HOKKAIDO UNIVERSITY

Title	Biochemical Composition and Lipid Compositional Properties of the Brown Alga Sargassum horneri
Author(s)	Hossain, Zakir; Kurihara, Hideyuki; Takahashi, Koretaro
Citation	Pakistan Journal of Biological Sciences, 6(17), 1497-1500 https://doi.org/10.3923/pjbs.2003.1497.1500
Issue Date	2003
Doc URL	https://hdl.handle.net/2115/42570
Type	journal article
File Information	takahashi_PJBS6_1497.pdf

Hokkaido University Collection of Scholarly and Academic Papers : HUSCAP

Pakistan Journal of Biological Sciences 6 (17): 1497-1500, 2003
ISSN 1028-8880
© 2003 Asian Network for Scientific Information

Biochemical Composition and Lipid Compositional Properties of the Brown Alga *Sargassum horneri*

Zakir Hossain, Hideyuki Kurihara and Koretaro Takahashi
Laboratory of Biomolecular Chemistry, Graduate School of Fisheries Sciences
Hokkaido University, Hakodate 041-8611, Japan

Abstract: Biochemical composition of brown alga *Sargassum horneri* was investigated by determination of moisture, protein, carbohydrate, ash, simple lipids and glycolipids. Fatty acid composition of simple lipids and glycolipids was determined by gas chromatography. The algal specimens were collected in two different months, January (sample-1) and February (sample-2). Moisture contents were 86.94 and 87.00% in sample-1 and sample-2, respectively. Protein, carbohydrate and ash contents were 22.94, 19.93 and 32.00% of the dry sample-1, respectively, while the contents of the dry sample-2 were 21.96, 20.81 and 33.58%, respectively. Glycolipids were 1.38 and 1.96% of the dry sample-1 and sample-2, respectively, whereas 2.45 and 2.75% when the samples were digested with the abalone *Haliotis discus* enzyme. Of the glycolipids obtained, MGDG, DGDG and SQDG were nearly 15, 15 and 68%, respectively. The major fatty acids in simple lipids were 16:0, 18:1 and 22:0. The major fatty acids in individual lipid class were 16:0, 18:1, 20:1, 20:4 and 20:5 in MGDG, 16:0, 20:0, 20:4 and 20:5 in DGDG and 16:0, 18:0, 18:1, 18:2 and 20:4 in SQDG. *S. horneri* is a potential source of valuable glycolipids.

Key words: Sargassum horneri, glycolipids, MGDG (monogalaclosyl diachlglycerol), DGDG (digalaclosyl diacyldlycerol), SGDG (sulfoquinovosyl diacylglycerol)

Introduction

Japan is favoured with a rich variety and abundance of seaweed. *Undaria*, *Hizikia*, *Sargassum*, *Eisenia* and *Ecklonia* are found abundantly in warmer water from Honshu to Kyushu islands in Japan. Surrounded by the sea, the Japanese have always relied heavily on seaweeds. Japanese use it in their diets more than any other people.

Sargassum is a large genus with more than 150 species described, occurring in tropical, subtropical and temperate zones of both hemispheres (Nizamuddin, 1962). It is the most conspicuous brown algas in the tropical and subtropical waters, ranging from mid-littoral to sublittoral zones. *Sargassum horneri* is found in the coast of Pacific ocean and the adjoining seas of Formosa, China, Korea and Japan (Tokida and Masaki, 1959).

Algae represent valuable sources of a wide spectrum of complex lipids with different potential applications. The lipids containing polyunsaturated fatty acids are especially interested in various applications. Essential fatty acids are precursors of postaglandins and, as such are becoming increasing by importance in the pharmaceutical industry (Borowitzka, 1988 and Becker, 1994). Some of these compounds are usually rare in terrestrial plants and animals but are present in relatively large amounts in some species of algae and fish (Richmond, 1990; Becker, 1994 and Caughey *et al*., 1996). They have beneficial effects on heart diseases, Parkinson disease, multiple sclerosis, inflammatory diseases, premenstrual syndrome, plasma cholesterol levels, cancers and others (Borowitzka, 1988; Callegari, 1989; Henrikson, 1989; Richmond, 1990 and Rodriguez and Guerrero, 1992). In this paper, the biochemical composition and fatty acid contents of the brown alga *S. horneri* are described.

