Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 103 (2007) 900–905
Food Chemistry
www.elsevier.com/locate/foodchem

Varieties of antioxidant and antibacterial properties of *Ecklonia stolonifera* and *Ecklonia kurome* products harvested and processed in the Noto peninsula, Japan

Takashi Kuda *, Taeko Kunii, Hideyuki Goto, Takamoto Suzuki, Toshihiro Yano

Department of Food Science, Ishikawa Prefectural University, Suemastu 1-308, Nonoichi, Ishikawa 921-8836, Japan

Received 20 June 2006; received in revised form 14 July 2006; accepted 25 September 2006

Abstract

Ecklonia stolonifera and *Ecklonia kurome* are traditional edible brown algae in the fisheries towns in Far East Asia. In the Noto Peninsula area, Ishikawa, Japan, both the *Ecklonia* are called 'kajime' and people believe that the algae improve the property of blood. To determine the varieties of antioxidant and antibacterial properties *E. stolonifera* and *E. kurome* products, assays for total phenolic content and antioxidant activities, including DPPH radical-scavenging activity, superoxide anion radical-generated by non-enzymatic system, ferrous-reducing power and WST-8, a tetrazolium salt, redox activity of four dried and two boiled *E. stolonifera*, and four dried and two raw *E. kurome* preparations were tested in this study. Furthermore, antibacterial activity of the products was tested. Though the total phenolic content, the antioxidant activity and the antibacterial activities of *E. stolorifera* and *E. kurome* products were high, these properties were varied by manufacturers or each product. Especially, two dried and two boiled products of *E. stolonifera* showed low activities. The results of this study indicate that the contents of functional compounds and their activities were affected or decreased greatly by the processing method.

Keywords: *Ecklonia stolonifera*; *Ecklonia kurome*; Antioxidant property; Antibacterial activity

1. Introduction

Ecklonia stolonifera and *Ecklonia kurome* are members of the family of Laminariaceae, belonging to the order Laminariales. It was reported that these *Ecklonia* have several bio-active compounds useful for human health. For example, phlorotannins (brown-algal polyphenols) extracted from these algae worked as antioxidants (Kang, Chung, Jung, Son, & Choi, 2003; Kang et al., 2004) and/or antibacterial compounds (Nagayama, Iwamura, Shibata, Hirayama, & Nakamura, 2002).

The antioxidants of edible plants interest many researchers. Oxidative modification of DNA, proteins, lipids 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, mutations and cancer (Byress & Guerrero, 1995; Kaur & Kapoor, 2001; Richardson, 1993).

There are several reports about fucoidan, a sulfated fucan, in *E. kurome* with anticoagulant and antithrombin activities (Nishino & Nagumo, 1992; Nishino, Fukuda, Nagumo, Fujihara, & Kaji, 1999). In recent years, fucoidan has been reported to be material having anti-tumor activity (Maruyama, Tamauchi, Hashimoto, & Nakano, 2003; Mastubara et al., 2005).

On the coasts of the Noto Peninsula, Ishikawa Prefecture, Japan, which faces the middle of the Sea of Japan (in the temperate zone), various edible algae thrive (Ikemori & Tajima, 2002; Kuda, Tsunekawa, Goto, & Araki, 2005; Kuda, Tsunekawa, Hishi, & Araki, 2005). The two *Ecklonia* are called 'kajime' in markets and are processed by small

* Corresponding author. Tel.: +81 76 227 7220; fax: +81 76 227 7410.
E-mail address: kuda@ishikawa-pu.ac.jp (T. Kuda).

0308-8146/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2006.09.042

manufacturers. From ancient times, people living in the area have believed that ingestion of 'kajime' can improve blood properties, such as glucose and lipid levels. Recently, 'kajime' has become a popular alga on account of its dietary functions. However, a general consumer cannot judge whether the product is made from *E. stolonifera* or *E. kurome*. Furthermore, the processing method and the preservation methods are different, depending on each manufacture.

In this study, to clarify the differences of functionality of materials and their functions between *E. stolonifera* and *E. kurome*, and/or among the several products made by different manufacturers, we determined the content of the fucoidan and phenolic compounds, and antioxidative and antibacterial activities.

