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Antioxidant properties of dried 'kayamo-nori', a brown alga *Scytosiphon lomentaria* (*Scytosiphonales, Phaeophyceae*)

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Abstract

Dried *Scytosiphon lomentaria*, a brown alga, is a traditional food in the Noto area in Japan. To determine the antioxidant properties of the dried *S. lomentaria*, assays for antioxidant activities, including suppression of hemoglobin-induced linoleic acid peroxidation, reducing power, ferrous ion chelating, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging and scavenging of a superoxide anion radical-generated non-enzymatic system were tested in this study. A water extract contained total phenols at about 5.5 mg catechin equivalents (CatE)/g dry sample (DS) and showed strong antioxidant activities in all five assays, especially in the linoleic acid peroxidation assay (about 22 mg CatE/g DS). On the other hand, the antioxidant activities of an ethanol extract were not detected or were very low compared with the water extract. The dietary fibres (crude fucoidan and alginate) showed antioxidant activity, not only in the ferrous chelating (binding) assay, but also in the superoxide anion radical scavenging assay. These results suggest that *S. lomentaria* is useful seafood and a healthy food having antioxidant activity.

Keywords: *Scytosiphon lomentaria*; Antioxidant property; Radical scavenging activity; Reducing power

1. Introduction

A brown alga, whip tube *Scytosiphon lomentarius* (Lyngbye) Link, called 'kayamo-nori' in Japan, is widely distributed all over the world, for example in the coasts of Japan, China, Taiwan, Australia, West of USA, the Atlantic Ocean and the Black Sea (Kamenarska, Dimitrova-Konaklieva, Stefanov, & Popov, 2003; Segawa, 1996). *Scytosiphon* is a very simple type of brown alga; for example, each cell contains only one chloroplast and one or two Golgi bodies that are always close to the centrosome (Cleyton & Beakes, 1983). The shape of *S. lomentaria* is an aggregate of dozens of leaves that are 3–8 mm in diameter and 20–50 mm in length with constrictions. Although *S. lomentaria* grows well in many coasts in Japan in spring, it is consumed as an edible alga only in a few areas. In the Noto area, Ishikawa, the dried product of the alga is consumed as a traditional food. Usually, the alga is eaten after drying and roasting lightly, like a dried product of nori *Porphyra* spp., a red alga.

Oxidative modification of DNA, proteins, lipid and small cellular molecules by reactive oxygen species (ROS) plays a role in a wide range of common diseases and age-related degenerative conditions (Borek, 1993). These include cardiovascular disease, inflammatory conditions, and neurodegenerative disease such as Alzheimer's disease (Rechardson, 1993), mutations and cancer (Byres & Guerrero, 1995). Furthermore, interest in employing antioxidants from natural sources to increase shelf life of foods is considerably enhanced by consumer preference for natural ingredients and concerns about the toxic effects of synthetic antioxidants (Schwarz et al., 2001).

There are publications about the antioxidant activity of seaweeds (Matsukawa et al., 1997; Yan, Nagata, & Fan, 1998). Though these studies are mainly confined to non-edible seaweeds, Jiménez-Escrig, Jiménez-Jiménez,

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Pulido, and Saura-Calixto (2001) reported antioxidant activity of fresh and processed edible seaweeds and indicated strong antioxidative activity of *Fucus vesiculosus*, a brown alga, using 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging assay and ferric-reducing antioxidant power (FRAP) assay. There are many reports about *S. lomentaria* in the research areas of biology, such as cell science, botanical biology and phycology (Nagasato & Motomura, 2002a, 2002b). However, there are hardly any reports about any functional compounds, such as antioxidants and dietary fibres, and other nutrients of *S. lomentaria*.

The aim of the present work was to evaluate the profitable properties of *S. lomentaria* for human food. We investigated the antioxidant activities of water extract, water-soluble low-molecular weight extract, ethanol extract, crude fucoidan (a sulfated fucan) and alginate of dried *S. lomentaria* by linoleic acid peroxidation assay, reducing power assay, ferrous ion chelating assay, DPPH radical assay, and non-enzymatic superoxide anion scavenging assay. These antioxidant assays employ methodology widely used for plant foods.

2. Material and methods

2.1. Material

S. lomentaria was harvested in Wajima, Ishikawa, Japan in March 2003. The harvested material was spread in a mesh bottom frame (about 30×30 cm) and dried like nori. The dried product was purchased from a retail shop in Noto and used in this study.

