Algae
Volume 18(1): 71-81, 2003

Antioxidant Activity of Enzymatic Extracts from Brown Seaweeds

Soo-Jin Heo, Ki-Wan Lee, Choon Bok Song and You-Jin Jeon*

Faculty of Applied Marine Science, Cheju National University, Jeju 690-756, Korea

This work was carried out to find water-soluble natural antioxidants from seaweeds. Seven species of brown seaweeds (*Ecklonia cava, Ishige okamurae, Sargassum fulvellum, Sargassum horneri, Sargassum coreanum, Sargassum thunbergii,* and *Scytosiphon lomentaria*) growing along Jeju-Do coasts were collected and enzymatically hydrolyzed to prepare water-soluble products. Enzymes used were five carbohydrases (Viscozyme, Celluclast, AMG, Termamyl, and Ultraflo) and five proteases (Protamex, Kojizyme, Neutrase, Flavourzyme, and Alcalase) commercially available from Novozyme Co. Antioxidant activities of enzymatic extracts from seaweeds were determined using two different assays, free radical scavenging activity by 1,1-diphenyl-2-pricrylhydrazyl (DPPH) decolorization assay and inhibitory capacity of lipid peroxidation in linoleic acid. Among all the enzymatic extracts enzymatically produced from the seven species of seaweeds, the enzymatic extracts of *E. cava* scavenged most effectively free radicals released from DPPH and especially Cellulast extract of *E. cava* caused around 80% scavenging activity at the extract concentration of 8 mg/ml. The antioxidant assay in terms of inhibitory capacity of lipid peroxidation revealed that Ultraflo and Alcalase extracts of *E. cava* and Neutrase extract of *S. lomentaria* had the highest inhibitory capacity. In particular it was noteworthy that the Neutrase extract of *S. lomentaria* had completely suppressed the lipid peroxidation during five days of the incubation period. In contrast to this result observed in the lipid peroxidation those extracts were shown to have poor radical scavenging activity. These results indicate that a large difference exists between the two antioxidant activity assays mentioned above. We demonstrated in the study that enzymatic extracts of seaweeds possess a potent antioxidant activity.

Key Words: antioxidant, enzymatic extract, lipid peroxidation, radical scavenging activity, seaweed

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. Additionally it is predominant cause of qualitative decay of foods which lead to rancidity, toxicity and destruction of biochemical components important in physiologic metabolism. ROSs are known to induce disorder of DNA, cell membranes, proteins and other cellular constituents. Thus they are potential inducers of various human diseases including atherosclerosis, rheumatoid arthritis, muscular dystrophy, cataracts, some neurological disorders and some types of cancer as well as aging (Kovatcheva *et al.* 2001; Ruberto *et al.* 2001; Lim *et al.* 2002). During the last decade, various synthetic [BHT (butylated hydroxytoluene) and BHA (butylated hydroxyanisol)] and natural antioxidants (vitamin C and E, carotenoids, flavonoids, phenolic compounds) have been used to prevent or to retard the lipid oxidation by ROSs. Due to some negative side effects and toxicity of the synthetic antioxidants, however, much higher attention of investigators and consumers are focusing on natural antioxidants. But there are some disadvantages of natural antioxidants. For example, vitamin E, carotenoids, and phenolic compounds are water-insoluble. Moreover vitamin C as well as E are greatly heat-sensitive and easily denatured.

Seaweeds or their extracts have been studied as potential natural antioxidants during the last decade (Anggadiredja *et al.* 1997; Matsukawa *et al.* 1997; Tutour *et al.* 1998; Yan *et al.* 1998; Duval *et al.* 2000; Hirata *et al.* 2000; Kovatcheva *et al.* 2001; Ruberto *et al.* 2001; Xue *et al.* 2001; Lim *et al.* 2002; Ruperez *et al.* 2002). 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

*Corresponding author (youjinj@cheju.ac.kr)

from any serious photodynamic damage *in vivo*. The observations suggest that their cells have protective antioxidative mechanisms as well as antioxidative compounds (Dykens *et al.* 1992; Sukenik *et al.* 1993; Matsukawa *et al.* 1997). Additionally seaweeds are rich in vitamins, minerals, natural bioactive compounds, and various functional polysaccharides. In particular, the polysaccharides plentifully present in brown seaweeds, such as alginates, fucans and laminarans are water-soluble dietary fibers and possess various bioactive properties. These polysaccharides are little or non-digested by digestive enzymes produced by human intestine. Instead the polysaccharides are considered to be digested by some types of carbohydrases derived from microorganisms. Those kinds of enzymes can convert water-insoluble seaweeds into water-soluble materials. It is thought that different bioactive properties including antioxidant activities will be expected with the resulting smaller materials, enzymatic extracts of seaweeds. Despite of the urgent need to screen seaweeds having the antioxidant activities few reports had dealt with producing enzymatic extracts of seaweeds and investigating their bioactivities.

In the present study, some edible seaweeds were enzymatically hydrolyzed with carbohydrases and proteinases to prepare water-soluble seaweed extracts, and then antioxidant activity was examined to evaluate the potential value as water-soluble antioxidants.

MATERIALS AND METHODS

Materials

Seaweeds were collected along Jeju-Do Island coast of Korea during March and October 2001. Of the Jeju coastal seaweeds, seven species of brown algae samples (Table 1) were collected, and then salt, epiphytes and sand, that had been attached to the surface of the samples, were removed using tap water. Finally the seaweeds were rinsed carefully in freshwater and stored in a medical refrigerator at –20°C. The frozen samples were lyophilized and homogenized with a grinder to carry out the enzymatic extraction. Carbohydrase (Viscozyme L, Celluclast 1.5L FG, AMG 300L, Termamyl 120L, Ultraflo L) and Protease (Protamex, Kojizyme 500 MG, Neutrase 0.8L, Flavourzyme 500 MG, Alcalase 2.4L FG) were donated from Novo Co. (Novozyme Nordisk, Bagsvaerd, Denmark). 1,1-Diphenyl-2-pricrylhydrazyl (DPPH), thiobarbituric acid (TBA), trichloroacetic acid (TCA), butylated hydroxyanysol (BHA), butylated hydroxytoluene (BHT), α-tocopherol and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO, USA). And other chemicals used were 99% or greater purity.