2. Material and methods

2.1. Material

Both the *E. stolonifera* and *E. kurome* were harvested in Noto Peninsula (located at Temperate Zone and facing the Sea of Japan), Ishikawa, Japan in March 2006. *E. stolonifera* and *E. kurome* were harvested and processed in Wajima city and Suzu city, respectively. Four dried and two boiled *E. stolonifera* products and four dried and two raw *E. kurome* products were purchased from retail shops in Wajima city and Suzu city.

2.2. Chemicals and bacterial strain

(+)-Catechin (Cat), Folin–Ciocalteu's phenol reagent, the stable radical DPPH, nitroblue tetrazolium salt (NBT), phenazine methosulphate (PMS), 3-(2-pyridyl)-5,6-di(*p*-sulfophenyl)-1,2,4-triazine, disodium salt (ferrozine) and ethylenediaminetetraacetic acid (EDTA) were purchased from Sigma–Aldrich Co. (St. Louis, MO). Phloroglucinol dihydrate (PG), ascorbic acid (AA) and potassium ferricyanide were purchased from Wako Chemicals Co., Ltd. (Osaka, Japan). Nutrient agar (NA) was purchased from Oxioid Ltd. (Hampshire, UK). Cell Counting Kit-8, containing 2-(2-methoxy-4-nitro-phenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium (WST-8) and 1-methoxy PMS, was purchased from Dojindo, Co., Kumamoto, Japan. Other reagents were of analytical grade.

Bacterial strains of *Escherichia coli* IAM12119, *Pseudomonas aeruginosa* IAM1514, *Staphylococcus aureus* IAM12544, *Bacillus cereus* IAM12605 and *Corynebacterium glutamicum* IAM12435 were obtained from IAM Culture Collection (Tokyo).

2.3. Preparation of sample extract and yield of water-soluble saccharides

The dried product sample (2 g) was weighed and 40 ml of distilled water or 80%(v/v) ethanol were added. The wet sample (10 g) was weighed and 30 ml of distilled water or 99% ethanol were added. The water extract (WE) and ethanol extract (EE) solutions were collected after shaking for 20 min at 75 °C and centrifugation ($2220g \times 10$ min). The sample preparation was replicated three times.

Crude fucoidan and crude alginate, in the precipitations after ethanol-extraction were extracted using alcohol precipitation methods described previously (Kuda, Taniguchi, Nishizawa, & Araki, 2002).

2.4. Determination of the amount of total phenolic compounds

Total phenolic content were determined as described previously (Oki et al., 2002; Kuda, Hishi, & Maekawa, 2006). 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 30 min at ambient temperature, and the absorbance was then measured at 750 nm. The phenolic content was expressed as PG equivalent (Eq).

2.5. DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Blois (1958) with slight modification (Kuda et al., 2005; Kuda, Tsunekawa, Hishi et al., 2005). Briefly, sample solution (0.2 ml) was put into a 96-well microplate and 1 mM DPPH/methanol solution (0.025 ml) was added. Before and 30 min after adding DPPH, absorbance was measured at 520 nm. Cat was used as positive control.

2.6. 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 15 min incubation with 0.025 ml of 0.03 mM PMS, the absorbance was again measured. Cat was used as a positive control.

2.7. Reducing power

Total reducing power was determined as described by Zhu, Hackman, Ensunsa, Holt, and Keen (2002), but modified slightly (Kuda et al., 2005; Kuda, Tsunekawa, Hishi et al., 2005). 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 10% trichloroacetic acid was added to the mixture. Finally, 0.125 ml of the mixture and 0.125 ml distilled water were put into a 96-well microplate and 0.02 ml of 0.1% $FeCl_3$ was added. Increased absorbance at 655 nm

of the reaction mixture indicated increased reducing power. Cat was used as a positive control.

2.8. WST-8 redox activity

Cell Counting Kit solution was diluted with four volumes of distilled water (WST-8 solution). Sample (0.02 ml) and 0.18 ml of 0.1 M phosphate buffer (pH 7.0) were placed in wells of a 96-well microplate and absorbance at 490 nm was measured. Then 0.025 ml of the WST-8 solution was added. The plate was incubated at 35 °C and the absorbance was measured every 15 min until 60 min. AA was used as a positive control.

2.9. Ferrous ion-chelating activity

The method of Decker and Welch (1990) was used. To a sample solution (0.1 ml), distilled water (0.1 ml) and 0.5 mM $FeCl_2$ (0.025 ml) were added. After measurement of absorbance at 550 nm, 2.5 mM ferrozine was added. After 20 min at room temperature, the absorbance was measured. EDTA was used as a positive control.