2.2. Chemicals

Catechin (Cat), Folin–Ciocalteu's phenol reagent, the stable free radical DPPH, nitroblue tetrazolium salt (NBT), phenazine methosulphate (PMS), 3-(2-pyridyl)-5,6-di (*p*-sulfophenyl)-1,2,4-triazine, disodium salt (ferrozine), ethylenediaminetetraacetic acid (EDTA) and ammonium thiocyanate were purchased from Sigma–Aldrich Co. (St. Louis, MO). Linoleic acid, ascorbic acid (AA) and hemoglobin were purchased from Wako Chemicals Co., Ltd. (Osaka, Japan). Other reagents were of analytical grade.

2.3. Preparation of sample extract

The dried product sample (2 g) was weighed and 50 ml of distilled water or ethanol were added. The water extract solution (WE) was collected after autoclave treatment (121 °C for 15 min) and centrifugation ($2220g \times 10$ min). The ethanol extract solution (EE) was collected after shaking for 4 h at room temperature and centrifugation. For collecting water-soluble and low molecular extract (LE), 5 ml of WE were added to 15 ml ethanol and centrifuged. The supernatant was concentrated to 5 ml with N_2 flashing. Dietary fibres, crude fucoidan (CF) and crude alginate (CA), in *S. lomentaria* were extracted using the alcohol precipitation method (Kuda, Taniguchi, Nishizawa, & Araki, 2002). CF and CA (0.5 g) were dissolved in distilled water (100 ml).

2.4. Determination of the total phenol content

Total phenol content of WE, LE and EE were determined by a modification of the Folin–Ciocalteu method (Oki et al., 2002). Briefly, 0.4 ml of 10% Folin–Ciocalteu solution was added to 0.2 ml of a sample solution. After an interval of 3 min, 0.8 ml of a 10% sodium carbonate was added. The mixture was allowed to stand for 1 h at ambient temperature, and the absorbance was then measured at 750 nm. The phenolic content was expressed as catechin equivalent (CatE).

2.5. Molecular ratio of constitution in the crude fucoidan

Molecular ratio of saccharide and SO_4 constitution in the CF were determined by HPLC and a colorimetric method, respectively, after hydrolysis by 1 mol/l trifluoroacetic acid (Kuda et al., 2002).

2.6. Antioxidant activity in a hemoglobin-induced linoleic acid system

The antioxidant activity was determined by a photometry assay (Kuo, Yeh, & Pan, 1999), with slight modification. The sample solution (0.1 ml) was mixed with 0.025 ml of 0.1 M linoleic acid/ethanol and 0.075 ml of 0.2 M phosphate buffer (pH 7.2). The autoxidation was started by adding of 0.05 ml of 0.08% hemoglobin. After incubation at 37 °C for 60 min, the lipid peroxidation was stopped by adding of 5 ml of 0.6% HCl/ethanol. The peroxidation value of the reacted mixture (0.2 ml) was measured in triplicate using the thiocyanate method, by reading the absorbance at 490 nm after colouring with 0.02 ml of 20 mM $FeCl_2$ and 0.01 ml of 30% ammonium thiocyanate. AA and Cat were used as positive controls.

2.7. Reducing power

Total reducing power was determined as described by Zhu, Hackman, Ensunsa, Holt, and Keen (2002), but modified slightly. Briefly, each 0.2 ml of the sample solution was mixed with 0.2 ml of phosphate buffer (0.2 M, pH 7.2) and 0.2 ml of 1% potassium ferricyanide. The mixture was then incubated at 50 °C for 20 min. Afterwards, 0.2 ml of trichloroacetic acid (10%) was added to the mixture. Finally, 0.125 ml of the mixture and 0.125 ml distilled water were put into a 96-well micro-

plate and 0.02 ml of 0.1% $FeCl_3$ was added. Increased absorbance at 655 nm of the reaction mixture indicated increased reducing power.

2.8. Ferrous ion chelating activity

The method of Decker and Welch (1990) was used. To a sample solutions (0.1 ml), distilled water (0.1 ml) and 0.5 mM $FeCl_2$ (0.025 ml) was added. After measurement of absorbance at 550 nm (Abs. 1), 2.5 mM ferrozine was added. After 20 min at room temperature, the absorbance was measured (Abs. 2). The activity is given as

Ferrous ion chelating activity [%]
= [(1-(sample Abs. 2-sample Abs. 1)/
(control Abs. 2-control Abs. 1)) × 100].

