radical scavenging activity; comet assay

INTRODUCTION

Lipid oxidation by reactive oxygen species (ROSs) such as superoxide anion, hydroxyl radicals, and hydrogen peroxide causes a decrease in nutritional value of lipids, in their safety and appearance. In addition, it is the predominant cause of qualitative decay of foods, which leads to rancidity, toxicity, and destruction of biochemical components important in physiologic metabolism. Free radicals-mediated modification of DNA, proteins, lipids, and small cellular molecules is associated with a number of pathological processes, including atherosclerosis, arthritis, diabetes, cataractogenesis, muscular dystrophy, pulmonary dysfunction, inflammatory disorders, ischemia−reperfusion tissue damage, and neurological disorders such as Alzheimer's disease (*1*). Therefore, antioxidants are important for bodily protection against oxidative stress. Generally, many synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), *tert*-butylhydroquinone (TBHQ), and propyl gallate may be added to food products to retard lipid oxidation (*2*). However, the use of synthetic antioxidants in food products is under strict regulation due to the potential health hazards caused by such compounds (*3*). Therefore, the search for natural antioxidants as alternatives to synthetic ones is of great interest among researchers.

Seaweeds or their extracts have also been studied as potential natural antioxidants in recent years (*4*−*15*). Although most photosynthesizing plants including seaweeds are exposed to a combination of light and high oxygen concentrations, which lead to the formation of free radicals and other strong oxidizing agents, they seldom suffer from any serious photodynamic damage in vivo. The observations suggest that their cells have protective antioxidative mechanisms as well as antioxidative compounds (*5*, *16*, *17*). In addition, seaweeds are rich in vitamins, minerals, natural bioactive compounds, and various functional polysaccharides. Therefore, it is important to extract or isolate useful bioactive compounds from seaweeds. Traditional extraction techniques for bioactive materials from seaweeds use different organic solvents or water. However, there have been several controversial points such as extremely low

* To whom correspondence should be addressed. Tel: +82-64-754-3475. Fax: +82-64-756-3493. E-mail: youjinj@cheju.ac.kr.
[†] Department of Biotechnology, Konkuk University.
[‡] Cheju National University.
[§] Kyungnam University.
[||] Pukyong National University.
[⊥] Nokyong Research Center, Konkuk University.

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recovery and strict regulations for use of organic solvents in the food industry and limited recovery of water soluble components in water extractions.

In our previous study (*15*), a novel extraction technique using digestive enzymes such as carbohydrases and proteases was employed in order to degrade seaweed tissues and to help in releasing a variety of bioactive compounds. This technique allowed successful production of water soluble materials from seaweeds with a relatively high yield of around 50% based on dry weight. In addition, scavenging of free radical and ROS and lipid peroxidation inhibition were possible using extracts of *Sargassum horneri* (Turner), a Sargassaceae brown alga (*17*).

In the present study, a seaweed *Sargassum thunbergii*, distributed over the shallow marine coast of Korea, was enzymatically hydrolyzed with different carbohydrases and proteases to prepare water soluble extracts, and then, the radical scavenging activity of the enzymatic extracts was evaluated on 1,1-diphenyl-2-picrylhydrazyl (DPPH), hydroxyl, and alkyl radicals using electron spin resonance (ESR) spectroscopy. In addition, the hydrogen peroxide scavenging activity and the preventive effect of extract from *S. thunbergii* against DNA damage were investigated.

MATERIALS AND METHODS

Materials. *S. thunbergii*, a marine brown macroalga, was collected along the Jeju island coast of Korea during a period from October 2002 to March 2003. The samples were washed three times using tap water to remove salt, epiphytes, and sand attached to the surfaces of the samples. Finally, the samples were rinsed carefully in freshwater and stored in a freezer at −20 °C. The frozen sample was lyophilized, homogenized with a grinder, and passed through a sieve (400 μm) to carry out the enzymatic extraction. Various carbohydrases such as AMG 300L (an exo-1,4-α-D-glucosidase), Viscozyme L (a multienzyme complex containing a wide range of carbohydrases, including arabanase, cellulase, β-glucanase, hemicellulase, and xylanase), Celluclast 1.5L (catalyzing the breakdown of cellulose into glucose, cellobiose, and higher glucose polymers), Termamyl 120L (a heat stable α-amylase), and Ultraflo L (a heat stable multiactive β-glucanase) and proteases such as Protamex (a bacillus protease complex), Kojizyme 500 MG (boosting of the soya sauce fermentation), Neutrase 0.8L (an endoprotease), Flavorzyme 500 MG (containing both endoprotease and exopeptidase activities), and Alcalase 2.4L (a endo protease) were donated by Novo Co. (Novozyme Nordisk, Bagsvaerd, Denmark). Peroxidase, 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid (ABTS), 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), 2,2-azobis (2-amidinopropane) hydrochloride (AAPH), α-(4-pyridyl-1-oxide)-*N*-*tert*-butylnitrone (4-POBN), Histopaque 1077, and DPPH were purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were of the highest grade available commercially.

