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In vitro determination of antioxidant activity of proteins from jellyfish *Rhopilema esculentum*

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Abstract

In this study, the antioxidant activity of proteins isolated from jellyfish, *Rhopilema esculentum* Kishinouye (*R. esculentum*), was determined by various antioxidant assays, including superoxide anion radical-scavenging, hydroxyl radical-scavenging, total antioxidant activity, reducing power and metal chelating activity. Butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), α-tocopherol, vitamin C and mannitol were used as standards in those various antioxidant activities. The crude protein (CP) and the protein fractions isolated by Sephadex chromatography, first peak (FP) and second peak (SP), had very low reductive power and metal chelating abilities compared to EDTA, but they showed strong scavenging effects on the superoxide anion radical, hydroxyl radical and varying total antioxidant activity. FP and SP exhibited stronger scavenging effects on the superoxide anion radical than BHA, BHT or α-tocopherol. The EC_{50} values of FP and SP were 6.12 and 0.88 μg/ml, respectively, while values EC_{50} of BHA, BHT and α-tocopherol were 31, 61 and 88 μg/ml, respectively. CP, FP and SP showed far higher hydroxyl radical-scavenging activities than did vitamin C or mannitol. The EC_{50} values of CP, FP and SP were 48.76, 45.42 and 1.52 μg/ml, but EC_{50} values of vitamin C and mannitol were 1907 and 4536 μg/ml, respectively. In a β-carotene–linoleate system, SP and CP showed antioxidant activity, but lower than BHA. Of the three samples, SP had the strongest antioxidant activity. So, SP may have a use as a possible supplement in the food and pharmaceutical industries.

Keywords: Antioxidant activity; Jellyfish; Protein; *Rhopilema esculentum* Kishinouye

1. Introduction

Reactive oxygen species (ROS), including the superoxide anion radical ($O_2^{-\bullet}$), hydroxyl radical ($^{\bullet}OH$), hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and nitric oxide ($NO^{\bullet}$), may contribute to a number of pathological events such as aging, cellular injury and DNA degradation when they are generated excessively or when the antioxidant defence systems are depressed (Söğüt et al., 2003). Of the reactive oxygen species, superoxide anion radical is generated first. Although it is a relatively weak oxidant, it decomposes to form stronger reactive oxidative species, such as singlet oxygen and hydroxyl radicals (Dahl & Richardson, 1978). Further, superoxide anion radicals are also known to indirectly initiate lipid peroxidation as a result of H_2O_2 formation, creating precursors of hydroxyl radicals (Meyer & Isaksen, 1995). Among the reactive

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oxygen species, the hydroxyl radical is the most active. Indeed, it is one of the most reactive chemical species known. The hydroxyl radical induces some oxidative damage to biomolecules, such as some proteins, DNA, PUFA, nucleic acid, and almost any biological molecule it touches, and this damage causes aging, cancer and several diseases (Aruoma, 1998). In order to reduce damage to the human body and prolong the storage stability of foods, synthetic antioxidants are used for industrial processing (Tepe, Sokmen, Akpulat, & Sokmen, 2005). The most commonly used antioxidants at the present time are butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), propyl gallate (PG) and *tert*-butylhydroquinone (TBHQ). However, BHA and BHT have been suspected of being responsible for liver damage and carcinogenesis (Grice, 1988; Williams, Iatropoulos, & Whysner, 1999; Witschi, 1986). Thus, it is essential to develop and utilize effective and natural antioxidants so that they can protect the human body from free radicals and retard the progress of many chronic disease (Gülçin, Şat, Beydemir, Elmastaş, & Küfrevioğlu, 2004).