Table 1. The list of brown seaweeds and collection site

Scientific name	Selection site*
Ecklonia cava	Hamdeok
Ishige okamurae	Seongsan
Sargassum fulvellum	Hamdeok
Sargassum horneri	Samyang
Sargassum coreanum	Samyang
Sargassum thunbergii	Hamdeok
Scytosiphon lomentaria	Seongsan

*Seaweeds were collected at the above places of Jeju-Do in Koera.

Approximate chemical compositions of seaweeds

Approximate chemical compositions of the seaweeds were determined according to AOAC method (1990). Crude carbohydrate was determined by phenol-sulfuric acid reaction (absorbance at 480nm, using glucose as the calibration standard), crude lipid was performed by Soxhlet method and crude ash was prepared at 550°C in the dry-type furnace. The amount of crude protein was determined by Lowry method, measuring absorbance at 540 nm using bovine serume albumin as the calibration standard (Lowry *et al.* 1951).

Preparation of enzymatic extracts from seaweeds

The seaweed samples were pulverized into powder using a grinder. A hundred m*l* of buffer solution was added to one gram of dried alga, and then 100 μl (or mg) of enzyme was mixed. The enzymatic hydrolysis reactions were performed for 12 h to achieve optimum hydrolysis. The hydrolysates were clarified by centrifugation at 3,000 rpm for 20 min to remove the residue unhydrolyzed. The degree of enzymatic hydrolysis was determined by subtracting the dried weight of the residue from one gram of seaweed samples dried and was expressed as a percentage. Enzymatic extracts of seaweeds was obtained after filtering the supernatant and were used for two assays of antioxidant activity, composed of radical scavenging activity and inhibitory capacity of lipid peroxidation. Concentrations of all the extracts were adjusted to 4 mg/m*l*.

Radical scavenging activity by DPPH decolorization

Free radical scavenging activity (RSA) of the enzymat-

Table 2. Chemical compositions of brown seaweeds (%)

Scientific name	Moisture	Ash	Protein	Carbohydrate	Lipid
Ecklonia cava	4.29	15.41	10.55	68.42	1.33
Ishige okamurae	4.49	13.26	18.91	60.69	2.66
Sargassum fulvellum	5.13	17.85	14.20	62.49	0.33
Sargassum horneri	5.19	20.83	17.23	55.45	1.33
Sargassum coreanum	4.30	12.77	14.40	67.20	1.33
Sargassum thunbergii	8.90	13.30	13.85	63.62	0.33
Scytosiphon lomentaria	4.62	24.37	16.85	53.49	0.67

ic extracts of seaweeds was determined by using stable free radical, DPPH, according to the modified method of Blois(1958). DPPH solution was prepared at the concentration of $4x10^{-4}$ M in ethyl alcohol. During the assay, the enzymatic extract of 0.1 m*l* was mixed with 2.9 m*l* DPPH solution. The mixture was incubated in the room temperature for 30 min. After standing for 30 min, absorbance was read at 516 nm, and the percentage of inhibition was defined by the absorbance at 516 nm in the absence of enzymatic extract to that measured with the sample. Commercial antioxidants such as α-tocopherol, BHA and BHT, whose concentrations were 2 mg/m*l*, respectively, were assayed together as controls.

Total phenolic assay

Phenolic contents were determined using the protocol similar to Chandler and Dodds (1983) described by Shetty *et al.* (1995). Each 1.0 m*l* of seaweed enzymatic extracts, 1.0 m*l* of 95% EtOH, 5.0 m*l* of distilled water, and 0.5 m*l* of 50% Folin-Ciocalteu reagent (Sigma Chemical, St. Louis, MO) were mixed and then were allowed to react for 5 min. 1.0 m*l* of 5% Na_2CO_3 was added, and the mixture was vortexed and placed in the dark for 1 h. Absorbance was measured at 725 nm using gallic acid as the calibration standard.

Inhibitory capacity of lipid peroxidation in linoleic acid

The assay for inhibition of lipid peroxidation in linoleic acid was carried out in linoleic acid system with and without (control group) added antioxidants according to the modified method of Mitsuda *et al.* (1996) and Sidwell *et al.* (1954). The substrate solution was made by mixing 0.1 M phosphate buffer (pH 7.0) and ethanol (4:1, v/v). Then 20 m*l* of substrate solution, 0.8 m*l* sample solution and 0.1 M phosphate buffer 19.2 m*l* were mixed and incubated in 40°C for 5 days. During incubation, 2 m*l* of each mixture was taken every 24 h interval. Then each 2.0 m*l* of mixture, 1.0 m*l* of 35% TCA and 2.0 m*l* of 0.75% TBA were mixed and placed in a boiling water bath for 40 min. The tubes were cooled and 1.0 m*l* of acetic acid and 2.0 m*l* of chloroform were added. Then the mixture was throughly mixed and centrifuged at 4,000 rpm for 5 min. Absorbance of the supernatant was measured at 532 nm and TBA values were calculated.

RESULTS

Approximate chemical compositions

Approximate chemical compositions of seven species of brown seaweeds, *Ecklonia cava, Ishige okamurae, Sargassum fulvellum, Sargassum horneri, Sargassum coreanum, Sargassum thunbergii* and *Scytosiphon lomentaria*, collected along Jeju-Do coasts were shown in Table 2. The major chemical component of the seaweeds tested was found to be carbohydrate whose content occupied over 60% of the total dry weight, except for *S. lomentaria* (53.49%). Protein contents determined from the seaweeds were between 10.6% and 18.9% showing *I. okamurae* has the highest protein content. Ash contents of most of the tested seaweeds were around 15% but *S. horneri* and *S. lomentaria* showed the value of 20.8% and 24.4%, respectively.