Preparation of Enzymatic Extracts from *S. thunbergii*. The enzymatic extracts were obtained according to the method described by Heo et al. (*16*). *S. thunbergii* was pulverized into a powder using a grinder and passed through a sieve (400 μm). One hundred milliliters of a buffer solution was added to 1 g of the lyophilized sample, and then, 10 mg of protein of each enzyme was added and allowed to mix. The optimum hydrolysis conditions of particular enzymes were as follows: Viscozyme, pH 4.5, 50 °C; Celluclase, pH 4.5, 60 °C; AMG, pH 4.5, 60 °C; Termamyl, pH 6.0, 60 °C; Ultraflo, pH 7.0, 60 °C; Protamex, pH 6.0, 40 °C; Kojizyme, pH 6.0, 40 °C; Neutrase, pH 6.0, 50 °C; Flavorzyme, pH 7.0, 50 °C; and Alcalase, pH 8.0, 50 °C, respectively. The enzymatic hydrolysis reactions were performed for 12 h to achieve a maximum hydrolytic level. The hydrolysates were clarified by centrifugation at 5000*g* for 20 min to remove any unhydrolyzed residue. Finally, the enzymatic extracts of *S. thunbergii* were obtained after filtering the supernatant by a filter paper (Whatman no. 41), followed by lyophilization and then storing at −20 °C until use.

DPPH Radical Assay. DPPH radical scavenging activity was measured using the method described by Nanjo et al. (*18*). An ethanol solution of 60 μL of each sample (or ethanol itself as a control) was added to 60 μL of DPPH (60 μmol/L) in ethanol solution. After they were mixed vigorously for 10 s, the solutions (50 μL) were then transferred into a 100 μL Teflon capillary tube and fitted into the cavity of the ESR spectrophotometer. The spin adduct was measured on an ESR spectrophotometer exactly 2 min later. Measurement conditions: central field, 3475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 5 mW; gain, 6.3×10^5; and temperature, 298 K.

Hydroxyl Radical Assay. Hydroxyl radicals were generated by Fenton reaction and reacted rapidly with nitrone spin trap DMPO: the resultant DMPO−OH adducts were detectable with an ESR spectrophotometer (*19*). The ESR spectrum was recorded 2.5 min after mixing in a phosphate buffer solution (pH 7.4) with 0.2 mL of 0.3 M DMPO, 0.2 mL of 10 mM $FeSO_4$, and 0.2 mL of 10 mM H_2O_2 using an ESR spectrophotometer (JEOL, Tokyo, Japan) set at the following conditions: central field, 3475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 1 mW; gain, 6.3×10^5; and temperature, 298 K.

Alkyl Radical Assay. Alkyl radicals were generated by AAPH. The phosphate-buffered saline (PBS, pH 7.4) reaction mixtures containing 10 mmol/L AAPH, 10 mmol/L 4-POBN, and indicated concentrations of tested samples were incubated at 37 °C in a water bath for 30 min (*20*) and then transferred to a 100 μL Teflon capillary tube. The spin adduct was recorded on a JES-FA ESR spectrophotometer. Measurement conditions: central field, 3475 G; modulation frequency, 100 kHz; modulation amplitude, 2 G; microwave power, 10 mW; gain, 6.3×10^5; and temperature, 298 K.

Hydrogen Peroxide Scavenging Activity. The hydrogen peroxide scavenging activity was determined according to the method of Muller et al. (*21*). One hundred microliters of 0.1 M phosphate buffer (pH 5.0) and sample solution were mixed in a 96 microwell plate. The final concentration was 2 mg/mL. Twenty microliters of hydrogen peroxide was added to the mixture and then incubated at 37 °C for 5 min. After incubation, 30 μL of 1.25 mM ABTS and 30 μL of peroxidase (1 unit/mL) were added to the mixture and then incubated at 37 °C for 10 min. The absorbance was read with an enzyme-linked immunosorbent assay reader at 405 nm.

Isolation and Cryoconservation of Human Peripheral Lymphocytes. Blood samples were obtained from two healthy male volunteers (nonsmokers, 27 and 35 years old, respectively). Five milliliters of fresh whole blood was added to 5 mL of PBS and layered onto 5 mL of Histopaque 1077. After centrifugation at 40*g* for 30 min at room temperature, the lymphocytes were collected from just above the boundary with the Histopaque 1077 and washed with 5 mL of PBS. Finally, they were resuspended in freezing medium (90% fetal calf serum, 10% dimethyl sulfoxide) at 6×10^6 cells/mL. The cells were stored in liquid nitrogen. The cells were thawed rapidly prior to each experiment in a water bath at 37 °C.