EDTA was used as positive control.

2.9. DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Blois (1958) with slight modification. Briefly, the sample solutions (0.2 ml) were mixed with equal volumes of ethanol. Thirty minutes after adding 1 mM DPPH/methanol solution (0.025 ml), absorbance was measured at 550 nm. If the mixture was turbid, the absorbance was measured after centrifugation.

2.10. Superoxide anion radical-scavenging activity

The non-enzymatic generation of superoxide anion was measured by the method of Robak and Gryglewski (1988). Sample solution (0.1 ml) was treated with 0.1 ml of 0.1 M phosphate buffer (pH 7.2), 0.025 ml of 2 mM NADH and 0.025 ml of 0.5 mM NBT, and absorbance at 550 nm was measured as a blank value. After a 3 min incubation with 0.025 ml of 0.03 mM PMS, the absorbance was again measured.

3. Results and discussion

3.1. Amount of total phenolic compounds and water-soluble saccharides

The total phenol contents in WE, EE and LE were 5.57, 0.42 and 4.73, respectively, as mg CatE/g dry sample (DS). The phenolic content in WE was similar to or higher than that of common algae, such as *Laminaria, Undaria* and *Porphyra* (Jiménez-Escrig et al., 2001), vegetables, such as onion, carrot and tomato (Kähkönen et al., 1999), and common beans (de Mejia, Castaño-Tostado, & Loarca-Piña, 1999).

Yields of CF and CA of the dried products were 53 and 546 as mg/g DS, respectively. The CF content was larger than that of dried products of common brown algae, such as Kombu *Laminaria* spp. and leaf of wakame *Undaria pinatifida* (Kuda, Goto, Yokoyama, & Fujii, 1998). Molecular ratio of fucose:galactose:glucose:SO_4 was 1:0.43:1.04:0.33. This result indicates that the CF contained fucose and galactose-rich fucoidan and also laminaran (β-1,3-glucan).

3.2. Antioxidant activity of dried product of S. lomentaria

The antioxidant activities of extract solutions of *S. lometaria* were determined by the method of Kuo et al. (1999) using the hemoglobin-induced linoleic acid system. This method could evaluate the results with only 1 h for oxidation time. Generally antioxidant assays with linoleic acid need more autooxidation for 5–6 days.

The antioxidant activities of WE and LE were dose-dependent and reached a plateau (about 95% inhibition) with a concentration of 10 mg DS per ml solvent (Fig. 1). From results of 50% inhibition concentration (IC50%) of the oxidation, antioxidant activities of WE and LE were 22.2 and 10 mg CatE /g DS, respectively. Clear antioxidant activities of EE, CF and CA were not detected at any concentration.

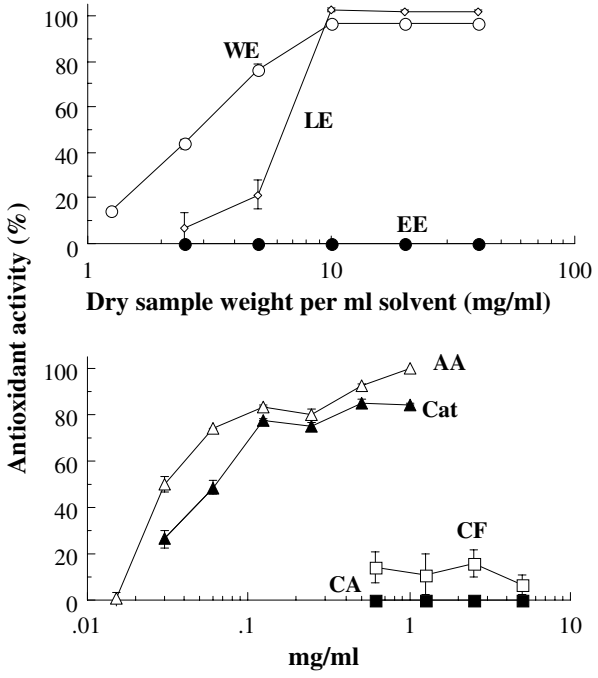

Fig. 1. Antioxidant activity of dried *S. lomentaria* against linoleic acid peroxidation induced by hemoglobin. The peroxide value was measured in triplicate by the thiocyanate method. WE: water extract solution; EE: ethanol extract solution; LE: water-soluble low-molecular extract solution; CF: crude fucoidan; CA: crude alginate; AA: ascorbic acid; Cat: catechin. AA and Cat were used as positive controls. Values are means and SE.