Preparation of enzymatic extracts from seaweeds

The seaweeds were enzymatically hydrolyzed by using the five types of carbohydrases (Viscozyme, Celluclast, AMG, Termamyl, and Ultraflo) and five proteases (Protamex, Kojizyme, Neutrase, Flavourzyme, and Alcalase) to produce enzymatic extracts as potential natural water-soluble antioxidants. As shown in Table 3, most of the seaweeds showed much higher susceptibility to the action of carbohydrases than proteases. Among the five kinds of carbohydrases, particularly, Viscozyme, Celluclast and AMG exhibited more than 30% of the hydrolytic degree for most seaweeds, except *S. lomentaria*. And these enzymes were able to hydrolyze around 40% for *E. cava*. The higher degree of enzymatic

Table 3. The degree of enzymatic hydrolysis* of the brown seaweeds (%)

		Ecklonia cava	*Ishige okamurae*	*Sargassum fulvellum*	*Sargassum horneri*	*Sargassum coreanum*	*Sargassum thunbergii*	*Scytosiphon lomentaria*
Carbo-hydrase	Viscozyme	39.59	33.79	24.51	29.99	30.30	31.48	15.01
	Celluclast	40.66	23.17	26.45	35.40	30.64	30.48	15.12
	AMG 300L	41.52	17.48	27.94	22.63	33.24	33.87	9.47
	Termarmyl	23.29	6.32	22.12	19.15	17.98	19.18	16.50
	Ultraflo	28.26	12.6	26.82	20.01	29.03	26.47	17.82
Protease	Protamex	28.12	9.62	16.97	27.43	21.30	24.97	18.37
	Kojizyme	20.94	9.51	14.06	25.33	15.46	13.07	2.80
	Neutrase	26.69	14.30	10.93	30.64	26.72	15.03	14.42
	Flavourzyme	32.81	17.01	19.07	35.51	19.51	15.30	14.26
	Alcalase	42.72	28.93	11.65	40.04	40.68	30.51	30.91

*The reaction for enzymatic hydrolysis was carried out under following conditions: a mixture of 1 gram algae dried and 100 μl(mg) enzyme in 100 m*l* buffer solution was incubated for 12 hr and the degree of enzymatic hydrolysis was expressed by percentage of hydrolyzed portion per total algae sample.

Table 4. RSA (Radical Scavenging Activity)*1 for enzymatic extracts of the seaweeds (%)

		Ecklonia cava	*Ishige okamurae*	*Sargassum fulvellum*	*Sargassum horneri*	*Sargassum coreanum*	*Sargassum thunbergii*	*Scytosiphon lomentaria*
Carbo-hydrase	Viscozyme	71.49	15.98	4.40	N.D*2	38.41	9.70	N.D
	Celluclast	72.46	12.77	11.62	N.D	33.79	6.48	N.D
	AMG 300L	70.17	12.40	3.34	N.D	32.56	8.41	N.D
	Termarmyl	62.90	23.03	19.91	11.18	37.15	16.78	16.00
	Ultraflo	28.66	N.D	N.D	N.D	7.97	N.D	N.D
Protease	Protamex	68.16	9.70	20.14	9.44	37.98	23.12	17.84
	Kojizyme	66.49	24.21	20.45	8.26	40.62	20.71	19.33
	Neutrase	66.64	25.42	20.42	11.70	40.56	20.14	18.70
	Flavourzyme	32.64	N.D	N.D	N.D	11.56	N.D	N.D
	Alcalase	2.61	N.D	N.D	N.D	N.D	N.D	N.D

*1RSA was determined by DPPH decolorization assay

*2N.D : Not Detected.

Activity of commercial antioxidants (Tocopherol 89.64%; Ascorbic acid 94.58%; BHA 87.38%; BHA 56.05%)

hydrolyses of *E. cava* seemed to reflect the higher content of carbohydrate in this seaweed. In contrust to this, *S. lomentaria* had the lowest content of carbohydrate. Enzymatic hydrolyses of the seaweeds by the proteases were less efficient than those by the carbohydrases, even though Alcalase showed similar hydrolytic levels compared to the three types of carbohydrases (Viscozyme, Celluclast and AMG). *E. cava* showed the highest susceptibility to the enzymes treated, which was followed by *S. horneri* and *S. coreanum*.

Radical scavenging activity measured by DPPH decolorization

Antioxidant activities of various enzymatic extracts from the different seaweed species were estimated by measuring RSA with DPPH decolorization (Table 4). Great variations in the activities among different seaweed species and among different enzymatic extracts were observed. The extracts prepared from enzymatic hydrolyses of *E. cava* other than the extracts with Ultraflo, Flavourzyme and Alcalase indicated very strong RSA, showing around 70% activity. Table 3 and 4 showed that the enzymatic extracts prepared with carbohydrases had a hydrolytic level-dependent increase in the RSA activities. In case of the hydrolyses by proteases, however, the results were quite contrasted to those obtained by carbohydrases. The extracts obtained by treating proteases, namely Protamex, Kojizyme and