The jellyfish, *Rhopilema esculentum* Kishinouye (*R. esculentum*), a cnidarian of the class Scyphozoa, the order Rhizostomeae, and the family Rhopilema, is widely distributed in the South China Sea, the Yellow Sea and the Bohai Sea and is abundant in late summer to early autumn (Hong, 2002). Among the several edible species of Scyphozoan jellyfish, it is the most abundant and most important species in the Asian jellyfish fishery which is a multi million-dollar seafood business (Omori & Nakano, 2000). *R. esculentum* contains some nutritional components such as amino acids and fatty acids. Chinese people have been eating jellyfish for more than a thousand years and have also regarded it as a treatment for high blood pressure, bronchitis, tracheitis, gastric ulcers and asthmas (Hsieh & Rudloe, 1994; Jiang & Zhang, 1994). Protein isolated from jellyfish, with a unique structure, has many bioactivities such as enzymatic activities, hemolysis, hepatocyte toxicity, myotoxicity, cardiac toxicity and neuro-toxicity (Cao et al., 1998; Chung, Ratnapala, Cooke, & Yanagihara, 2001; Gusman, Avian, & Galil, 1997; Radwan, Gershwin, & Burnett, 2000). However, no report on the antioxidant activity of jellyfish protein has yet been made.

The body of jellyfish is composed of a hemispherical transparent umbrella and oral arms. Generally, the oral arms are discarded as waste because of their high content of water and sting of venom in tentacles and nematocysts. In the present study, the antioxidant activity of proteins isolated from the oral arms of *R. esculentum* was determined using various antioxidant assays, including superoxide anion radical-scavenging, hydroxyl radical-scavenging, antioxidant activity, reducing power and metal chelating activity.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), butylated hydroxyanisole (BHA), nicotinamide adenine dinucleotide-reduced (NADH), nitro blue tetrazolium (NBT), phenazine methosulphate (PMS), hydrogen peroxide (H_2O_2), ethylene diamine tetra-acetic acid (EDTA), potassium ferricyanide and ferric chloride were purchased from Sigma. Sephadex (G-100, G-50, G-200) were purchased from Amersham. All other chemicals and reagents used were of analytical grade.

2.2. Protein isolation

Jellyfish, *R. esculentum*, were collected in the Aoshan Bay in Qingdao, Shandong Province, China, in August 2003. The oral arms were manually excised in vivo, packed in polythene bags, and frozen immediately at −20 °C. The frozen oral arms were then sonicated in cold (4 °C) phosphate buffer solution (0.01 M, pH 6) eight times for 30 s each time at 100 mv. The resultant fluids were clarified by centrifugation (13,000 rpm) for 20 min at 4 °C and used as crude protein (CP). Sample protein concentrations were determined by the method of Bradford (1976), using bovine serum albumin (BSA) as a standard. For chromatography of the crude protein, the following procedure was used. Sephadex G-100 was allowed to swell in 0.05 M phosphate buffer (pH 6.0) for 2 days at room temperature. Each day, the fines were decanted and the buffer replenished. Swollen gel was degassed and poured as a slurry into a buffer solution (0.05 M, pH 6) jacketed column measuring 40 cm in length and 1.0 cm in internal diameter. The column was equilibrated at a flow rate of 25 ml/h with phosphate buffer (0.05 M, pH 6). After the gel had settled, column packing was checked using a 2 mg/ml solution of Blue Dextran 2000 (mol. Wt = 2×10^6). At the same time, the void volume was determined. Samples (1 ml) of crude protein from the oral arms were applied to the top of the Sephadex G-100 column, care being taken to prevent disturbance of the bed. Each sample was allowed to drain into the top of the bed and then elution with phosphate buffer (0.05 M, pH 6) was commenced. Absorbance, at 280 nm, of the eluate was determined by an ultraviolet detector.