Antioxidant activity of tea extract has been observed to correlate with anti mutagenicity (Yen & Chen, 1995). It is thought that the oxidative DNA damage, mediated by active oxygen radical, induces carcinogenesis (Borek, 1993). Hasegawa et al. (1995) reported that green tea solution reduced hepatic lipid peroxide levels and effectively blocked oxidative DNA damage in liver of rats.

3.3. Reducing power

The reducing powers of the extracts of dried *S. lomentaria* and ascorbic acid (to reduce ferric ions) were determined in this study (Fig. 2). The highest amount of reducing power was observed in WE. The reducing power in LE was about 30–50% that of WE. According to the concentration reached for OD 1.0, the reducing powers of WE and LE were 7.7 and 3.4 mg CatE/g DS, respectively. Though the reducing powers of EE and CF were low, these were dose-dependent.

In most cases, irrespective of the stage in the oxidative chain in which the antioxidant action is assessed, most non-enzymatic antioxidative activity, such as scavenging of free radicals or inhibition of peroxidation, is mediated by redox reactions (Zhu et al., 2002). Jiménez-Escrig et al. (2001) reported that the reducing power measured by FRAP assay was detected in *Fucus, Laminaria* and *Porphyra*, but not detected in *Undaria* and *Chondrus*.

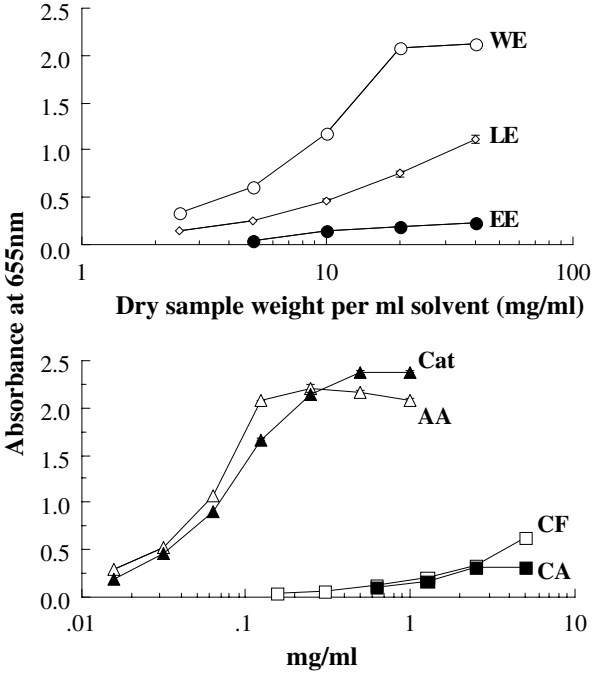

Fig. 2. Ferrous-reducing power of dried *S. lomentaria*. WE: water extract solution; EE: ethanol extract solution; LE: water-soluble low-molecular extract solution; CF: crude fucoidan; CA: crude alginate; AA: ascorbic acid; Cat: catechin. Values are means and SE ($n = 3$).