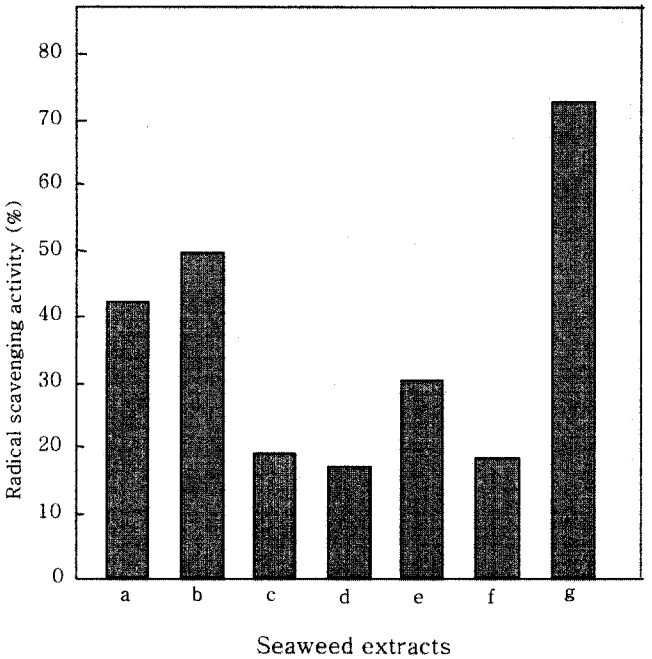

Fig. 1. Comparisons on radical scavenging activities between various organic solvent extracts and enzymatic extract of *E. cava*. a: EtOH ext., b: MeOH ext., c: Ether ext., d: Chloroform ext., e: Acetone ext., f: Hexane ext., g: Celluclast ext.

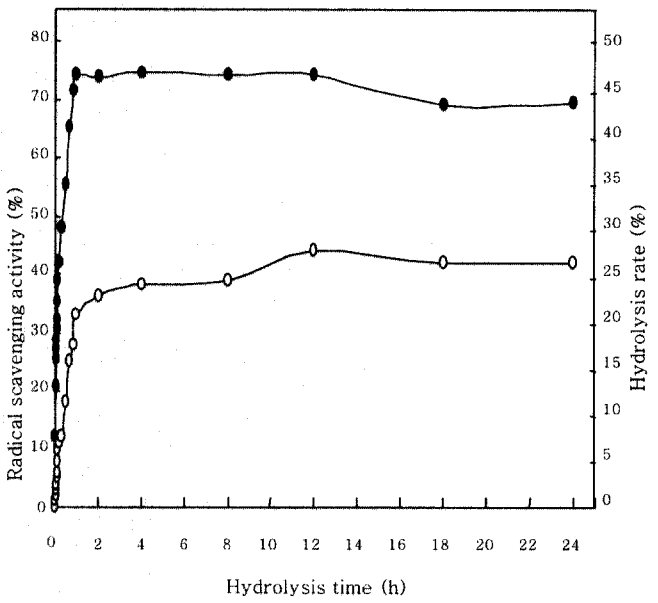

Fig. 2. Changes in hydrolysis rate and radical scavenging activity of *E. cava* Celluclast extract during enzymatic hydrolysis at 50°C. (—●— Radical scavenging activity, —○— Degree of hydrolysis)

Neutrase exhibited relatively high values of RSA, in spite of a lower degree of hydrolysis, which ranged between 20% and 30%. Meanwhile, the extract prepared with Alcalase, a protease, showed very low level of RSA (2.6%), even though relatively high degree of hydrolysis was achieved (42.7%).

The seaweed which possessed the second highest level of RSA next to *E. cava* was *S. coreanum*, whose activities were quite comparable to those of *E. cava*. The activity values were at the range of 30% to 40% of RSA for those with proteases other than Ultraflo, Flavourzyme and Alcalase, whose activities were less than 12%. The enzymatic extracts from the other seaweed species listed in Table 4 did not show any activity, regardless of enzyme types used for enzymatic hydrolysis. The results indicated that the enzymatic extracts of different seaweeds prepared with different enzymes exhibited quite different values of RSA and that the enzymatic extracts of *E. cava* obtained by Viscozyme, Celluclast, and AMG was the most effective antioxidants in terms of RSA. These values were by about 20% lower than those of representative natural antioxidants, namely, tocopherol and ascorbic acid.

The Celluclast extract of *E. cava*, which was found to be the most effective enzymatic extract of *E. cava*, was compared to the extracts produced with various organic solvents in terms of their RSA (Fig. 1). Among them the methanol extract of *E. cava* exhibited RSA value as much as almost 50%. This value is around 20% lower than Celluclast extract of *E. cava*.

Fig. 2 shows the change of RSA with increase in the degree of hydrolysis. RSA values of Celluclast extract of *E. cava* considerably increased in parallel with the increase of hydrolysis rate, which sharply increased within 2 min of incubation time. No further increase in RSA was observed during the incubation time period when the hydrolysis rate achieved a steady state. RSA levels of the enzymatic extract were dependent on the concentration of the enzymes reaching around 80% level of the activity at 8 mg/ml of the extract concentration.

Further analysis was carried out in terms of phenolic content of the extracts to clarify their effectiveness in the antioxidant activity (Table 5). *E. cava, I. okamurae, S. fulvellum S. coreanum*, and *S. thunbergii* unhydrolyzed had phenolic compounds at a higher level than 10 mg/g, especially *E. cava, S. coreanum* and *S. thunbergii having* the content of the compounds higher than 15 mg/g. The enzymatic extracts having higher values of RSA contained distinctly larger amounts of phenolic compounds. In particular, Viscozyme, Celluclast and AMG, which are carbohydrases, extracted approximately 90% of the phe-

Table 5. Phenolic contents in enzyme extracts of seaweeds. (mg phenolic componud / g seaweed)

		Ecklonia cava	*Ishige okamurae*	*Sargassum fulvellum*	*Sargassum horneri*	*Sargassum coreanum*	*Sargassum thunbergii*	*Scytosiphon lomentaria*
	Unhydrolysed	15.6	14.1	10.7	3.3	15.6	15.2	2.1
Carbo-hydrase	Viscozyme	13.5	2.7	2.5	1.8	11.2	3.8	1.4
	Celluclast	13.5	2.3	2.3	1.6	10.6	3.1	1.1
	AMG 300L	13.4	2.2	2.6	1.6	10.9	3.5	1.1
	Termarmyl	11.6	2.1	2.4	2.7	9.4	2.6	1.1
	Ultraflo	10.3	2.7	3.1	3.8	7.4	2.6	1.4
Protease	Protamex	12.4	2.3	2.3	2.4	9.9	2.9	1.2
	Kojizyme	12.1	1.9	1.9	1.9	9.9	2.3	1.1
	Neutrase	12.3	2.4	2.2	2.5	9.5	2.7	1.3
	Flavourzyme	10.8	2.8	2.2	3.1	8.1	2.5	1.4
	Alcalase	10.9	4.2	3.6	5.3	9.6	4.1	2.0

