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Antioxidant and fermentation properties of aqueous solutions of dried algal products from the Boso Peninsula, Japan

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ABSTRACT

The mineral and saccharide contents, and antioxidant properties in aqueous extract solutions of eleven dried algal products obtained from the Boso Peninsula, Japan, were investigated. Potassium content was high in the brown alga *Sargassum fusiforme*. Polysaccharides content and viscosity were high in the red algae *Gloiopeltis furcata*, *Chondrus ocellotus* and *C. elatus*. Total phenolic compound content, 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging capacity, and Fe-reducing power were high in brown algae *Eisenia bicyclis*, *S. fusiforme* and the red alga *Pyropia* sp. Superoxide anion (O_2^-) radical-scavenging capacity was high in *G. furcata*, *C. ocellotus*, *C. elatus* and the green alga *Monostroma nitidum*. *Lactobacillus plantarum* strains isolated from the coast could ferment *G. furcata*, *C. elatus* and *M. nitidum*. The O_2^- radical-scavenging capacities of the red algae were increased by fermentation. These results suggest that some macroalgal beach-cast brown algae without fermentation and red algae with fermentation can be utilized as natural resources for functional foods.

1. Introduction

As of the last decade of the 20th century, there were more than 200 different kinds of marine algae used world-wide; the amount of marine algae collected and cultured world-wide in one year has been reported to be more than 2 million tons dry weight (Zemle-White & Ohno, 1999). Far East Asian countries including China, Japan, Korea, and the Philippines, produce a high quantity of marine algal products (Lüning & Pang, 2003). Among them, brown algae production was the highest in China (about 75% of the world-wide total) and red algae production was the highest in the Philippines (about 31% of the world-wide total). According to the Food and Agriculture Organization of the United Nations (FAO, 2014), about 99% of the farmed marine algae are obtained from just seven Asian countries: China (54%), Indonesia (27%), the Philippines (7%), South Korea (4%), North Korea (2%), Japan (2%), and Malaysia (1%). In 2012, the total production value of the marine algae in aquaculture was about US $6.4 billion (FAO, 2014). In the case of European countries, captured production is high in Norway, France, Ireland, and Iceland, whereas aquaculture production is high in Denmark.

From ancient times, various algae have been used as food sources in Japan (Murara & Nakazoe, 2001). For example, the oldest literature relating to the use of edible red alga *Pyropia* sp (nori) as a tribute was described in the *Taiho Ritsuryo* Code promulgated in AD 701 (Ichi, 2015). Although nori, *Saccharina japonica* (Makombu), and *Undaria pinnatifida* (Wakame) are very popular edible algae all over the world, at least fifty more algal species are still used as human food in local, mainly coastal, areas in Japan (Ohno, 2004). Examples of these locales include the Noto Peninsula and the Boso Peninsula in Ishikawa and Chiba Prefectures, which are on the Sea of Japan and the Pacific Ocean, respectively. People in these *satoumi* areas have a culture of eating various marine algae as food, and have traditionally performed nori farming. However, depending on the season, a large amount of algae, including edible and non-edible, can also be deposited on beaches following storms. In this case, a large amount of unutilized algal beach casts are incinerated and/or transported to a landfill at cost.

Not only in East Asian countries, but also in Europe, the US, and other countries, the consumption of several types of marine algae is associated with health benefits (Brown et al., 2014; Fleurence & Levine, 2016). Algae are known to be good sources of minerals, including potassium and magnesium, and several water-soluble dietary fibers, such as alginate, laminaran, and fucoidan (Kuda & Ikemori, 2009; Nakata, Kyoui, Takahashi, Kimura, & Kuda, 2016); they also contain other chemical compounds, including phenolic compounds.

Reports of antioxidant activities in edible algae have increased recently (Balboa, Conde, Moure, Falqué, & Domínguez, 2013). It has been well reported that reactive oxygen species correlate with inflammation (Mittal, Siddiqui, Tran, Reddy, & Malik, 2014), cancer (Waris & Ahsan, 2006), and ageing (Ludovico & Burhans, 2013). For this reason, there have been many reports about the presence of antioxidants in foods

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(Lobo, Patil, Phatak, & Chandra, 2010). In our previous studies, the antioxidant capacities of several traditional algal products obtained from the Noto Peninsula coasts were reported (Eda, Kuda, Kataoka, Takahashi, & Kimura, 2016; Kuda et al., 2016). Furthermore, fermentation with lactic acid bacteria (LAB) has been reported to increase superoxide anion (O_2^-) radical-scavenging capacity in several food materials, including algae (Nemoto et al., 2017). In some LAB species, compared with the strains isolated from dairy products, several isolates obtained from algal beach casts or fish intestines showed a higher resistance against salt, bile, and acid (Kawahara et al., 2015).

In the present study, we sought to examine the beneficial properties of hot aqueous extract solution (AES) of edible algae obtained from the Boso Peninsula, which has species of algae that are different from those of the Noto Peninsula. In particular the mineral, polysaccharide, and antioxidant properties of the AES were measured. Furthermore, the fermentation ability of LABs isolated from this coastal area and the antioxidant properties of the algal extracts after fermentation were determined.

2. Materials and methods

2.1. Chemicals

Folin-Ciocalteu's phenol reagent, the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical phenazine methosulfate (PMS), 3-(2-pyridyl)-5,6-di(p-sulfophenyl)1,2,4-triazine disodium salt (ferrozine), β-nicotinamide adenine dinucleotide (NADH), and nitroblue tetrazolium salt (NBT) were from Sigma-Aldrich (St. Louis, MO, USA). Phloroglucinol dehydrate (PG), potassium ferricyanide, trichloroacetic acid (TCA), $FeCl_3$ and bile (Oxgall) were from Wako Chemicals (Osaka, Japan), while 1,10-phenanthroline was from Nacalai Tesque (Kyoto, Japan). The other reagents were of analytical grade.