2.3. Superoxide anion radical-scavenging assay

The superoxide anion radical-scavenging ability of protein was assessed by the method described by Gülçin et al. (2004) with slight modification. Superoxide anion radicals are generated in PMS–NADH systems by oxidation of NADH and assayed by the reduction of NBT. In this experiment, superoxide anion radicals were

generated in 2.5 ml of Tris–HCl buffer (16 mM, pH 8.0) containing 0.5 ml of NBT (300 μM) solution, 0.5 ml of NADH (468 μM) solution and protein samples (0.47–290μg/ml). The reaction was started by adding 0.5 ml of PMS (60 μM) solution to the mixture. The reaction mixture was incubated at room temperature for 5 min and the absorbance was read at 560 nm by a spectrophotometer against blank samples. Decreased absorbance of the reaction mixture indicated increased superoxide anion-scavenging activity. The capability of scavenging the superoxide anion radicals was calculated using the following equation:

$$\% \text{ Scavenging effect} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 is the absorbance of the control (without protein samples) and A_1 is the absorbance of the mixture containing protein samples.

2.4. Hydroxyl radical-scavenging assay

The reaction mixture, total volume 4.5 ml, containing protein samples (0.62–65.10 μg/ml), was incubated with EDTA–Fe^{2+} (220 μM), safranine O (0.23 μM) and H_2O_2 (60 μM) in potassium phosphate buffer (150 mM, PH 7.4) for 30 min at 37 °C (Wang et al., 1994). The absorbance of the mixture was measured at 520 nm. Hydroxyl radical bleached the safranine O, so decreased absorbance of the reaction mixture indicated decreased hydroxyl radical-scavenging ability.

$$\% \text{ Scavenging effect} = [(A_1 - A_B)/(A_C - A_B)] \times 100,$$

where A_B is the absorbance of the blank (distilled water instead of protein samples), A_C is the absorbance of the control (distilled water instead of H_2O_2) and A_1 is the absorbance of mixture containing protein samples.

2.5. Total antioxidant assay using β-carotene–linoleate model system

The total antioxidant activity of protein samples was evaluated by the β-carotene–linoleate model system (Hidalgo, Fernández, Quilhot, & Lissi, 1994) with slight modification. β-Carotene (0.2 mg), linoleic acid (20 mg) and Tween-40 (200 mg) were mixed in 0.5 ml of chloroform. Chloroform was removed at 40 °C under vacuum using a rotary evaporator. The resulting mixture was immediately diluted with 10 ml of triple-distilled water and was mixed well for 1–2 min. The emulsion was further made up to 50 ml with oxygenated water. Aliquots (4 ml) of this emulsion were transferred into different test tubes containing 0.2 ml of protein sample. BHA was used for comparative purposes. A control, containing 0.2 ml distilled water and 4 ml of the above emulsion, was prepared. The tubes were placed, at 50 °C, in a water bath. Absorbances of all the samples at 470 nm were taken at zero time ($t = 0$). Measurement of absorbance was continued until the colour of β-carotene disappeared in the control reaction ($t = 270$ min) at 30 min intervals (Fig. 1). A mixture prepared as above, without β-carotene, served as blank. All determinations were carried out in triplicate.

2.6. Determination of reducing power

The reducing power of the protein samples was determined by the method of Yen and Duh (1993).

