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Analysis of functional components and radical scavenging activity of 21 algae species collected from the Japanese coast

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ABSTRACT

The functional chemical substances and the antioxidant activity of lipids in 21 marine algae along the Japanese coast were investigated. Principal component analysis was performed to detect any correlation between the chemical substances and algae phylum. Chlorophyta contained a high level of β-carotene. Rhodophyta contained high amounts of cholesterol, β-sitosterol, and saturated fatty acids. Phaeophyta were rich in fucosterol, α-tocopherol, fucoxanthin, and polyphenol. Phaeophyta algae also showed the highest antioxidant activity compared with other phylum. This suggests that Phaeophyta has the greatest potential to be used as a functional food. Consumption of the beneficial Phaeophyta species, such as *Eisenia arborea* Areschoug and *Ecklonia cava* Kjellman should be encouraged as not only as food products but also as nutraceuticals and dietary supplements. These beneficial ingredients should be encouraged to be studied in depth with the possibility to develop specific formulated products target to special consumer's population with added nutritional value.

1. Introduction

Approximately 2000 kinds of algae inhabit the Japanese coast. Algae have been consumed since ancient times. Among the traditional Japanese foods, algae are generally used as a seasoning or a sea vegetable, and account for 10–25% of the average food intake of Japanese (Skibola, 2004). Daily consumption of algae has been suggested to be a key factor in lowering postmenopausal breast cancer incidence and mortality rates in Japan (Teas, 1981). Algae, such as *Monostroma nitidum*, *Undaria pinnatifida*, and *Sargassum fusiforme* are indispensable food resources for the Japanese cuisine, and are consumed every day. They contain several nutrients and functional ingredients, making algae a remarkably healthy food source. Algae synthesize many polymers, such as agars, alginates, carrageenans, fucans, and phlorotannins, which are not found in terrestrial plants (Khan et al., 2009). These polymers are bioactive in algae and play an important role in the host defense mechanism (Myers et al., 2010). The Phaeophyta contain polysaccharides, such as alginate, which is a polymer composed of several different kinds of fucose and fucoidan, which acts as an anti-neoplastic drug (Riou et al., 1996). In addition, Phaeophyta are rich in polyphenols such as phlorotannins (Craigie, 2011). Phlorotannins are oligomers of phloroglucinol and are found in several marine plants, especially Phaeophyta and Rhodophyta (Pal Singh & Bharate, 2006). Furthermore, phlorotannins purified from several Phaeophyta have been reported to possess strong antioxidant activity, which may be associated with their unique molecular skeleton (Ahn et al., 2007).

Algae are also rich in phytosterols. Fucosterol is a low molecular weight phytosterol and is the primary sterol found in seaweed and algae, particularly in Phaeophyta (Sánchez-Machado, López-Hernández, Paseiro-Losada & López-Cervantes, 2004). It has antifungal, antibacterial, anti-inflammatory, anti-tumor, antioxidant, and anti-ulcerative properties (Malcolm, 2000). In recent years, investigations into the effects of phytosterol on medical conditions, such as benign prostatic hypertrophy, rheumatoid arthritis, allergy, and colon cancer have increased (Yankah, 2006). However, fewer studies have been conducted on phytosterols derived from algae than those derived from land plants. We, therefore, developed a method for the simultaneous analysis of phytosterols (stigmasterol, β-sitosterol, campesterol, ergosterol, and fucosterol) and cholesterol using high-performance liquid chromatography (HPLC) with fluorescence detection (Ito, Ishimaru, Shibata,

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Hatate & Tanaka, 2017). We utilized this method to analyze algae phytosterols.

In this study, we evaluated phytosterols and other functional chemical components of the commercial edible and unused algae found growing along the Japanese coast. To determine if the variation in chemical components and algae phylum followed a specific pattern, principal component analysis (PCA) was performed. Previously, Andrade and colleagues reported a strong correlation between the chemical composition of algae (fatty acid, sterols, mannitol, and phloroglucinol) and their biological activity (radical scavenging activity and enzyme activity) in 18 macroalgae of the Portuguese coast (Andrade et al., 2013). However, they investigated only three kinds of phytosterols and did not include tocopherols and carotenoid. Similarly, another study analyzed the seasonal changes in the composition of fat-soluble substances of New Zealand Phaeophyta, *Undaria pinnatifida* (Boulom, Robertson, Hamid, Ma, & Lu, 2014). However, they focused only on fatty acids, two kinds of sterols, and α-tocopherol of the algae. Here, we conducted a more comprehensive analysis of the functional constituents of edible and unused algae. We determined the contents of phytosterols, tocopherols, fatty acids, polyphenol, and carotenoids, and also determined the antioxidant ability. These data were then used to assess the potential of algae as a functional food for human health.

2. Materials and methods

2.1. Algae samples

Algae samples consisted of four Chlorophyta, seven Rhodophyta, nine Phaeophyta, and a Spermatophyta species, and collection site and sampling day of these samples were shown in Table 1. The collection sites were also shown in Fig. 1. These samples were confirmed free from any visible grazing or other tissue damage in the sea from each coast, and then the sample were collected by knife, washed with filtered seawater, air dried in the shade, and pulverized using blender (Vita-Mix Blender ABSOLUTE-3, OSAKA Chemical Co. Ltd., Osaka, Japan). Previously washed, boiled, and dried edible samples of *M. nitidum*, *S. fusiforme*, and *U. pinnatifida* were purchased from a local food store in Japan and pulverized using blender in the laboratory. All algal powders were stored at −30 °C until further analysis.