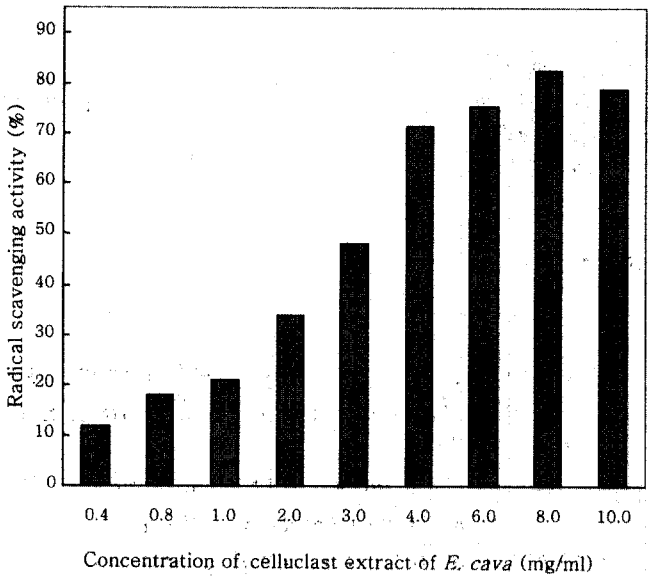

Fig. 3. Changes in radical scavenging activity of Celluclast extract from *E. cava* as affected by concentration of the extract.

nolic compounds present in the original seaweeds when the hydrolysis was completed. Ultraflo, Flavourzyme and Alcalase, which are proteases, also extracted around 60% of the phenolic compounds, although they showed lower activities of RSA. This fact suggested that phenolic compounds of seaweeds might not be a sole factor influencing the level of RSA, even though they have a significant effect on the activity. Fig. 3 revealed that RSA of Celluclast extract of *E. cava* was dependent on the increased concentration of samples and reached around 85% at a concentration of 8 mg/ml.

Inhibitory capacity of lipid peroxidation in linoleic acid

Another assay for the antioxidant activity was performed in linoleic acid system to evaluate the capacity of the enzymatic extracts to inhibit lipid peroxidation in linoleic acid and the amount of lipid peroxides generated from linoleic acid was expressed as TBA value which is considered to the level of antioxidant activity.

Fig. 4 shows the TBA values of lipid peroxidation against linoleic acid by the enzymatic extracts prepared using the carbohydrases. Fig. 5 shows the TBA values obtained by applying the extracts produced with the proteases. The control group in which no seaweed extract or synthetic antioxidant was added rapidly oxidized to produce lipid peroxides during the incubation period of 5 days, while the respective commercial antioxidants such as tocopherol, BHA and BHT completely inhibited the oxidation. Among the enzymatic extracts prepared from seven species of seaweeds, Ultraflo and Alcalase extract of *E. cava*, Celluclast extract of *S. horneri* and Neutrase extract of *S. lomentaria* could effectively suppress the lipid peroxidation. In particular Neutrase extract of *S. lomentaria* almost completely inhibited the lipid peroxidation to a comparable level with tocoperol, BHA or BHT.

Apart from this observation, a remarkable difference was found between the both assay methodologies for the antioxidant activity; the extracts which were found to possess antioxidant activities when measured by the TBA-based assay showed no or very little RSA level, when were measured by the method of DPPH decolorization. For example, although Ultraflo or Alcalase