Incubation of Lymphocytes. Each lyophilized extract was dissolved in PBS and diluted into concentrations 0, 1, 10, 25, and 50 μg/mL. Diluted extract aliquots of 1 mL with a lymphocyte suspension containing 2×10^4 cells/mL were incubated for 60 min at 37 °C in a dark incubator together with untreated control samples. After preincubation, samples were centrifuged at 380*g* for 5 min at 4 °C. The incubated cells were resuspended in PBS with 50 μM hydrogen peroxide (H_2O_2) for 5 min at 0 °C. The untreated control sample was resuspended only in PBS without H_2O_2. Cells were centrifuged as described above and then washed with 1 mL of PBS. All of the experiments were repeated twice with lymphocytes from each of two donors.

Determination of DNA Damage (Comet Assay). The alkaline comet assay was conducted according to Singh et al. (*22*) with a slight modification. The cell suspension was mixed with 75 μL of 0.5% low melting agarose (LMA) and added to the slides precoated with 1.0% normal melting agarose (NMA). After solidification of the agarose, slides were covered with another 75 μL of 0.5% LMA and then immersed in lysis solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, and 1% sodium laurylasarcosine; 1% Triton X-100 and 10% DMSO) for 1 h at 4 °C. The slides were next placed into an electrophoresis tank containing 300 mM NaOH and 10 mM Na_2EDTA (pH 13.0) for 40 min for DNA unwinding. For electrophoresis of the DNA, an electric

current of 25 V/300 mA was applied for 20 min at 4 °C. The slides were washed three times with a neutralizing buffer (0.4 M Tris, pH 7.5) for 5 min at 4 °C and then treated with ethanol for another 5 min before staining with 50 μL of ethidium bromide (20 μg/mL). Measurements were made by image analysis (kinetic Imaging Komet 5.0, UK) and fluorescence microscope (LEICA DMLB, Germany), determining the percentage of fluorescence in the tail (tail intensity, TI; 50 cells from each of two replicate slides).

Statistical Analysis. Data were analyzed using the SPSS package for Windows (Version 10). Values were expressed as means ± standard errors (SE). The mean values of the tail intensity from each treatment were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. A P value of less than 0.05 was considered significant.

RESULTS AND DISCUSSION

In general, the enzyme-assisted extraction of vegetable compounds has been commercially used for the extraction of vegetable oil, protein, and other valuable compounds. In this study, the alga was applied for extraction of the bioactive compounds, and the enzymatic water soluble extracts from the brown marine alga, *S. thunbergii*, were successfully prepared with high extraction yield using five different types of proteases such as Alcalase, Flavorzyme, Kojizyme, Neutrase, and Protamex and another five different carbohydrases such as AMG, Celluclast, Termamyl, Ultraflo, and Viscozyme, respectively, according to Heo et al. (*15*). Free radical scavenging activity of the enzymatic extracts was investigated on DPPH, alkyl, and hydroxyl radicals. DPPH is stable free radical, which has been used to evaluate free radical scavenging activity of natural antioxidants (*23*). In this study, the DPPH radical scavenging activity of various protease and carbohydrase extracts is shown in **Figure 1**, and the extracts (25.0 μg/mL) hydrolyzed by the five kinds of carbohydrases such as AMG, Celluclast, Termamyl, Ultraflo, and Viscozyme from *S. thunbergii* scavenged 93.8, 83.9, 62.9, 95.6, and 61.1% against DPPH radical, respectively (**Figure 1B**). In addition, the radical scavenging activity was concentration-dependent. It was observed that Alcalase, Flavorzyme, Kojizyme, Neutase, and Protamex were 95.9, 75.1, 80.4, 71.8, and 59.4% at 25.0 μg/mL against DPPH radical, respectively (**Figure 1C**). These results indicate that all enzymatic extracts were found to possess DPPH radicals. In addition, the activities of AMG and Alcalase extracts were the highest, and the IC_{50} values of AMG and Alcalase extracts were 1.56 and 1.35 μg/mL, respectively. The alkyl radical spin adduct of 4-POBN/free radicals was generated from AAPH at 37 °C for 30 min, and the decrease of ESR signals was observed with the dose increment of all enzymatic extracts (**Figure 2**). All tested carbohydrase extracts exhibited the alkyl radical scavenging activities, and the scavenging activities of AMG, Celluclast, Termamyl, Ultraflo, and Viscozyme extracts were 94.6, 85.7, 55.7, 81.3, and 17.9% at 25 μg/mL, respectively (**Figure 2B**). In addition, 25 μg/mL of the extracts hydrolyzed from *S. thunbergii* by the five types of proteases such as Alcalase, Flavorzyme, Kojizyme, Neutrase, and Protamex also scavenged 98.2, 48.2, 55.4, 67.9, and 73.2%, respectively (**Figure 2C**). AMG and Alcalase extracts showed the highest alkyl radical scavenging activities, and the IC_{50} values were 4.14 and 3.82 μg/mL, respectively. Hydroxyl radicals generated in the Fe^{2+}/H_2O_2 system were trapped by DMPO, forming a spin adduct detected by an ESR spectrophotometer, and the typical 1:2:2:1 ESR signal of the DMPO−OH adduct was observed as shown in **Figure 3A**. Additionally, background signals are present in **Figure 3A**. They may be due to the paramagnetic impurities contained in unpurified commercial DMPO (*24*). The