2.10. Antibacterial activity

The bacterial strains were grown overnight at 37 °C in PYG-broth (peptone, 5 g; yeast extract, 2.5 g; glucose, 1 g/l, pH 7.2). The cultured broth (0.01 ml) was put on NA containing EE (1 ml/20 ml medium agar) and incubated at 37 °C for 24 h (*E. coli*, *P. aeruginosa*, *S. aureus* and *B. cereus*) or 72 h (*C. glutamicum*). After the incubation periods, the samples that completely prevented the colony forming on the agar were assumed to be antibacterial activity positives.

3. Results and discussion

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

The total phenolic contents in *E. stolonifera* (ES1–ES6) and *E. kurome* (EK1–EK6) are shown in Fig. 1. The phenolic contents, in WE and EE of the dried products, were 300–600 and 150–460 μmol PGEq/g, respectively. The phenolic content was higher in *E. kurome* than in *E. stolonifera* preparations. In a half of the products (ES3, ES4, EK1 and EK2), the content of EE was lower than those of WE. Particularly, in EE of ES3 and ES4, the content was below 180 μmol PGEq/g. The content of boiled *E. stolonifera* was below 50 μmol PGEq/g.

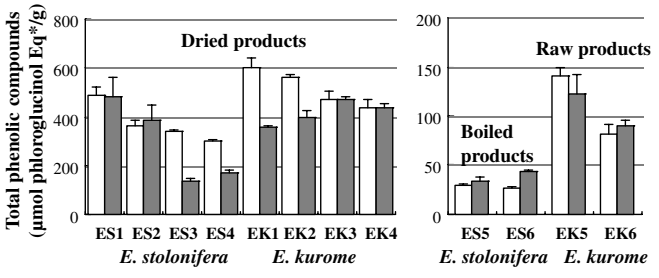

Fig. 1. Total phenolic compounds in *E. stolonifera* and *E. kurome* products. The samples were extracted with distilled water (open squares) or 80% ethanol (closed squares). *Eq: equivalent. Values are means and SEs ($n = 3$).

Yields of water-soluble polysaccharides are summarized in Table 1. Yield of crude fucoidan in the dried products varied from 3 to 34 mg/g. The yield in ES3 and ES4 was low. Yield of crude alginate did not differ between the dried products. Only a small content of crude fucoidan (0.05 and lower as mg/g) was detected in boiled *E. stolonifera*.

These results suggested that a part of dried products, such as ES3 and ES4, was boiled before the drying process and the boiling process took away a part or most of the functional compounds, such as phlorotannins and fucoidan.

3.2. Radical-scavenging activities

DPPH has been used extensively as a radical to evaluate reducing substances (Cotelle et al., 1996). The activities in EE of two dried *E. stolonifera* (ES3, ES4) and in WE of two boiled *E. stolonifera* were low (Fig. 2). ES1, ES2, EK1 and EK2 showed higher activity in WE rather than EE. The other samples showed high activity in EE. In the case of raw *E. kurome* samples (EK5, EK6), the activity of EE was twice higher than that of WE.

On the other hand, superoxide anion radical-scavenging activity was high in WE rather than EE in all products (Fig. 3). The activity was not detected in EE of ES3 and ES4, or boiled *E. stolonifera*. In general organisms, super-

Table 1
Yield of crude fucoidan and crude alginate from *Ecklonia stolonifera* and *E. kurome*

	Fucoidan	Alginate
Dried products		
Ecklonia stolonifera		
ES1	27.7 ± 1.1	346 ± 46
ES2	19.2 ± 0.6	493 ± 19
ES3	10.4 ± 1.5	423 ± 19
ES4	2.9 ± 0.4	353 ± 39
Ecklonia kurome		
EK1	34.2 ± 0.5	306 ± 16
EK2	30.1 ± 1.8	429 ± 57
EK3	22.1 ± 0.5	443 ± 20
EK4	16.6 ± 1.8	358 ± 14
Wet products		
Ecklonia stolonifera		
ES5	0.51 ± 0.25	32.9 ± 6.3
ES6	0.04 ± 0.01	43.4 ± 5.8
Ecklonia kurome		
EK5	1.75 ± 0.08	53.5 ± 3.4
EK6	1.72 ± 0.34	44.0 ± 5.1

Values are means and SD ($n = 3$).