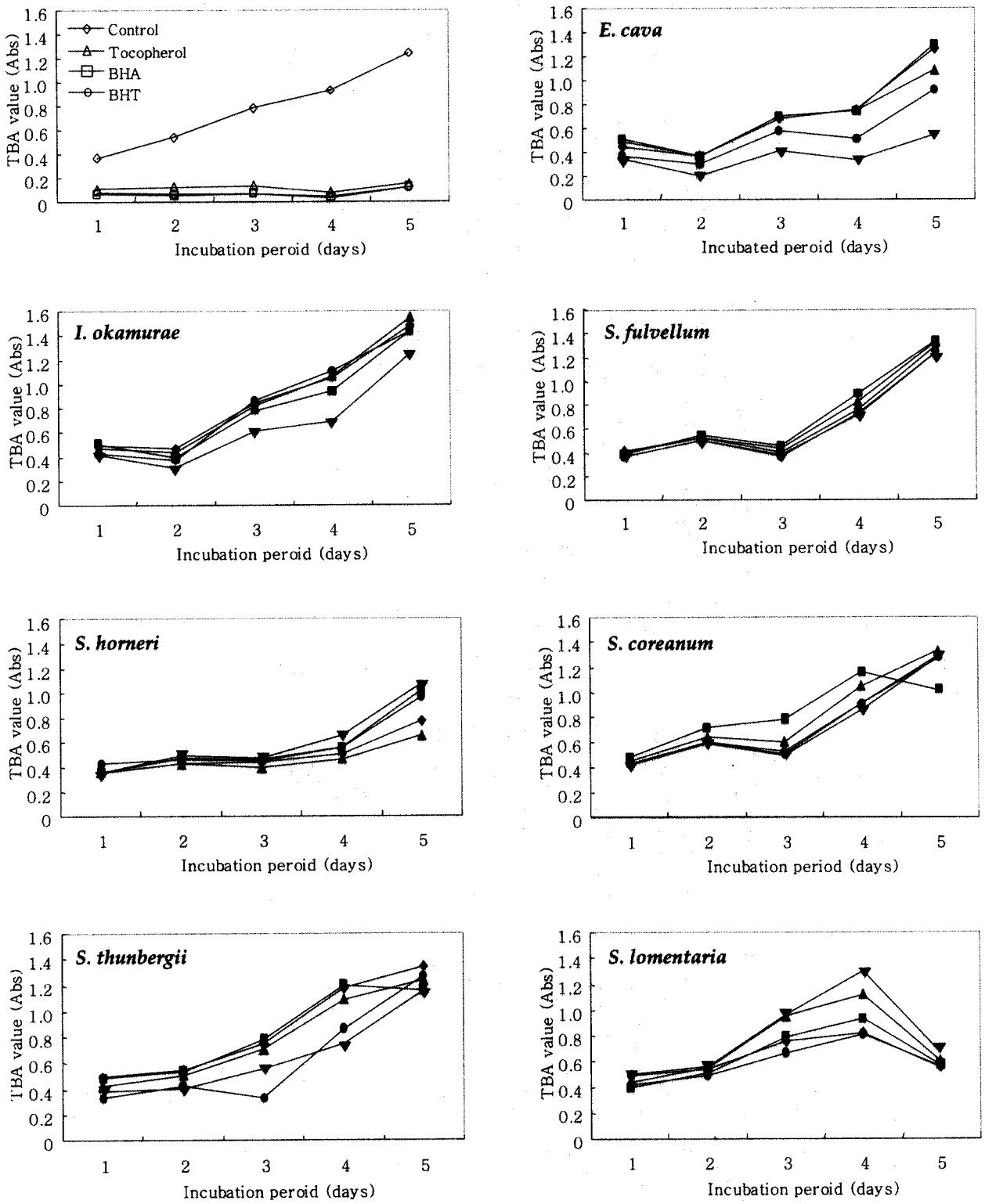

Fig. 4. Antioxidant effect of carbohydrase extracts from seaweeds on inhibition of lipid peroxidation in linoleic acid with and without (control) antioxidants. The activity was carried out by TBA assay at 532 nm.
—◆— Viscozyme ext.; —▲— Celluclast ext.; —■— AMG ext.; —●— Termamyl ext., —▼— Ultraflo ext.

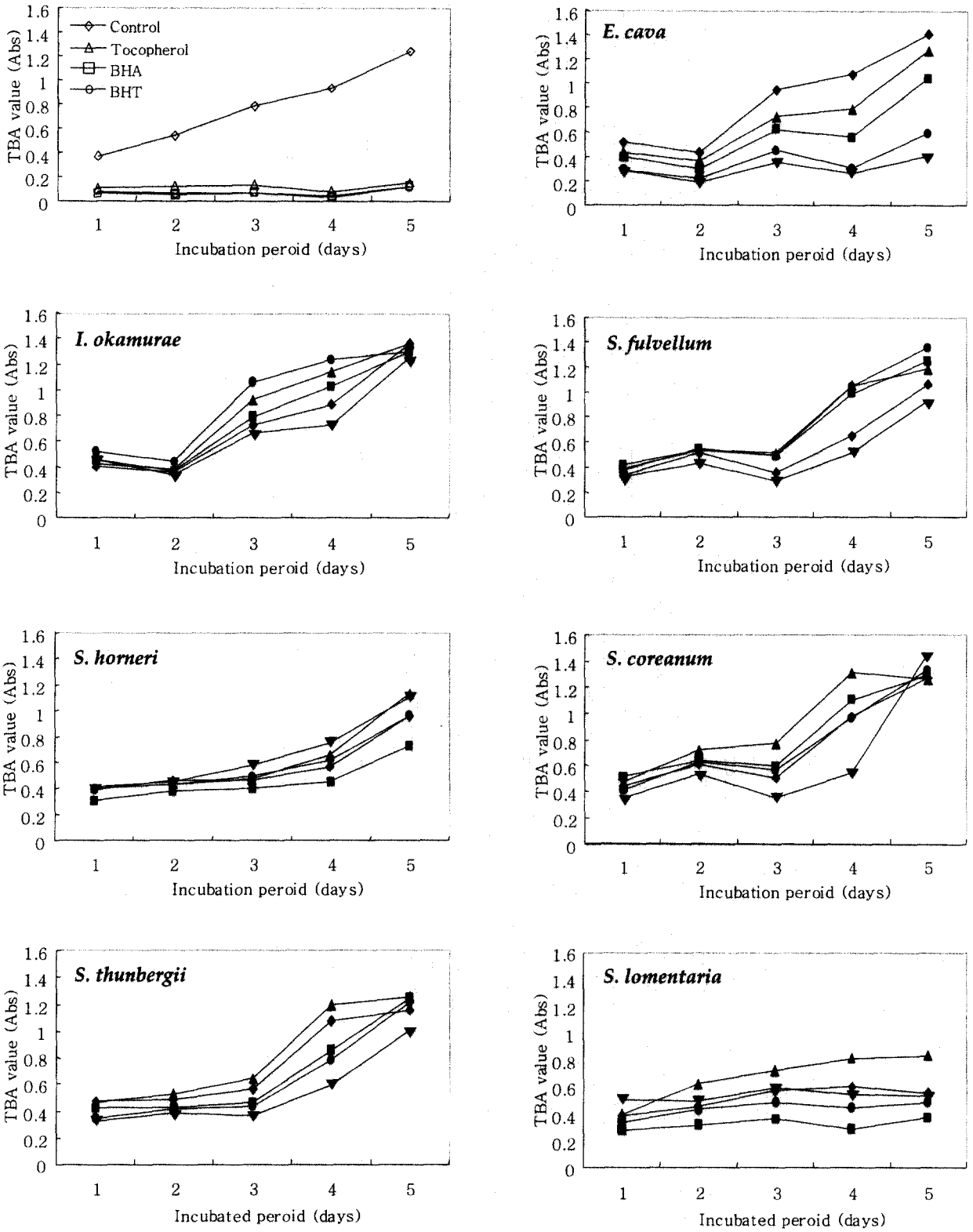

Fig. 5. Antioxidant effect of protease extracts from seaweeds on inhibition of lipid peroxidation in linoleic acid with and without (control) antioxidants. The activity was carried out by TBA assay at 532 nm.