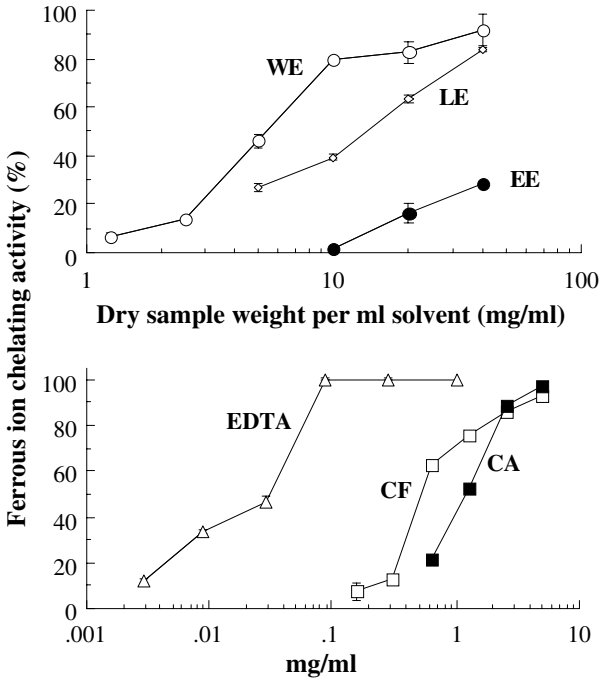

Fig. 3. Ferrous ion chelating effect of the *S. lomentaria*. WE: water extract solution; EE: ethanol extract solution; LE: water-soluble low-molecular extract solution; CF: crude fucoidan; CA: crude alginate. EDTA was used as a positive control. Values are means and SE ($n = 3$).

3.4. Ferrous ion chelating activity

Ferrous ion chelating activities of the extracts of dried *S. lomentaria* and EDTA, a strong chelator, are shown in Fig. 3. The activities of WE and LE were dose-dependent and were 6.0 and 2.7 mg EDTA equivalent/g DS, respectively. EE also showed a lower effect, though that showed a dose-dependent chelating activity. Interestingly, the crude dietary fibres, CF and CA, showed strong activities in this assay system. Their activities were 40 mg CetE/g for CF and 10 mg EDTA equivalent/g for CA.

The metal binding capacities of dietary fibres are well known and the inhibitory effects on ferrous absorption of algal dietary fibres, such as carrageenan, agar and alginate, have also been reported (Harmuth-Hoene & Schelenz, 1980). The ferrous binding capacities of CF and CA may cause decrease of ferrous ion in the assay system, in this study. However, the result with LE indicates that there are water-soluble low-molecular compounds in the dried *S. lomentaria* having ferrous chelating activity.

3.5. DPPH radical-scavenging activity

DPPH has been used extensively as a free radical to evaluate reducing substances (Cotelle et al., 1996). Percent DPPH-scavenging activities of WE and LE were dose-dependent (Fig. 4). These scavenging activities

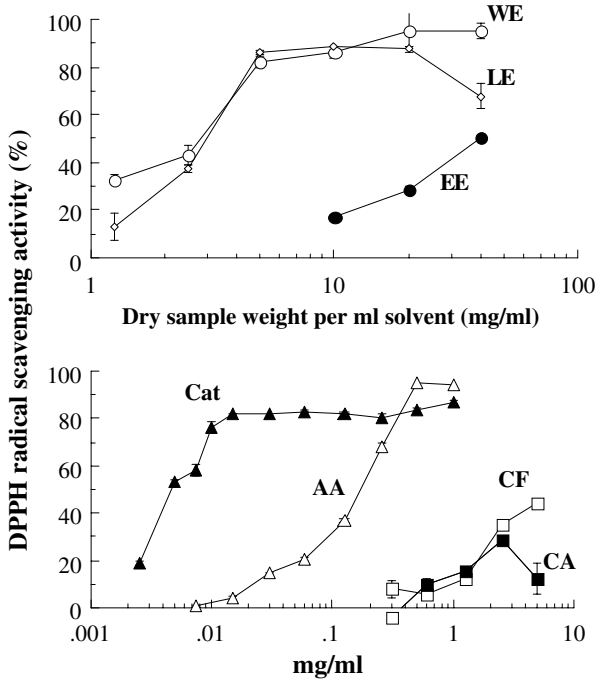

Fig. 4. Scavenging effect of dried *S. lomentaria* on DPPH radicals. WE: water extract solution; EE: ethanol extract solution; LE: water-soluble low-molecular extract solution; CF: crude fucoidan; CA: crude alginate; AA: ascorbic acid; Cat: catechin. Values are means and SE ($n = 3$).