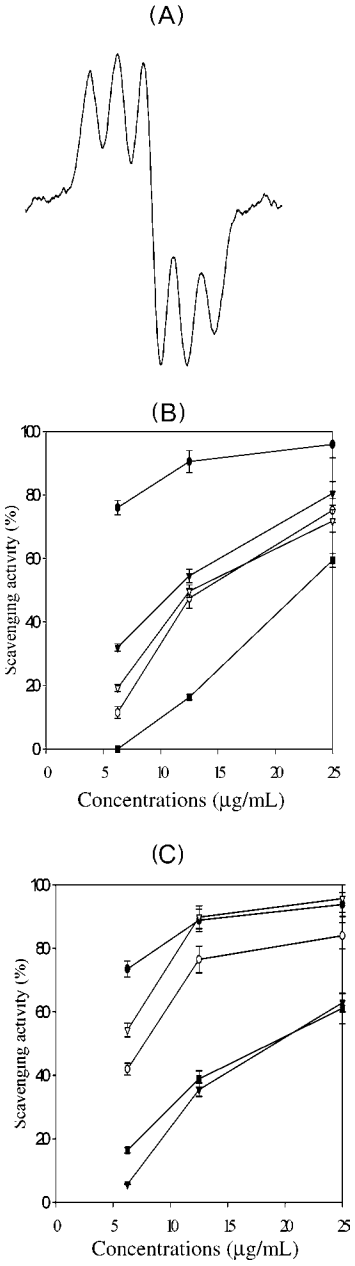

Figure 1. DPPH radical scavenging activity of the extracts prepared using the various carbohydrases (**B**) and proteases (**C**) from *S. thunbergii*. Panel **A** shows the ESR spectrum of DPPH radical. Where it is said (**B**); ●, extracts hydrolyzed with AMG; ○, extracts hydrolyzed with Celluclast; ▼, extracts hydrolyzed with Termamyl; □, extracts hydrolyzed with Ultraflo; and ■, extracts hydrolyzed with Viscozyme. Ehrtr iy dhoulf br dsif z9Vz0; ●, extracts hydrolyzed with Alcalase; ○, extracts hydrolyzed with Flavorzyme; ▼, extracts hydrolyzed with Kojizyme; □, extracts hydrolyzed with Neutrase; and ■, extracts hydrolyzed with Protamex. Means ± SE of determinations were made in triplicate experiments.

height of the third peak of the spectrum represents the relative amount of DMPO−OH adduct. As shown in **Figure 3B**, it was observed that the hydroxyl radical scavenging activities of AMG, Celluclast, Termamyl, Ultraflo, and Viscozyme extracts were 90.1, 92.8, 81.0, 73.0, and 91.9% at 25 μg/mL, respectively. As shown in **Figure 3C**, it was observed that 25 μg/mL of the extracts prepared with five types of proteases such as Alcalase, Flavorzyme, Kojizyme, Neutrase, and Protamex also scavenged 81.1, 69.0, 83.8, 83.4, and 82.0% of hydroxyl radical, respectively. In addition, the AMG and Alcalase extracts were the highest activity by basis on IC_{50} values, and the values were