—◆— Protamex ext.; —▲— Kojizyme ext.; —■— Neutrase ext.; —●— Flavourzyme ext., —▼— Alcalase ext.

extract exhibited lower levels of RSA, their inhibitory capacity of lipid peroxidation displayed higher activities. The result suggest that there seems to be some contradictions in the evaluation of the antioxidant activity of seaweeds between the assay of TBA value and RSA.

DISCUSSION

Recently many researchers are interested in finding any natural antioxidants having safety and effectiveness, which can be substituted for current commercial synthetic antioxidants, BHA and BHT. Seaweeds have become good candidates for the source of natural antioxidants due to a number of studies recently revealed (Fujimoto and Kaneda 1984; Cahyana *et al.* 1992; Lee *et al.* 1996; Yan *et al.* 1996; Matsukawa *et al.* 1997; Yan *et al.* 1998; Yan *et al.* 1999; Ruperez *et al.* 2002; Lim *et al.* 2002)

In this study, seven species of brown seaweeds inhabiting the costal area of Jeju-Do coasts were collected and enzymatically hydrolyzed to produce water-soluble seaweed extracts having effective antioxidant activities. Most seaweeds tested showed a possibility to enzymatically produce the water-soluble extract from seaweeds, with enzymatic extracts of *E. cava* being the most effective (the hydrolytic levels between about 20% and 40%). This fact implies that seaweed extracts will be able to be produced in commercial scale. Most of reports so far have dealt with organic solvent extracts of seaweeds which are water-insoluble materials in a small amount.

Seven kinds of enzymatic extracts from *E. cava*, that showed higher hydrolytic degrees, exhibited higher antioxidant activities (around 70%) in RSA assay by DPPH decolorization. The results implied that higher degree of hydrolysis induced higher antioxidant activity, except for the Alcalase extract.

Above all Celluclast extract had the highest and superior antioxidant activity to various organic extracts of *E. cava*, whose activities was less than 50%. The content of phenolic compounds in the enzymatic extracts as shown in Table 5 suggested that phenolic compound could be a positive effector of the antioxidant activity when examined by RSA assay. The enzymatic extracts of *E. cava* were the richest in phenolic compounds and those of *S. coreanum* ranked the second most abundant. The results are consistent with the observation that the extracts of *E. cava* showed the highest activity of antioxidation, followed by those of *S. coreanum*. Previous reports proposed that phytophenolic compounds are closely associated with antioxidative action in biological systems, acting as scavengers of singlet oxygen and free radicals (Rice-Evans *et al.* 1995; Jorgensen *et al.* 1999; Duval *et al.* 2000). Considering the fact that some enzymatic extracts of *E. cava* and *S. coreanum*, especially Alcalase extracts, did not have any antioxidant activity, despite they contain as much phenolic compounds as the other extracts of *E. cava*, it is noted that the amount of phenolic compounds might not play a sole role in determining the activity. It is thought that unknown compounds which might be produced from seaweeds during enzymatic reaction, such as small molecular weight polysaccharides, proteins or some organic compounds, probably influence the activity.

When the antioxidant activity was assayed in terms of inhibitory effect of lipid peroxidation in linoleic acid, Ultraflo and Alcalase extract of *E. cava* were the most excellent. The results were quite contrasting to the RSA assay for these extracts, in which no or only a little activity of antioxidation was observed. Additionally not only Neutrase extract of *S. lomentaria* completely inhibited lipid peroxidation, but some enzymatic extracts prepared with proteases such as Flavourzyme, Protamex and Alcalase also displayed considerably excellent effects. However, it was quite remarkable that these extracts did not show any antioxidant activity when determined by RSA assay. These results suggest that there is no correlation between the evaluation system of antioxidant activity based on RSA assay and that based on the inhibitory effect of lipid peroxidation in linoleic acid. Matsukawa *et al.* (1997) also pointed out a lack of correlation between lipoxygenase inhibition, which corresponds to inhibition of lipid peroxidation formation discussed in the current study, and RSA assay, noting that the two assay systems of the activity had unrelated mechanisms. Additionally they mentioned that antioxidant activity measured by lipoxygenase inhibition might block the enzymatic addition of oxygen to the fatty acid substrate and the formation of the hydroperoxide. In our results, the two types of assays for determining antioxidant activities of seaweed enzymatic extracts were carried out in different media systems, with RSA in polar system and inhibition of lipid peroxidation in non-polar system, respectively. The extracts in RSA assay act as electron or hydrogen donors for DPPH and those in lipid peroxidation assay act as inhibitors for the formation of lipid peroxides.

The present study demonstrated that the enzymatic extracts of seaweeds, especially carbohydrase extracts of *E. cava* showed positive effect for RSA by DPPH decol-

orization assay and that protease extracts of *S. lomentaria* and *E. cava* showed the strongest activity of antioxidant when measured by inhibition of lipid peroxidation in linoleic acid. There is no report so far on the study of antioxidant activities with enzymatic extracts of seaweeds. Enzymatic extraction of seaweeds for the purpose of obtaining natural antioxidant substances would provide several potential advantages: water solubility, and simple and large scale production process of antioxidant extracts from seaweeds. Further study is required for identification of antioxidant active compounds from enzymatic extracts of seaweeds.

ACKNOWLEDGEMENTS

This work was supported by the Brain Korea 21 Project in 2002.

REFERENCE

AOAC. 1990. *Official Method of Analysis of the Association of Official Analytical Chemists* (15th ed.), Washington D.C.