Materials and Methods

Source of sample: The brown alga *S. horneri* was collected from Japan sea of Toyama Prefecture, Japan, twice on 10th January (sample-1) and on 6th February (sample-2) in 2002. The algal specimens were maintained at –20°C until use.

Measurement of biochemical composition: Moisture was determined by drying 100g samples in a freeze dryer (FD-5, Tokyo Rikakikai, Co. Ltd., Japan) for 72h. Other determinations were carried out using the freeze-dried samples. Protein content was determined according to the method of Kjeldhal. Carbohydrate was analysed by anthrone-sulfuric acid method with glucose as a standard. Total lapid was extracted by the method of Bligh and Dyer (1959). For ash determination, 0.5 g samples were maintained at 600°C for 8 h in a muffle furnace (FO200, Yamato Scientific Co. Ltd. Japan).

Separation of glycolipids and simple lipids from total lipids: The extracted total lipids was applied to silica gel column chromatography and eluted with chloroform to afford simple lipids. Lipids were monitored on thin layer chromatography (TLC) by comparing each spol with authentic lipid standards. TLC plates were developed with hexane/diethyl ether/acetic acid (70:30:1, v/v/v) for simple lipids and chloroform/methanol/water (65:25:4, v/v/v) for glycolipid analysis, respectively. Acetone was then applied to the same silica gel column to collect simple lipids and glycolipids. But simple lipid was absent and negligible amount of MGDG was found in acetone fraction. Finally, methanol was used to the same silica gel column to afford glycolipids.

Removal of chlorophyll from simple lipids: Silica gel Sep-Pak vac-12cc (Water Corporation, Massachusetts, USA) was used for removal of chlorophyll. Firstly, Sep-Pak cartridges were washed with n-hexone (20 ml n-hexane/cartridge). Hexane solution of the simple lipid-containing fraction was subjected on silica gel cartridge and eluted with n-hexane. During the elution, firstly only simple lipids came out. And then a mixture of simple lipids and chlorophyll were eluted off. The simple lipids and the mixture of simple lipids and chlorophyll were preserved separately. The mixture was separated seven times in the same way to divide both the simple lipids and chlorophyll completely.

Separation of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) in glycolipid fraction: MGDG, DGDG and SQDG were separated by TLC on 20 X 20 cm^2 preparative glass plates coated with silica gel 60 (Merck). Chromatography was carried out in glass jar under an air atmosphere in darkroom. Lipids were separated with a developer of chloroform/methanol/water (65:25:4, v/v/v). The bands of lipids were visualized under UV light in dark room. The lipid individual bands were scraped off and immediately eluted with methanol. Methanol elution was evaporated and dissolved in chloroform/methanol/water (10:5:3, v/v/v) in separatory funnel for over night at 4°C. Chloroform layer was collected, evaporated and each lipid was recovered, then the percentage of samples were calculated.

Digestion of *S. horneri* with abalone enzyme: Crude enzyme was collected from the digestive tract of fresh abalone. Shell was opened and digestive tract was cut by scissor. After cutting the digestive tract, inner fluid containing enzymes was started to come out drop by drop and consequently tract was washed with phosphate-buffered saline. Collected enzyme solution was preserved with adding 0.1% sodium azide at 4°C. One gram of each *S. horneri* sample was taken in test tube and was dipped in abalone enzyme solution. Tubes were placed in water bath at 37°C with shaking rate of 50 shakes min^{-1} for 48 h. After 48 h, total lipids were extracted according to the Bligh and Dyer (1959) method with slight modification. Total lipids were then eluted over Sep-Pak cartridges with chloroform, acetone and methanol. All of chloroform, acetone and methanol fractions were monitored on TLC. Methanol fraction was mainly composed of glycolipids and percentages of glycolipids were calculated.