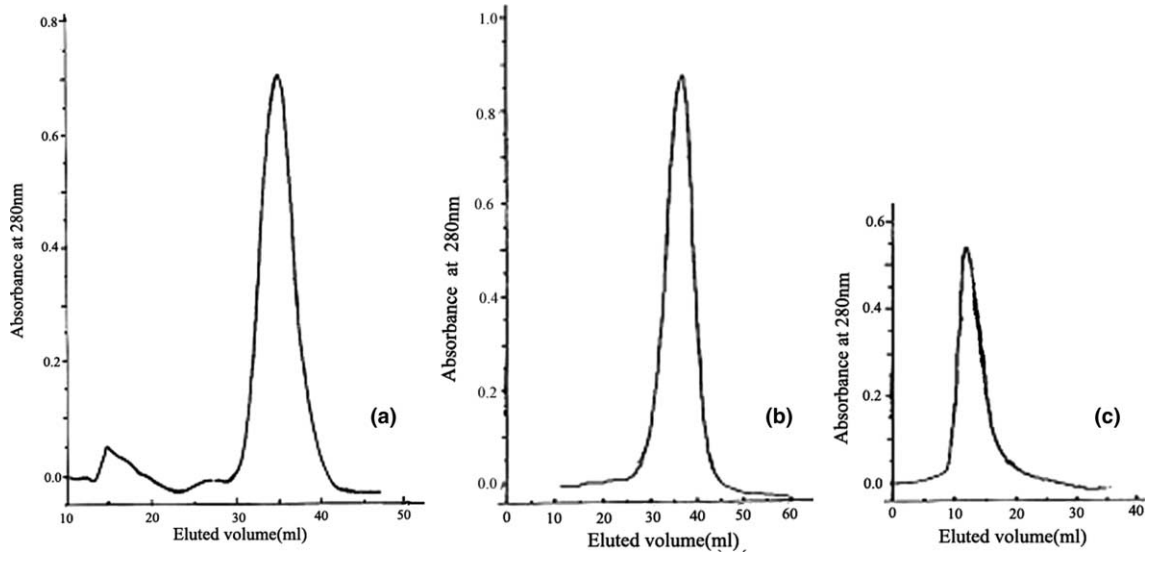

Fig. 1. Chromatogram of *R. esculentum* protein by Sephadex chromatography. (a) Chromatogram of crude protein by Sephadex G-100 chromatography, two major protein peaks were obtained at the flow rate of 25 ml/h. (b) Chromatogram of the second peak by Sephadex G-50 chromatography, one protein peak was obtained at the flow rate of 25 ml/h. (c) Chromatogram of the first peak by Sephadex G-200 chromatography, one protein peak was obtained at the flow rate of 25 ml/h.

Different concentrations of protein samples in 3.5 ml phosphate buffer (0.2 M, pH 6.6) were mixed with 2.5 ml of 1% potassium ferricyanide in 10 ml test tubes. The mixtures were incubated for 20 min at 50 °C. At the end of the incubation, 2.5 ml of 10% trichloroacetic acid were added to the mixtures, followed by centrifugation at 5000 rpm for 10 min. The supernatant fluid (2.5 ml) was mixed with 2.5 ml of distilled water and 0.5 ml of 0.1% ferric chloride and the absorbance was measured at 700 nm. The reducing power tests were run in triplicate. Increase in absorbance of the reaction mixture indicated the reducing power of the protein samples.

2.7. Metal chelating activity

The chelating of ferrous ions by the samples and standard was estimated by the method of Dinis, Madeira, and Almeida (1994). Samples were added to a solution of 2 mM $FeCl_2$ (0.05 ml). The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. After the mixture had reached equilibrium, the absorbance of the solution was then measured spectrophotometrically at 562 nm. All analyses were done in triplicate. The percentage inhibition of ferrozine–Fe^{2+} complex formation is given by the formula:

$$\% \text{ Chelating percent} = [(A_0 - A_1)/A_0] \times 100,$$

where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the samples and standard. The control contained $FeCl_2$ and ferrozine, complex formation molecules.

2.8. Statistical analysis

All data were expressed as means ± SD of three parallel measurements. Data were analysed by student *t*-test and all tests were considered statistically significant at $P < 0.05$.

3. Results and discussion

3.1. Protein isolation

Two major protein peaks appeared in the elution of Sephadex G-100 (Fig. 1(a)). The eluted volume of the first peak was 14.8 ml and almost equal to the void volume (14 ml), The eluted volume of the second peak was 36 ml. In order to get better results, Sephadex G-50 and Sephadex G-200 were applied to isolate the second peak and first peak, respectively, and one peak was obtained by Sephadex G-50 chromatography and Sephadex G-200 chromatography (Fig. 1(b) and (c)). The eluted volume of the protein peak was almost equal to the void volume of the Sephadex G-200 chromatography, indicating that the molecular weight of the first peak was too high to overstep the isolating range of Sephadex G-200 chromatography and gel filtration chromatographfy could not isolate the first protein peak further.