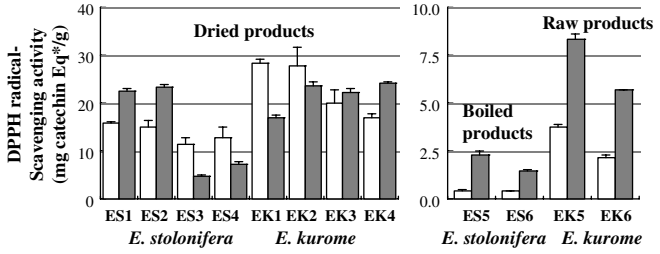

Fig. 2. DPPH radical-scavenging activities of *E. stolonifera* and *E. kurome* products. The samples were extracted with distilled water (open squares) or 80% ethanol (closed squares). *Eq: equivalent. Values are means and SEs ($n = 3$).

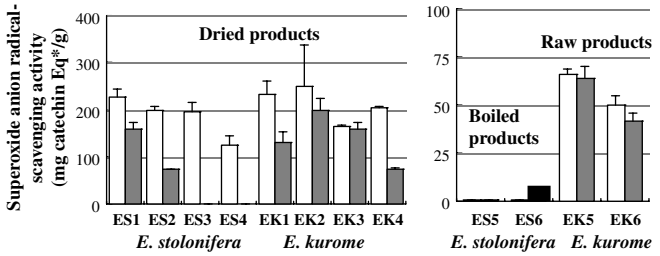

Fig. 3. Superoxide anion radical-scavenging activity of *E. stolonifera* and *E. kurome* products. The samples were extracted with distilled water (open squares) or 80% ethanol (closed squares). *Eq: equivalent. Values are means and SEs ($n = 3$).

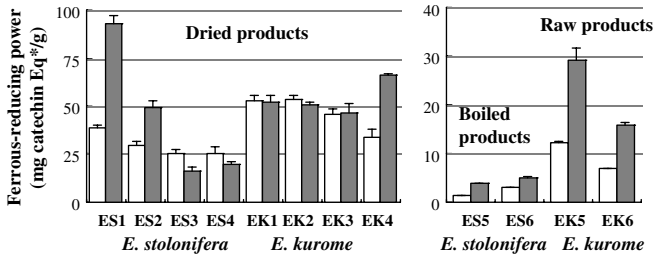

Fig. 4. Ferrous-reducing powers of *E. stolonifera* and *E. kurome* products. The samples were extracted with distilled water (open squares) or 80% ethanol (closed squares). *Eq: equivalent. Values are means and SEs ($n = 3$).

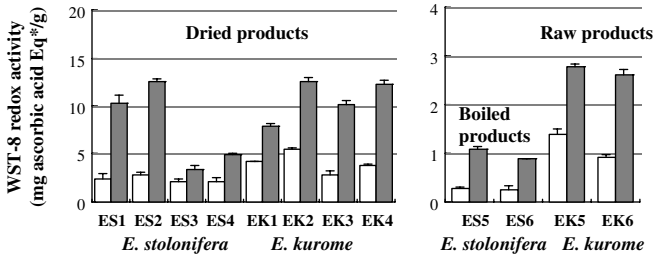

Fig. 5. WST-8 redox activity of *E. stolonifera* and *E. kurome* products. The samples were extracted with distilled water (open squares) or 80% ethanol (closed squares). *Eq: equivalent. Values are means and SEs ($n = 3$).

oxide anion radical is converted to hydrogen peroxide by the enzyme 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 (toxic) than are superoxide anions.

3.3. Reducing power and WST-8 redox activity

Most non-enzymatic antioxidative activity, such as scavenging of free radicals and inhibition of peroxidation, is mediated by redox reactions (Zhu et al., 2002). Jiménez-Escrig, Jiménez-Jiménez, Pulido, and Saura-Calixto (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. 4 shows ferrous-reducing power of the samples. In ES1, ES2, EK5 and EK6; the activity in EE was higher by about two times that in WE. The activities of two dried (ES3, ES4) and two boiled *E. stolonifera* were low. These results are similar to the result of DPPH radical scavenging activity.