2.2. Reagents

Reagents, including campesterol, cholesterol, ergosterol, fucosterol, β-sitosterol, stigmasterol, 1-hexacosanol, various fatty acids, and quinuclidine were obtained from Sigma-Aldrich Ltd. (Tokyo, Japan). Various tocopherols, including α-, β-, γ-, and δ-tocopherol were obtained from Eisai Co. Ltd. (Tokyo, Japan). Analytical grade chemicals, including acetone, acetonitrile, 1-anthroyl cyanide (ACN), benzene, 14% boron trifluoride (BF_3) in methanol, chloroform, diethyl ether, 2,2-diphenyl-1-picrylhydrazyl (DPPH), ethyl acetate, Folin–Denis reagent, *n*-hexane, methanol, potassium hydroxide, phloroglucinol, pyrogallol, sodium carbonate, and sodium chloride were obtained from Wako Pure Chemical Industries Ltd. (Osaka, Japan).

2.3. Extraction of lipids from dry marine algae

Lipids were extracted from dried, pulverized algal tissue as described (Folch, Lees, & Stanley, 1957). Briefly, 1 g of algal samples were weighed in separate 50 mL glass centrifuge tubes and mixed with 30 mL of chloroform:methanol (2:1, v/v). The mixture was sonicated using supersonic waves for 10 min. Subsequently, 7.5 mL of distilled water was added to each sample. Samples were mixed and centrifuged at 1500*g* for 10 min (KN-70, KUBOTA Co., Tokyo, Japan). The upper layer was removed using an aspirator and the lower layer was filtered using a cotton plug. The solution was evaporated using a rotary evaporator (N-1110, Tokyo Rikakikai Co. Ltd., Tokyo, Japan), and the residue was reconstituted in chloroform/methanol (2:1, v/v) to a final volume of 10 mL. The lipid solutions were stored at − 30 °C until further analysis.

2.4. Analysis of phytosterols

Phytosterol contents of marine algae were determined as described

Table 1
Marine algae species used in this study.

Phylum	No	Species	Abbreviation	Collection site	Sampling day
Chlorophyta	1	*Monostroma nitidum* Wittrock	*M. nitidum*	Local store*	–
	2	*Enteromorpha linza* (Linnaeus) J. Agardh	*E. linza*	Hakata bay[a]	Jun. 2015
	3	*Codium fragile* (Suringar) Hariot	*C. fragile*	Hakata bay[a]	Jun. 2015
	4	*Caulerpa brachypus* Harvey	*C. brachypus*	Hayataura[e]	Jul. 2015
Rhodophyta	5	*Gracilaria vermiculophylla* (Ohmi) Papenfuss	*G. vermiculophylla*	Hakata bay[a]	Jun. 2015
	6	*Pterocladiella tenuis* (Okamura) Sa. Shimada, T. Horiguchi & Masuda	*P. tenuis*	Hakata bay[a]	Jun. 2015
	7	*Palisada intermedia* (Yamada) Nam	*P. intermedia*	Hakata bay[a]	Jun. 2015
	8	*Chrysymenia wrightii* (Harvey) Yamada	*C. wrightii*	Hakata bay[a]	Jun. 2015
	9	*Gelidium elegans* Kuezing	*G. elegans*	Hakata bay[a]	Jun. 2015
	10	*Grateloupia asiatica* Kawaguchi & H.W. Wang	*G. asiatica*	Hakata bay[a]	Jun. 2015
	11	*Laurencia okamurae* Yamada	*L. okamurae*	Hakata bay[a]	Jun. 2015
Phaeophyta	12	*Eckloniopsis radicosa* (Kjellman) Okamura	*E. radicosa*	Hayataura[e]	Jul. 2015
	13	*Sargassum thunbergii* (Mertens ex Roth) Kuntze	*S. thunbergii* (Hakata)	Hakata bay[a]	Jun. 2015
	14	*Sargassum thunbergii* (Mertens ex Roth) Kuntze	*S. thunbergii* (Miyama)	Miyama[d]	Jul. 2015
	15	*Ecklonia kurome* Okamura	*E. kurome*	Amakusa[b]	May 2014
	16	*Eisenia arborea* Areschoug	*E. arborea*	Mugisaki[c]	Nov. 2014
	17	*Sargassum piluliferum* (Turner) C. Agardh	*S. piluliferum*	Miyama[d]	Jul. 2015
	18	*Sargassum fusiforme* (Harvey) Setchell	*S. fusiforme*	Local store*	–
	19	*Undaria pinnatifida* (Harvey) Suringar	*U. pinnatifida*	Local store*	–
	20	*Ecklonia cava* Kjellman	*E. cava*	Mugisaki[c]	Nov. 2014
Spermatophyta	21	*Zostera marina* Linnaeus	*Z. marina*	Hakata Bay[a]	Jun. 2015

[a] Hakata bay.
[b] Amakusa.
[c] Mugisaki.
[d] Miyama.
[e] Hayataura, these superscripts means sample collection sites shown in Fig. 1.
* Previously washed, boiled, and dried edible samples were purchased from a local store in Japan.