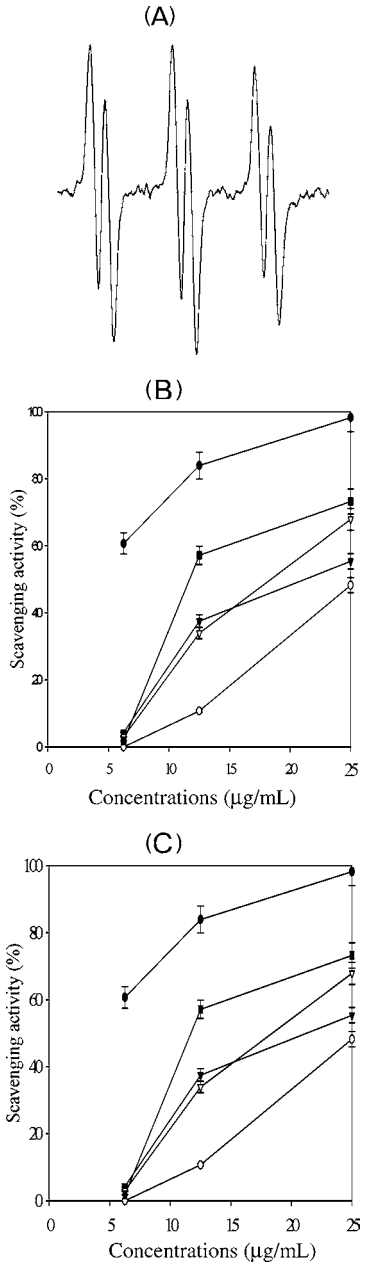

Figure 2. Alkyl radical scavenging activity of the extracts prepared using the various carbohydrases (**B**) and proteases (**C**) from *S. thunbergii*. Panel **A** shows the ESR spectrum of alkyl radical. Where it is said (**B**); ●, extracts hydrolyzed with AMG; ○, extracts hydrolyzed with Celluclast; ▼, extracts hydrolyzed with Termamyl; □, extracts hydrolyzed with Ultraflo; and ■, extracts hydrolyzed with Viscozyme. Ehrtr iy dhoulf br dsif z9Vz0; ●, extracts hydrolyzed with Alcalase; ○, extracts hydrolyzed with Flavorzyme; ▼, extracts hydrolyzed with Kojizyme; □, extracts hydrolyzed with Neutrase; and ■, extracts hydrolyzed with Protamex. Means ± SE of determinations were made in triplicate experiments.

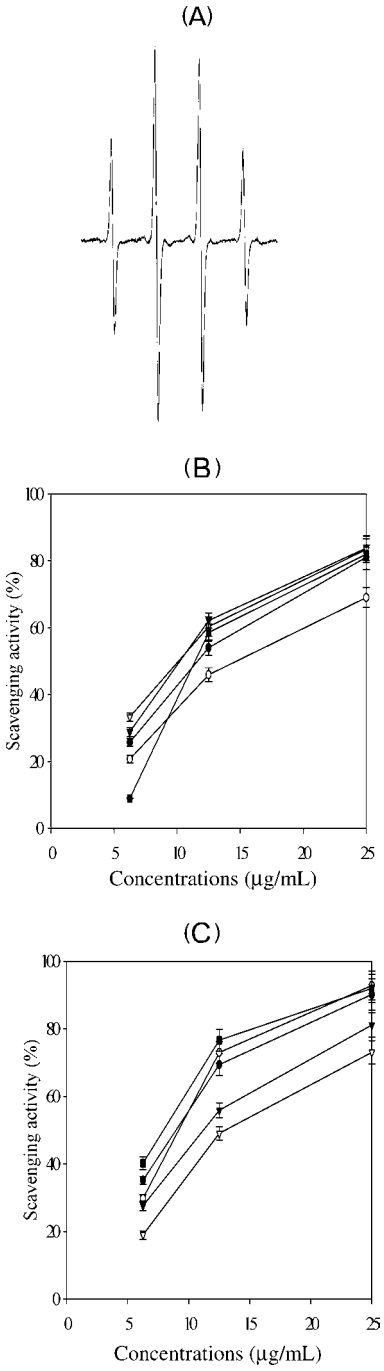

Figure 3. Hydroxyl radical scavenging activity of the extracts prepared using the various carbohydrases (**B**) and proteases (**C**) from *S. thunbergii*. Panel **A** shows the ESR spectrum of hydroxyl radical. Where it is said (**B**); ●, extracts hydrolyzed with AMG; ○, extracts hydrolyzed with Celluclast; ▼, extracts hydrolyzed with Termamyl; □, extracts hydrolyzed with Ultraflo; and ■, extracts hydrolyzed with Viscozyme. Ehrtr iy dhoulf br dsif z9Vz0; ●, extracts hydrolyzed with Alcalase; ○, extracts hydrolyzed with Flavorzyme; ▼, extracts hydrolyzed with Kojizyme; □, extracts hydrolyzed with Neutrase; and ■, extracts hydrolyzed with Protamex. Means ± SE of determinations were made in triplicate experiments.