WST-8 redox activity of the samples is shown in Fig. 5. In all of the samples, the activity of EE was about 2–5 times higher than those of WE. The activity was still low in ES3, ES4 and boiled *E. stolonifera*. This result showed that the redox indicator was useful for examination of reducing power of foodstuffs, though the redox indicators are reagents normally used for cell toxicity and cell growth examinations (Kuda, Shimizu, & Yano, 2004).

3.4. Ferrous ion-chelating activity

Ferrous ion-chelating activities of the samples were below 1 and 0.1 mg EDTA Eq/g dried and wet product, respectively (data are not shown). 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). We have found that WE of brown algae *Scytociphon lomentaria* and *Petalonia binghamiae* showed activities of about 5.9 and 2.3, respectively, of mg EDTA Eq/g dried sample.

3.5. Antibacterial activity

Antibacterial properties of the *E. stolonifera* and *E. kurome* products are summarized in Table 2. ES1, ES2 and all of the *E. kurome* products showed antibacterial activity against various bacterial species. On the other hand, the bacterial growths were not affected by ES3, ES4, ES5 or ES6 and these samples had low contents of phenolic compounds (Fig. 1). It is known that *Ecklonia* sp. have bactericidal activity. Nagayama et al. (2002) reported that phlorotannins, brown-algal phenolic compounds, such as eckol and eckol-related compounds, from *E. kurome*, have strong bactericidal activity.

Table 2
Antibacterial properties of *Ecklonia stolonifera* and *E. kurome*

	Escherichia Coli	*Pseudomonas aeruginosa*	*Coryne-bacterium Glutamicum*	*Staphylo-coccus aureus*	*Bacillus cereus*
Dried products[a]					
E. stolonifera					
ES1	–	+	+	+	+
ES2	+	+	+	+	+
ES3	–	–	–	–	–
ES4	–	–	–	–	–
E. kurome					
EK1	–	+	+	+	+
EK2	–	+	+	+	+
EK3	–	+	+	+	+
EK4	–	+	+	+	+
Wet products[b]					
E. stolonifera(boiled products)					
ES5	–	–	–	–	–
ES6	–	–	–	–	–
E. kurome					
EK5	+	+	+	+	+
EK6	–	+	+	+	+

+: Sample prevented cell growth.
[a] Nutrient agar contained 3 mg dried products equivalent/ml.
[b] Nutrient agar contained 15 mg wet products equivalent/ml.

In conclusion, the concentrations of functional compounds, such as polyphenols and fucoidan, antioxidant activity and antibacterial activity of *E. stolorifera* and *E. kurome* products were varied by manufacturers or each product. It is necessary to recognize that the content of functional compounds and their activities decrease greatly by each processing method and/or preservation condition. Especially, it is thought that the boiling process affects the content and the functions, and then the useful compounds, dissolved in the boiling soup, are thrown away. On the other hand, results in this study showed that the boiling process spoiled the antioxidant activities of EE rather than of WE. Although more experiments are necessary, it may be noted that many low-molecular weight compounds extracted in EE were spoiled easily by heating rather than were the high-molecular weight compounds extracted in WE. We are now studying processing methods that can maintain the bio-active compounds, their functionality and a recycling method for the boiling soup.

References

Blois, M. S. (1958). Antioxidant determination by the use of a stable free radical. *Nature, 26*, 1199–1200.

Borek, C. (1993). Molecular mechanisms in cancer induction and prevention. *Environmental Health Perspective, 101*, 151–160.

Byress, T., & Guerrero, N. (1995). Epidemiologic evidence for vitamin C and vitamin E in cancer prevention. *American Journal of Chemical Nutrition, 62*, 1385–1392.

Cotelle, N., Bemier, J. L., Catteau, J. P., Pommery, J., Wallet, J. C., & Gaydou, E. M. (1996). Antioxidant properties of hydroxyl flavones. *Free Radical Biology and Medicine, 20*, 35–43.

Decker, E. A., & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and Chemistry, 38*, 674–677.

Harmuth-Hoene, A. E., & Schelenz, R. (1980). Effects of dietary fiber on mineral absorption in growing rats. *Journal of Nutrition, 110*, 1774–1784.

Ikemori, T., & Tajima, M. (2002). List of marine plants from the coast of Ishikawa Prefecture. *Bulletin of Ishikawa Prefecture Fisheries Research Center, 3*, 1–11.

Jiménez-Escrig, A., Jiménez-Jiménez, I., Pulido, R., & Saura-Calixto, F. (2001). Antioxidant activity of fresh and processed edible seaweeds. *Journal of the Science of Food and Agriculture, 81*, 530–534.