3.2. Superoxide anion radical-scavenging assay

In the PMS/NADH–NBT system, superoxide anion radicals derived from dissolved oxygen by the PMS-NADH coupling reaction reduce NBT. The decrease of absorbance, at 560 nm, with antioxidants indicates the consumption of superoxide anion radicals in the reaction mixture (Gülçin et al., 2004; Oktay, Gülin, & Küfrevioğlu, 2003). Table 1 shows the % scavenging effect on superoxide anion radicals of crude protein (CP), first peak (FP) and second peak (SP). Protein samples had strong superoxide anion radical-scavenging activities. For CP, at a concentration from 19.34 to 290 μg/ml, the % scavenging effect on the superoxide anion radical was from 21.0% to 89.5%. The scavenging effect depended on concentration and the correlation between them was strong; correlation coefficient (*r*) was 0.9861 (Table 3). For FP, at a concentration from 3.13 to 31.3 μg/ml, the % scavenging effect was

Table 1
Scavenging effect of protein samples on superoxide anion radical

Sample protein	Concentration (μg/ml)	A_{560}	Scavenging effect (%)
Crude protein	19.4	0.683 ± 0.001	21.0 ± 0.11
	38.7	0.591 ± 0.001	31.7 ± 0.11
	77.4	0.486 ± 0.006	43.8 ± 0.66
	116	0.391 ± 0.006	54.8 ± 0.66
	155	0.312 ± 0.008	64.0 ± 0.88
	194	0.241 ± 0.005	72.2 ± 0.55
	290	0.091 ± 0.001	89.5 ± 0.11
Control		0.864 ± 0.002	
First peak	3.13	0.748 ± 0.004	27.9 ± 0.41
	6.27	0.615 ± 0.031	40.6 ± 3.00
	9.40	0.280 ± 0.023	73.0 ± 2.21
	12.5	0.214 ± 0.025	79.4 ± 2.39
	18.8	0.105 ± 0.003	89.9 ± 0.27
	25.1	0.054 ± 0.014	94.8 ± 1.36
	31.34	0.040 ± 0.023	96.2 ± 2.25
Control		1.04 ± 0.003	
Second peak	0.47	0.659 ± 0.019	34.7 ± 1.80
	0.94	0.644 ± 0.005	36.2 ± 0.49
	1.87	0.347 ± 0.008	65.7 ± 0.77
	2.81	0.132 ± 0.004	86.9 ± 0.33
	3.75	0.079 ± 0.001	92.2 ± 0.070
	7.49	0.019 ± 0.001	98.2 ± 0.070
	15.0	0.012 ± 0.002	98.9 ± 0.14
	18.7	0.012 ± 0.001	98.9 ± 0.070
Control		1.01 ± 0.002	

Values are means ± SD of three determinations.

from 27.9% to 96.2%. For SP, at a concentration from 0.47 to 18.7 μg/ml, the % scavenging effect was from 34.7% to 98.9%. When the concentration of SP was above 3.75 μg/ml, the scavenging effect (>98%) was nearly unchanged. As far as FP and SP were concerned, the natural logarithm of concentration and scavenging effect was correlative and *r*s were 0.9675 and 0.9183, respectively (Table 3). FP and SP exhibited far higher superoxide radical-scavenging activities than did BHA, BHT, or α-tocopherol. The EC_{50} values (efficient concentration, defined as the concentration to inhibit 50% radical generation or scavenge 50% radical generated was usually used to express the radical-scavenging activity and was obtained by interpolation from regression analysis) of FP and SP were 6.12 and 0.88 μg/ml, respectively (Table 3), while values EC_{50} of BHA, BHT and α-tocopherol were 31, 61 and 88 μg/ml respectively (Oktay et al., 2003). Superoxide anion radical-scavenging effects of protein samples followed the order: SP > FP > CP.