9.47 and 7.15 μg/mL, respectively. All enzymatic extracts scavenged hydroxyl radicals, and the scavenging activity increased with the concentrations of the extracts. All enzymatic extracts exhibited scavenging activities of DPPH, alkyl, and hydroxyl radicals. Vitamin C was used as a reference antioxidant in this study, and its IC_{50} values of DPPH, alkyl, and hydroxyl radicals were 0.85, 6.23, and 12.18 μg/mL, respectively (data not shown). These results indicate that the scavenging values of the enzymatic extracts from *S. thunbergii* are higher or similar on alkyl and hydroxyl radicals and lower on DPPH radicals as compared with vitamin C. Alcalase extracts strongly scavenged the radicals examined as compared with extracts of other enzymes. There is an increasing interest in finding natural antioxidants, which are safe and effective in order to replace commercial synthetic antioxidants such as BHA and BHT. Athukorala et al. (*14*) reported that antioxidants from a marine red *Grateloupia filicina*, extracted with various solvents such as hot water, methanol, ethanol, acetone, ethyl acetate, chloro-

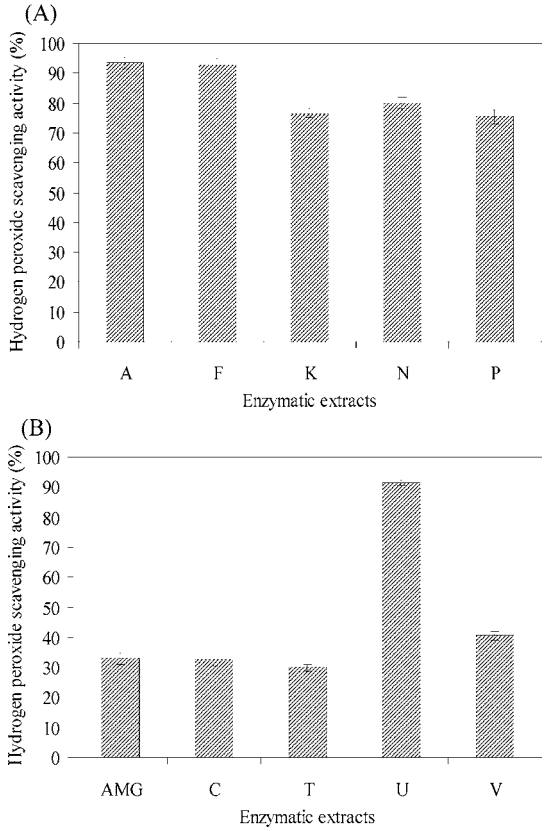

Figure 4. Hydrogen peroxide scavenging activity of the extracts hydrolyzed with various carbohydrases (**A**) and proteases (**B**) from *S. thunbergii*. P, Protamex; K, Kojizyme; N, Neutrase; F, Flavorzyme; A, Alcalase; V, Viscozyme; C, Celluclast; T, Termamyl; and U, Ultraflo.

form, ether, hexane, and carbon tetrachloride, inhibited lipid peroxidation more effectively than commercial antioxidants such as BHA, BHT, and α-tocopherol in a linoleic acid model system. Zheng et al. (*25*) reported that polysaccharide extracts from the plant *Saussurea involucrate* exerted free radical scavenging effects. Ahn et al. (*26*) extracted various enzymatic extracts with free radical scavenging activity from a brown seaweed *S. iomentaria*, and their free radical scavenging activities were examined in different systems using an ESR spectroscopy. Seaweeds are also good candidates as a natural source of antioxidants (*5*, *7*, *12*, *13*, *27*−*29*). In general, the cells have protective antioxidative mechanisms and antioxidative compounds. In this study, the contents of moisture, ash, protein, carbohydrate, and lipid from *S. thunbergii* are 8.9, 13.35, 63.62, and 0.33% (data not shown). In addition, the contents of protein and carbohydrate of Viscozyme, Celluclase, AMG, Termamyl, Ultraflo, Protamex, Kojizyme, Neutrase, Flavorzyme, and Alcalase extracts were 10, 8, 13, 9, 9, 7, 6, 7, 8, and 17%, respectively. Therefore, the results of free radical scavenging activity and chemical composition of enzymatic extracts may indicate that the type of bioactive compounds presumably present in the extract is oligosaccharide or peptide. The hydrogen peroxide scavenging activity of various enzymatic extracts was shown in **Figure 4**. All tested enzymatic extracts exhibited hydrogen peroxide scavenging activity, and the activity was different on each sample. The extracts prepared from *S. thunbergii* by five types of proteases exhibited very high hydrogen peroxide scavenging activities, and the activity was over 80%. In the extracts hydrolyzed from *S. thunbergii* by carbohydrases, Ultraflo extracts showed the highest scavenging activity, and the value was about 90%. Generally, hydrogen