Kang, H. S., Chung, H. Y., Jung, H. A., Son, B. W., & Choi, J. S. (2003). A new phlorotannins from the brown alga *Ecklonia stolonifera*. *Chemical and Pharmaceutical Bulletin, 51*, 1012–1014.

Kang, H. S., Chung, H. Y., Kim, J. Y., Son, B. W., Jung, H. A., & Choi, J. S. (2004). Inhibitory phlorotannins from the edible brown alga *Ecklonia stolonifera* on total reactive oxygen species (ROS) generation. *Archives of Pharmacal Research, 27*, 194–198.

Kaur, C., & Kapoor, H. C. (2001). Antioxidants in fruits and vegetable. *International Journal of Food Science and Technology, 36*, 703–725.

Kuda, T., Hishi, T., & Maekawa, S. (2006). Antioxidant properties of dried product of 'haba-nori', an edible brown alga, *Petalonia binghamiae* (J. Agaradh) Vinogradova. *Food Chemistry, 98*, 545–550.

Kuda, T., Shimizu, K., & Yano, T. (2004). Comparison of rapid and simple colorimetric miacroplate assays as an index of bacterial count. *Food Control, 15*, 421–425.

Kuda, T., Taniguchi, E., Nishizawa, M., & Araki, Y. (2002). Fate of water-soluble polysaccharides in dried Chorda filum a brown alga during water washing. *Journal of Food Composition and Analysis, 15*, 3–9.

Kuda, T., Tsunekawa, M., Goto, H., & Araki, Y. (2005). Antioxidant properties of four edible algae harvested in the Noto Peninsula, Japan. *Journal of Food Compsition and Analysis, 18*, 625–633.

Kuda, T., Tsunekawa, M., Hishi, T., & Araki, Y. (2005). Antioxidant properties of dried 'kayamo-nori', a brown alga *Scytosiphon lomentaria* (Scytosiphonales, Phaeophyceae). *Food Chemistry, 59*, 617–622.

Macdonald, J., Galley, H. F., & Webster, N. R. (2003). Oxidative stress and gene expression in spices. *British Journal of Anesthesia, 90*, 221–232.

Maruyama, H., Tamauchi, H., Hashimoto, M., & Nakano, T. (2003). Antitumor activity and immune response of Mekabu fucoidan extracted from Sporophyll of *Undaria pinatifida*. *In Vivo, 17*, 245–249.

Mastubara, K., Xue, C., Zhao, X., Mori, M., Sugawara, T., & Hirata, T. (2005). Effects of middle molecular weight fucoidans on *in vitro* and ex vivo angiogenesis of endothelial cells. *International Journal of Molecular Medicine, 15*, 695–699.

Nagayama, K., Iwamura, Y., Shibata, T., Hirayama, I., & Nakamura, T. (2002). Bactericidal activity hlorotannins from the brown alga *Ecklonia kurome*. *Journal of Antimicrobial Chemotheraphy, 50*, 889–893.

Nishino, T., Fukuda, A., Nagumo, T., Fujihara, M., & Kaji, E. (1999). Inhibition of the generation of thrombin and factor Xa by a fucoidan from the brown sea weed *Ecklonia kurome*. *Thrombosis Research, 96*, 37–49.

Nishino, T., & Nagumo, T. (1992). Anticoagulant and antithrombin activities of oversulfated fucans. *Carbohydrate Research, 229*, 355–362.

Oki, T., Masuda, M., Furuta, S., Nishiba, Y., Terahara, N., & Suda, I. (2002). Involvement of antocyanines and other phenolic compounds in radical-scavenging activity of purple-fleshed sweet potato cultivars. *Journal of Food Science, 67*, 1652–1655.

Richardson, S. J. (1993). Free radical in the genesis of Alzheimer's disease. *Annals of the New York Academy of Sciences, 695*, 73–76.

Robak, J., & Gryglewski, R. J. (1988). Flavonoids are scavenging of superoxide anions. *Biochemical Pharmacology, 37*, 841–937.

Zhu, Q. T., Hackman, R. M., Ensunsa, J. L., Holt, R. R., & Keen, C. L. (2002). Antioxidative activities of oolong tea. *Journal of Agricultural and Food Chemistry, 50*, 6929–6934.