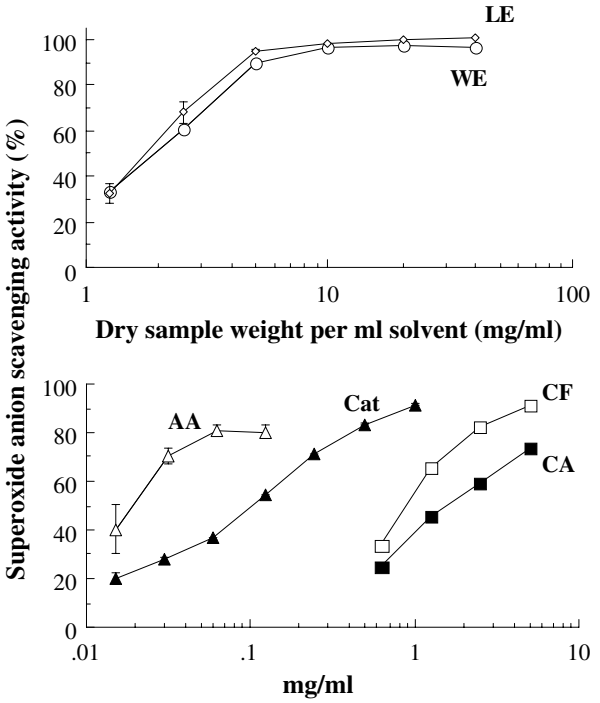

Fig. 5. Effect of dried *S. lomentaria* on NBT reduction induced by superoxide radical generated in a NADH/phenazine methosulphate system. WE: water extract solution; LE: water-soluble low-molecular extract solution; CF: crude fucoidan; CA: crude alginate; AA: ascorbic acid; Cat: catechin. Values are means and SE ($n = 3$).

were 3.2 and 2.3 mg CatE/g DS, respectively. The activities of EE and CF were also dose-dependent, though the activities were low.

The DPPH radical-scavenging activities of methanol extracts of some brown algae have been reported (Han et al., 2002). Jiménez-Escrig et al. (2001) reported that the radical scavenging activity of a brown alga *Fucus* was 98% which decreased by drying at 50 °C for 48 h. In the case of hoshi-nori, a dried product of a red alga *Porphyra*, not only ascorbic acid but also carotenoids and chlorophylls are decreased during shade storage (Araki, 1983). We believe that the drying and storage processes may destroy the alcohol extracted antioxidants rather than the water-soluble antioxidants.

3.6. Superoxide anion-scavenging activity

The capacity of the extract solutions of *S. lomentaria* to scavenge superoxide radicals was confirmed when these radicals were generated by a chemical system composed of PMS, NADH and oxygen (Fig. 5). The scavenging activities of WE, LE, CF and CA were dose-dependent. The activities of WE and LE were 56 and 50 mg CatE/g DS, and those of CF and CA were 110 and 83 mg CetE/g extract, respectively. Scavenging activity of EE was not shown.

In most organisms, superoxide anion radical is converted to hydrogen peroxide by superoxide dismutase. In the absence of transition metal ions, hydrogen peroxide is fairy stable. However, hydroxyl radicals can be formed by the reaction of superoxide with hydrogen peroxide in the presence of metal ions, usually ferrous or copper (Macdonald, Galley, & Webster, 2003). Hydroxyl free radicals are much more reactive (toxicity) than superoxide anions. Superoxide anion-scavenging activity of WE, shown in this study, suggests that *S. lometaria* has benefits for decreased toxicity of not only superoxide anions but also of hydroxyl radicals.

In this study, the antioxidant activities of extract solutions of dried *S. lomentaria* varied with the five test models. WE and LE, containing polyphenols at about 5 mg CatE/g DS, showed strong antioxidant activities in all five assays. EE showed lower activity in ferrous reducing assay, ferrous chelating assay and DPPH radical-scavenging assay, though the antioxidant activity was not shown in linoleic acid peroxidation assay or superoxide anion-scavenging assay. The crude dietary fibres CF and CA, showed activity not only in ferrous chelating (binding) assay, but also in superoxide anion radical-scavenging assay.

There are several reports about antioxidant activity of brown algae. However, most of the algae were fresh and raw materials. As mentioned above, it is reported that drying and storage decreased the antioxidant

compounds and activities (Araki, 1983). We believe that the algal foods circulating as dried product should be examined after the drying process. Although antioxidant activities of dried products of some brown algae, kombu *Laminaria* and wakame *Undaria pinatifida*, have been reported, usually, these dried algae are eaten after swelling with 20 to 40 volumes of water. On the other hand, *S. lomentaria* is eaten after only light roasting. From the results of this study and the processes, we consider that *S. lomentaria* is a useful healthy seafood having antioxidant activity. It is necessary to investigate changes of antioxidant capacity during the processing and storage of *S. lomentaria*. Furthermore, studies of antioxidants in WE and LE, using in vitro and in vivo assays, are necessary. These studies are now in progress.

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