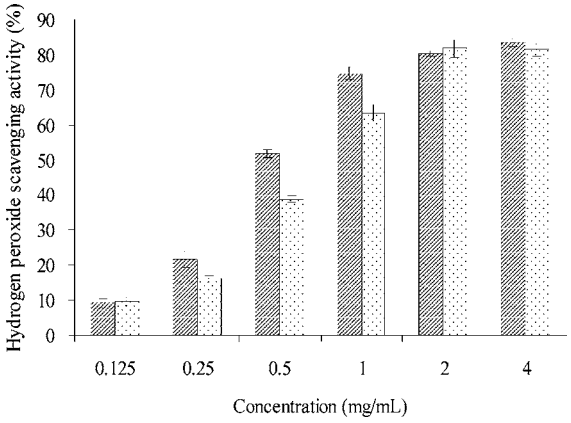

Figure 5. Hydrogen peroxide scavenging activity of Alcalase (white bars) and Ultraflo (shaded bars) extracts from *S. thunbergii* at various concentrations.

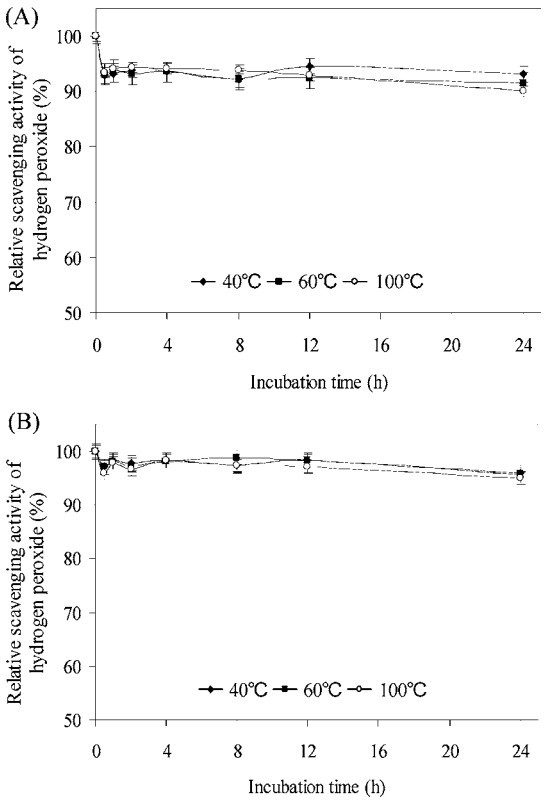

Figure 6. Thermal stability on hydrogen peroxide scavenging activity of Alcalase (**A**) and Ultraflo (**B**) extracts from *S. thunbergii*.

peroxide is able to damage a lot of cellular components, and it initiates a lipid peroxidation reaction or is toxic to cells since it generates hydroxyl radical by a Fenton reaction. In this study, Alcalase and Ultraflo extracts showed the highest hydrogen peroxide scavenging activity as compared to those of other protease and carbohydrase extracts. In addition, the hydrogen peroxide scavenging activities of Alcalase and Ultraflo extracts on various concentrations were shown in **Figure 5**. The Alcalase extracts showed a higher hydrogen peroxide scavenging activity as compared to that of Ultraflo extracts at below 1.0 mg/mL and similar at over 2.0 mg/mL, respectively. The thermal stabilities of Alcalase and Ultraflo extracts were slightly decreased as the incubation time increased, and the activity remained over 90% at 100 °C until 24 h (**Figure 6**). In general, the measurement of hydrogen peroxide scavenging activity is a useful method to determine the ability of antioxidants