3.3. Hydroxyl radical-scavenging assay

The hydroxyl radical, known to be generated through the Fenton reaction in this system, was scavenged by protein samples. Table 2 shows the % scavenging effect of protein samples and standards. For CP, at a concentration from 13.0 to 65.1 μg/ml, the % scavenging effect was from 10.6% to 69.3%. For FP, at a concentration from 1.12 to 44.9 μg/ml, the % scavenging effect was from 3.46% to 68.6%. For SP, at a concentration from 0.62 to 9.99 μg/ml, the % scavenging effect was from 17.2% to 94.5%. The correlation between scavenging effect and concentration of CP and FP was linear and *r*s were 0.9968 and 0.9957, respectively; as for SP, the correlation was logarithmic and r was 0.9722 (Table 3). Three sample proteins showed far higher hydroxyl radical-scavenging activities than did vitamin C or mannitol. The EC_{50} values of CP, FP and SP were 48.8, 45.4 and 1.52 μg/ml, but EC_{50} values of Vitamin C and mannitol was 1907 and 4536 μg/ml, respectively. The hydroxyl radical-scavenging effect of protein samples and standards followed the sequence: SP > FP > CP > Vitamin C > mannitol.

Table 2
Scavenging effect of protein samples on hydroxyl radical

Sample protein	Concentration (μg/ml)	A_{520}	Scavenging effect (%)
Crude protein	13.0	0.076 ± 0.017	10.6 ± 2.37
	26.0	0.165 ± 0.001	23.0 ± 0.14
	39.1	0.258 ± 0.002	36.0 ± 0.28
	52.1	0.396 ± 0.011	55.3 ± 1.54
	65.1	0.496 ± 0.002	69.3 ± 0.28
Control		0.716 ± 0.003	
First peak	1.12	0.022 ± 0.001	3.46 ± 0.17
	2.25	0.106 ± 0.004	16.7 ± 0.67
	4.49	0.130 ± 0.006	20.6 ± 1.00
	8.98	0.166 ± 0.001	26.1 ± 0.17
	18.0	0.230 ± 0.006	36.1 ± 1.00
	22.5	0.289 ± 0.004	45.5 ± 0.67
	44.9	0.436 ± 0.004	68.6 ± 0.56
Control		0.635 ± 0.002	
Second peak	0.62	0.109 ± 0.014	17.2 ± 2.23
	1.25	0.294 ± 0.011	46.2 ± 1.67
	2.50	0.469 ± 0.011	73.8 ± 1.67
	4.99	0.564 ± 0.004	88.7 ± 0.56
	6.24	0.575 ± 0.001	90.5 ± 0.11
	9.99	0.600 ± 0.003	94.5 ± 0.44
Control		0.635 ± 0.002	
Mannitol	200	0.022 ± 0.004	3.10 ± 0.51
	400	0.051 ± 0.006	7.35 ± 0.82
	800	0.106 ± 0.007	15.3 ± 1.02
	1600	0.201 ± 0.001	28.9 ± 0.10
	3200	0.320 ± 0.001	46.0 ± 0.10
	6400	0.431 ± 0.004	62.0 ± 0.51
Control		0.694 ± 0.003	
Vitamin C	200	0.003 ± 0.001	0.40 ± 0.14
	400	0.029 ± 0.001	4.19 ± 0.14
	800	0.161 ± 0.002	23.2 ± 0.29
	1600	0.388 ± 0.001	55.9 ± 0.14
	3200	0.540 ± 0.003	77.8 ± 0.43
Control		0.694 ± 0.003	

Values are means ± SD of three determinations.