2.2. Preparation of aqueous extract solutions from dried algal solutions

A total of eleven dried products (Table 1) from two species of brown algae Phaephyta, *Eisenia bicyclis* (Arame: E1 and E2) and *Sargassum fusiforme* (Hijiki: S), five species of red algae Rhodphyta *Pyropia* sp. (Amanori: P), *Gloiopeltis furcata,* (Funori: Gf1 and Gf2), *Chondrus ocellatus* (Tsunomata: Co), *C. elatus* (Kotoji-tusunomata: Ce), *Gelidiaser* sp. (Tengusa: Ge), as well as two species of green algae Chlorphyta *Monostroma nitidum* (Hitoegusa: M) and *Ulva* sp.(Aosa: U) were obtained from Suzuki Nori Co., Choshi, Chiba, Japan. Among these algal samples, *E. bicyclis* (Arame), *G. furcata* (Funori), *C. ocellatus* (Tsunomata), and *C. etatus* (Kotoji-tsunomata) are predominantly seaweeds washed ashore on Boso Peninsula beaches. These algae were therefore collected from beach casts. *M. nitidum* (Hitoegusa) and *Ulva* sp. (Aosa) were collected from the intertidal zone. *Pyropia* sp. (Amanori) was obtained from an aquafarm. These fresh algae were all dried under the sun at the coast.

The dried samples were milled using a blender (Oster 16 Speed Blender; Osaka Chemical Co., Japan) and sieved through a 1-mm^2 mesh. The algae powder (5 g) was added to 200 mL of distilled water and heated at 105 °C for 15 min using an autoclave. After cooling with tap water, the algae suspension was centrifuged at 3000 × *g* for 10 min at 4 °C. The collected supernatant was used as the algal aqueous extract solutions (AES) and stored at − 30 °C until used for analysis.

2.3. Determination of minerals in the sample solutions

Among the major five cations (Na^+, K^+, NH_4^+, Ca^{2+}, and Mg^{2+}), and three anions (Cl^-, PO_4^{3-} and SO_4^{2-}), K^+, NH_4^+, Ca^{2+}, Cl^-, PO_4^{3-}, and SO_4^{2-} were measured using commercially available kits from the series of Reagent Set for Water Analyzer (LR-K, LR-NH4-A, LR-Ca-B, LR-Cl, LR-PO4 and LR-SO4) respectively (Kyoritsu Chemical-Check Lab., Corp., Tokyo, Japan). Na^+ was measured using a Na^+-ion meter (LAQUA Twin B-721, Horiba, Kyoto, Tokyo). Mg^{2+} was determined using a diagnosis commercial kit (Magnesium B-Test Wako, Wako Pure Chemical).

2.4. Saccharide contents and viscosity of the AES

The total water-soluble saccharide and polysaccharide contents were determined by the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and the alcohol precipitation method (Kuda, Goto, Yokoyama, & Fujii, 1998). The relative viscosity of the sample solutions was directly determined using an oscillation viscometer (Viscomate VM-1G; Yamaichi Electronics; Osaka, Japan) under ice-cold conditions (Kuda & Ikemori, 2009). The relative viscosity was calculated as the quotient of the AES viscosity divided by the distilled water viscosity.

2.5. Phenolic content and antioxidant properties

Total phenolic content, as the polyphenol content in the AES, was determined by the Folin-Ciocalteu method, as described previously (Eda et al., 2016).

DPPH radical-scavenging capacity was determined as described previously (Kuda & Ikemori, 2009). Briefly, the diluted sample (0.1 mL) and ethanol (0.1 mL) were put into a 96-well microplate, and

Table 1
Cations and anions in aqueous extract solutions (AES) of dried algal products (mmol/L).

Name of algae			Cations						Anions		
Scientific	Japanese	Abbreviation	Na	K	NH_4	Ca	Mg	K/Na	Cl	PO_4	SO_4
Phaeophyta											
Eisenia bicyclis	Arame	E1	17.1	26.6	1.98	14.7	26.2	1.56	0.52	0.08	11.9
		E2	39.4	26.5	1.53	14	24.5	0.67	1.62	0.12	–
Sargassum fusiforme	Hijiki	S	25.7	110.8	0.28	20	23.7	4.31	4.06	0.06	0.8
Rhodphyta											
Pyropia sp.	Amanori	P	8.6	2.3	2.39	7.7	20.5	0.27	–	0.36	–
Gloiopeltis furcata	Funori	Gf1	18.8	–	0.25	10.9	22.0	–	0.84	0.07	–
		Gf2	15.4	1.6	0.02	19.1	24.9	0.10	0.18	0.04	54.0
Chondrus ocellatus	Tsunomata	Co	46.2	2	1.69	13.2	6.54	0.04	4.24	0.05	1.7
Chondrus elatus	Kotoji-tsunomata	Ce	32.5	11.5	1.71	10.8	5.21	0.35	2.97	0.06	–
Gelidiaceae	Tengusa	Ge	6.8	–	0.46	7.8	26.4	–	0.01	0.05	1.3
Chlorophyceae											
Monostroma nitidum	Hitoegusa	M	30.8	0.1	0.08	19.4	26.5	0.00	1.35	0.04	1.0
Ulvaceae	Aosa	U	27.4	0.8	1.51	42.2	29.8	0.03	0.45	0.08	63.8

Values are mean of triplicate measurement.