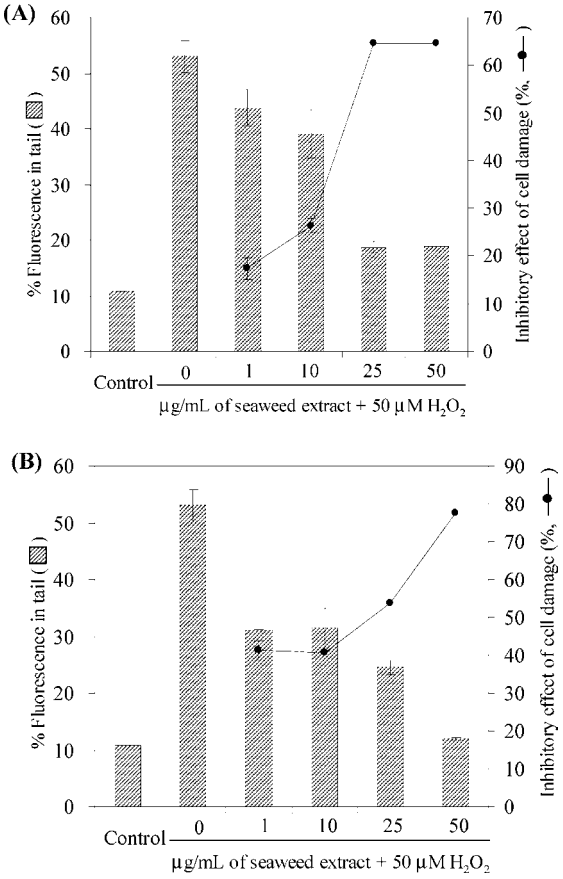

Figure 7. Effect of supplementation in vitro with different concentrations of the Alcalase (**A**) and Ultraflo (**B**) extracts from *S. thunbergii* on H_2O_2-induced human lymphocytes DNA damage. Values are means with standard errors of duplicate experiments with lymphocytes from each of two different donors. Significant differences to values for samples treated with 50 μM H_2O_2 only using LSD: **, $p < 0.01$; ***, $p < 0.001$.

decreasing the level of prooxidants (*30*). Therefore, the scavenging capacity on ROS and thermal stability of the enzymatic extracts from *S. thunbergii* suggest that they are a good antioxidative source. In addition, the effect of Alcalase extracts from *S. thunbergii* on H_2O_2-induced DNA damage in human lymphocytes was investigated. **Figure 7** shows the effect of Alcalase extracts on H_2O_2-treated human lymphocytes, determined by the comet assay. The percent fluorescence tail DNA intensity of lymphocytes treated for 30 min with negative control was significantly different from the positive control (H_2O_2 preincubated in PBS, 50 μM). This increase of DNA damage induced by H_2O_2 was significantly inhibited in a dose-dependent manner by preincubating H_2O_2 together with the negative cells of Alcalase extracts at the concentrations of 1, 10, 25, and 50 μg/mL in PBS (**Figure 7A**). The tail intensity began to significantly decrease at the lowest concentration (1 μg/mL) by about 30% of the positive control. The maximum inhibition was about 79% at 50 μg/mL concentration, showing no statistical difference from the PBS-treated negative control. In addition, the results of Ultraflo extracts on H_2O_2-induced DNA damage in human lymphocytes were similar to those of Alcalase extracts (**Figure 7B**). The understanding of the role of nutrition in health promotion and disease prevention has improved during the past 20 years. The focus of concern about human nutrition has shifted from issues of deficiencies to issues of health maintenance throughout all phases of life cycle and of chronic disease prevention. In a recent report by Adams et al. (*31*), it was recommended that healthy individuals should consume 5−7 servings of fruits and vegetables daily and should change to a healthy lifestyle to reduce the risk of heart disease. Park et al. (*32*) reported the effect of grape juice supplementation on reducing cellular DNA damage and free radical release in healthy human subjects including smokers. In another study, Muller et al. (*33*) have shown that DNA damage in mononuclear cells, which circulate in the blood vessels of the skin, was higher during summer than winter. In the present study, the enzymatic extracts from *S. thunbergii* showed the inhibitory effect on H_2O_2-induced DNA damage in human lymphocytes. Therefore, the enzymatic extracts from *S. thunbergii* have the potential to inhibit the DNA damage induced by hydrogen peroxide.

In conclusion, the scavenging effects on ROS of enzymatic extracts from *S. thunbergii*, a brown seaweed, were evaluated by examining radical scavenging effect using an ESR spectrophotometer and hydrogen peroxide scavenging effect using the comet assay in human lymphocytes. The water soluble enzymatic extracts were prepared by enzymatic hydrolysis with various carbohydrases such as AMG, Celluclast, Termamyl, Ultraflo, and Viscozyme and proteases such as Alcalase, Flavorzyme, Kojizyme, Neutrase, and Protamex, respectively. The Alcalase extract showed the highest scavenging activity on DPPH, alkyl, and hydroxyl radicals, and the IC_{50} values were 1.35, 3.82, and 7.15 μg/mL, respectively. In addition, the IC_{50} values on alkyl and hydroxyl radicals were higher or similar, and the value on DPPH radical was lower as compared to that of vitamin C used as a natural antioxidant. The hydrogen peroxide scavenging activity of Alcalase and Ultraflo extracts was 93 and 91%, respectively, and the two extracts showed significant thermal stabilities at 100 °C. In addition, it is proven that both extracts have the potential inhibitory activity on H_2O_2-induced DNA damage in human lymphocytes.

ABBREVIATIONS USED

ESR, electron spin resonance; ROS, reactive oxygen species; BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; TBHQ, *tert*-butylhydroquinone; DPPH, 1,1-diphenyl-2-picrylhydrazyl; ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline)-6-sulfonic acid; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; AAPH, 2,2-azobis-(2-amidinopropane)hydrochloride; 4-POBN, α-(4-pyridyl-1-oxide)-N-*tert*-butylnitrone; PBS, phosphate-buffered saline; LMA, low melting agarose; NMA, normal melting agarose.

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