3.4. Total antioxidant assay using the β-carotene–linoleate model system

The total antioxidant activity of protein samples and BHA at 50 μg/ml, measured by the bleaching of β-carotene, is presented in Fig. 2. CP and SP exhibited varying degrees of total antioxidant activity, and total antioxidant activity of SP was stronger than that of CP. But

Table 3
EC_{50} values, linear or logarithmic regression equations and correlation coefficients

	EC_{50} (μg/ml)		Regression equation		Correlation coefficient (*r*)	
	$O_2^{-\bullet}$	·OH	$O_2^{-\bullet}$	·OH	$O_2^{-\bullet}$	·OH
CP	112	48.8	$Y = 0.25X + 22.38$	$Y = 1.15X - 6.03$	0.9861	0.9968
FP	6.12	45.4	$Y = 32.33\text{LnX} - 8.46$	$Y = 1.90X - 18.25$	0.9675	0.9957
SP	0.88	1.52	$Y = 19.54\text{LnX} + 52.50$	$Y = 28.49\text{LnX}+38.05$	0.9183	0.9722
Vitamin C		1907		$Y = 0.027X - 0.75$		0.9665
Mannitol		4536		$Y = 0.0094X + 7.36$		0.9623

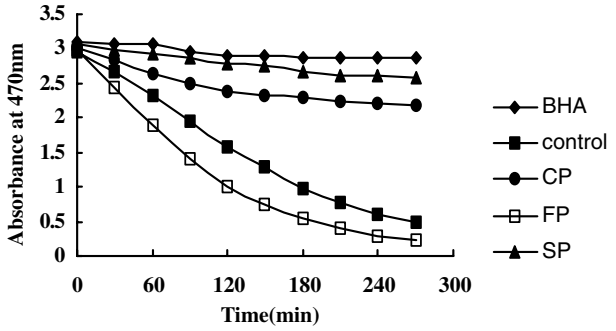

Fig. 2. Total antioxidant activity of sample proteins and BHA at 50 μg/ml in α-carotene–linoleate model system.

in this system, FP did not exhibit total antioxidant activity. The mechanism of bleaching of β-carotene is a free radical-mediated phenomenon, resulting from the hydroperoxides formed from linoleic acid. β-Carotene, in this model system, undergoes rapid discolouration in the absence of an antioxidant. The linoleic acid-free radical, formed upon the abstraction of a hydrogen atom from one of its diallylic methylene groups, attacks the highly unsaturated β-carotene molecules. As β-carotene molecules lose their double bonds by oxidation, the compound loses its chromophore and characteristic orange colour, a change which can be monitored spectrophotometrically (Jayaprakasha, Singh, & Sakariah, 2001). The presence of protein samples can hinder the extent of β-carotene-bleaching by scavenging the linoleate-free radical and other free radicals formed in the system.

3.5. Reducing power

For the measurements of the reductive ability, Fe^{3+}–Fe^{2+} transformation in the presence of protein samples was investigated. Table 4 shows the reductive capability of protein samples. In this system, reductive capability of three samples was very low, and all absorbance at 700 nm was below 0.07.

3.6. Metal chelating activity

Ferrozine can quantitatively form complexes with Fe^{2+}. In the presence of other chelating agents, the complexes formation is disturbed, with the result that the red colour of the complex is decreased. Therefore, measurement of the rate of colour reduction estimates the chelating activity of the coexisting chelator (Oktay et al., 2003; Yamaguchi, Ariga, Yoshimura, & Nakazawa, 2000). The chelating abilities of protein samples and EDTA are shown in Table 5. Of the three samples, chelating ability of FP was strongest but far lower than that of EDTA. Chelating ability was not concentration-dependent.

Table 4
Reductive power of protein samples

Sample protein	Concentration (μg/ml)	A_{700}
Crude protein	19.4	–
	38.7	0.007 ± 0.002
	77.4	0.013 ± 0.001
	116	0.014 ± 0.002
	155	0.002 ± 0.001
	194	–
First peak	1.57	0.016 ± 0.002
	3.13	0.020 ± 0.001
	6.27	0.020 ± 0.001
	12.5	0.023 ± 0.001
	18.8	0.028 ± 0.002
	25.1	0.037 ± 0.006
Second peak	0.47	0.012 ± 0.001
	0.94	0.013 ± 0.001
	1.87	0.015 ± 0.001
	3.75	0.024 ± 0.001
	7.49	0.041 ± 0.004
	15.0	0.066 ± 0.002