Anggadiredja J., Andyani R., Hayati and Muawanah. 1997. Antioxidant activity of *Sargassum polycystum* (Phaeophyta) and *Laurencia obtusa* (Rhodophyta) from Seribu Islands. *J. Appl. Phycol.* **9:** 477-479.

Blois M.S. 1958. Antioxidant determination by the use of a stable free radical. *Nature* **181:** 1199-1120.

Cahyana A.H., Shuto, Y. and Kinoshita, Y. 1992. Pyropheophytin *a* as an antioxidative substance from the marine alga, Arame (*Eisenia bicyclis*). *Biosci. Biotechnol. Biochem.* **56:** 1533-1535.

Chandler S.F. and Dodds J.H. 1983. The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum laciniatum*. *Plant Cell Rep.* **2:** 105-110.

Duval B., Shetty K. and Thomas W.H. 2000. Phenolic compounds and antioxidant properties in the snow alga *Chlamydomonas nivalis* after exposure to UV light. *J. Appl. Phycol.* **11:** 559-566.

Dykens J.A., Shick J.M., Benoit C., Buettner G.R., and Winston G.W. 1992. Oxygen radical production in the sea anemone *Anthopleura elegantissima* and its endosymbiotic algae. *J. Exp. Biol.* **168:** 219-241.

Fujimoto K. and Kaneda T. 1984. Separation of antioxygenic (antioxidant) compounds from marine algae. *Hydrobiologia* **116/117:** 111-113.

Hirata T., Tanaka M., Ookie M., Tsunomura T. and Sakaguchi M. 2000. Antioxidant activities of phycocyanobilin prepared from *Spirulina platensis*. *J. Appl. Phycol.* **12:** 435-439.

Jorgensen L.V., Madsen H.L., Thomsen M.K., Dragsted L.O. and Skibsted L.H. 1999. Regulation of phenolic antioxidants from phenoxyl radicals: An ESR and electrochemical study of antioxidant hierarchy. *Free Rad. Res.* **30:** 207-220.

Kovatcheva E.G., Koleva I.I., Ilieva M., Pavlov A., Mincheva M. and Konushlieva M. 2001. Antioxidant activity of extracts from *Lavandula vera* MM cell cultures. *Food Chem.* **72:** 295-300.

Lee J.H., Park J.C. and Choi J.S. 1996. The antioxidant activity of *Eclonia stolonifera*. *Arch. Pharm. Res.* **19:** 223-227.

Lim S.N., Cheung P.C.K., Ooi V.E.C. and Ang P.O. 2002. Evaluation of antioxidative activity of extracts from a brown seaweed, *Sargassum siliquastrum*. *J. Agric. Food Chem.* **50:** 3862-3866.

Lowry O.H., Rosebrough N.J., Farr, L. and Rindall, R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193:** 256-259.

Matsukawa R., Dubinsky Z., Kishimoto E., masak, K., Masuda Y., Takeuchi T., Chihara M., Yamamoto Y., Niki E. and Karube, I. 1997. A comparison of screening methods for antioxidant activity in seaweeds. *J. Appl. Phycol.* **9:** 29-35.

Mitsuda H., Yasumoto K. and Iwami K. 1996. Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Nippon Eiyo Shokuryo Gakkaishi.* **49:** 210-217.

Rice-Evans C.A., Miller N.J., Bolwell P.G., BramLey P.M. and Pridham J.B. 1995. The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Rad. Res.* **22:** 375-383.

Ruberto G., Baratta M.T., Biondi D.M. and Amico V. 2001. Antioxidant activity of extracts of the marine algal genus *Cystoseira* in a micellar model system. *J. Appl. Phycol.* **13:** 403-407.

Ruperez P., Ahrazem O. and Leal J.A. 2002. Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosus*. *J. Agric. Food.* **50:** 840-845.

Shetty K., Curtis O.F., Levin R.E., Witkowsky R. and Ang, V. 1995. Prevention of vitrification associated with the in vitro shoot culture of oregano (*Origanum vulgare*) by *Pseudomonas spp.* *J. Plant. Physiol.* **147:** 447-451.

Sidwell C.G., Salwin H.M. and Mitchell Jr.J.H. 1954. The use of thiobarbituric acid as a measure of fat oxidation. *J. Am. Oil. Chem. Soc.* **31:** 603-609.

Sukenik A., Zmora O. and Carmeli Y. 1993. Biochemical quality of marine unicellular algae with special emphasis lipid composition: II. *Nannochloropsis* sp. *Aquaculture* **117:** 313-326.

Tutour B.L., Benslimane F., Gouleau M.P., Gouygou J.P., Saadan B. and Quemeneur F. 1998. Antioxidant and pro-oxidant activities of the brown algae, *Laminaria digitata, Himanthalia elongata, Fucus vesiculosus, Fucus serratus* and *Ascophyllum nosum*. *J. Appl. Phycol.* **10:** 121-129.

Xue C.-H., Fang Y., Lin H., Chen L., Li Z.-J., Deng D. and Lu C.-X. 2001. Chemical characters and antioxidative properties of sulfated polysaccharides from *Laminaria japonica*. *J. Appl. Phycol.* **13:** 67-70.

Yan X.J., Chuda Y., Suzuki M. and Nagata T. 1999. Fucoxanthin as the major antioxidant in *Hijikia fusiformis*, a common edible seaweed. *Biosci. Biotechnol. Biochem.* **63:** 605-607.

Yan X.J., Li X.C., Zhou C.X. and Fan X. 1996. Prevention of fish oil rancidity by phlorotannins from *Sargassum kjellmanianum*. *J. Appl. Phycol.* **8:** 201-203.

Yan X.J., Nagata T. and Fan X. 1998. Antioxidative activities in some common seaweeds. *Plant Foods for Hum. Nutrit.* **52:** 253-262.

Accepted 10 February 2003