Table 5
Metal chelating effect of protein samples

Sample protein	Concentration (μg/ml)	A_{562}	Chelating percent (%)
Crude protein	8.08	1.23 ± 0.054	12.2 ± 3.81
	16.2	1.20 ± 0.001	14.4 ± 0.10
	24.2	1.088 ± 0.031	22.5 ± 2.18
	48.5	1.17 ± 0.004	16.6 ± 0.25
	64.6	1.04 ± 0.004	25.8 ± 0.25
	80.8	1.14 ± 0.019	18.6 ± 1.37
Control		1.40 ± 0.002	
First peak	2.35	0.864 ± 0.004	35.3 ± 0.26
	4.70	0.846 ± 0.030	36.6 ± 2.22
	9.40	0.829 ± 0.008	37.9 ± 0.53
	14.1	0.784 ± 0.078	41.3 ± 5.87
	18.8	0.790 ± 0.003	40.8 ± 0.21
	23.5	0.746 ± 0.028	44.1 ± 2.12
Control		1.34 ± 0.004	
Second peak	1.41	1.22 ± 0.004	8.39 ± 0.30
	2.81	1.22 ± 0.009	8.39 ± 0.67
	5.62	1.19 ± 0.007	11.2 ± 0.52
	8.43	1.10 ± 0.004	17.4 ± 0.30
	11.2	1.02 ± 0.002	23.3 ± 0.15
	14.1	0.973 ± 0.003	27.1 ± 0.22
Control		1.34 ± 0.004	
EDTA	30	1.50 ± 0.007	33.3 ± 0.31
	60	1.07 ± 0.006	52.4 ± 0.27
	120	0.341 ± 0.001	84.8 ± 0.04
	240	0.257 ± 0.002	88.6 ± 0.09
	480	0.169 ± 0.001	92.2 ± 0.04
	960	0.175 ± 0.001	92.5 ± 0.04
Control		2.24 ± 0.006	

The antioxidant activities of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides,

prevention of continued hydrogen abstraction, reductive capacity and radical-scavenging (Diplock, 1997). For the three protein samples, we can eliminate the mechanism of reductive capacity because of their weak reductive ability (Table 4). The antioxidation may be explained by radical-scavenging due to the strong scavenging effect on superoxide anion radicals and hydroxyl radicals (Tables 1 and 2). For hydroxyl radical, there are two type of antioxidation mechanism; one suppresses the generation of the hydroxyl radical, and the other scavenges the hydroxyl radicals generated. In the former, the antioxidant may ligate to the metal ions which react with H_2O_2 to give the metal complexes. The metal complex thus formed can not further react with H_2O_2 to give a hydroxyl radical (Ueda, Saito, Shimazu, & Ozawa, 1996). The chelating ability of samples was low, which perhaps indicates that the samples belong to the latter.

Reactive oxygen species can damage the protein. However, some antioxidants in organisms belong to protein and these proteins play an important role in the antioxidant defence system. A dietary deficiency of protein not only impairs the synthesis of antioxidant enzymes but also reduces tissue concentrations of antioxidants, thereby resulting in a compromised antioxidant status (Fang, Yang, & Wu, 2002; Sies, 1999). Antioxidant activity of protein is mainly attributed to the content of –SH, protein enzymes such as superoxide dismutase, catalase and peroxidase. The properties of protein samples from *R. esculentum* are under study.

4. Conclusion

SP shows strong superoxide anion radical-scavenging, hydroxyl radical-scavenging and total antioxidant activity. The results of this study show that SP may have a use as a possible supplement in the food and pharmaceutical industries. The antioxidant activity of protein samples may explain that jellyfish have curative effects for bronchitis, tracheitis, gastric ulcers and asthmas.

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