Fatty acid composition analysis: Fatty acid methyl esters were analysed by gas chromatography with a 3 mm x 300 cm glass column packed with Unisole 3000 (GL Sciences, Tokyo, Japan) on uniport C (80-100 mesh) and with flame ionization detection. The used instrument was Hitachi 163 gas chromatograph (Ibaraki, Japan). The temperatures of column, detector and injector were 210, 250 and 240°C, respectively. The identification of fatty acids was determined by comparing the peak retention times with authentic standards (Sigma).

Results and Discussion

The proximate compositions of *Sargassum horneri* are shown in Table 1. Moisture contents in sample-1 (86.94%) and sample-2 (87.0%) were nearly similar. Protein contents were 22.38% and 21.96% of dry sample-1 and sample-2, respectively. Vagas *et al.* (1998) reported that the protein content was about 40% for filamentous N_2-fixing cyanobacteria. In the present experiment, carbohydrate contents in both samples were about 20%, nearly similar to that of protein. But carbohydrate content was about half to that of protein for most of the strains of N_2-fixing cyanobacteria (Vagas *et al.*, 1998). Ash contents accounted 32% and 33.58% in sample-1 and sample-2, respectively, whereas Vagas *et al.* (1998) accounted 5.5% to 11.2% ash for most of the strains of N_2-fixing cyanobacteria. There were little differences in protein, carbohydrate and ash contents in sample-1 and sample-2. Ricketts (1966) reported that protein ranged from 24-46%, lipid 14-48% and carbohydrate 5-57% of various golden brown algae in dry weight basis. Lipids and carbohydrate contents should obviously be very variable depending upon the state of nutrition of cells (Ricketts, 1966). The findings of present experiment coincide well with that of protein and carbohydrate contents reported by Ricketts (1966) who worked with golden brown algae. But lipid was 10-30 folds higher than that of Ricketts (1966). Simple lipids (with chlorophyll) were 0.82% and 0.96% of dry sample-1 and sample-2, respectively, while 0.19% and

Table 1: Proximate compositions of the brown algae *S. horneri*

Property	Condition of samples	% Sample-1	% Sample-2
Moisture	W[a]	86.94	87.00
Protein	D[a]	22.38	21.96
Carbohydrate	D[a]	19.93	20.81
Ash	D[a]	32.00	33.58
Simple lipid (with Chlorophyll)	D[a]	0.82	0.96
Simple lipid (with Chlorophyll)	W[a]	0.19	0.20
Simple lipid (without Chlorophyll)	W[a]	0.05	0.05
Glycolipids	D[a]	1.38	1.96
Glycolipids assisted With abalone digestive enzymes	D[a]	2.45	2.75

[a] W, Wet weight basis; D, dry weight basis

Table 2: Fatty acid composition of simple lipid, MGDG, DGDG and SQDG of *S. Horneri*

Fatty acid	% Simple lipid	MGDG	DGDG	SQDG
$C_{10:0}$	2.10	--	--	--
$C_{11:1}$	1.64	--	--	--
$C_{13:0}$	5.29	--	--	--
$C_{14:0}$	6.00	3.04	3.70	1.67
$C_{14:1}$	3.16	--	--	--
$C_{16:0}$	10.77	14.79	9.95	45.26
$C_{16:1}$	5.24	2.87	6.94	2.78
$C_{17:1}$	3.13	--	--	--
$C_{17:2}$	1.76	--	--	--
$C_{18:0}$	7.73	--	1.98	12.27
$C_{18:1}$	11.54	6.35	4.76	6.21
$C_{18:2}$	9.30	5.77	4.29	9.79
$C_{18:3}$	5.00	4.71	3.44	--
$C_{20:0}$	6.14	--	13.40	--
$C_{20:1}$	--	31.25	2.76	5.86
$C_{20:3}$	7.38	--	--	--
$C_{20:4}$	--	10.62	17.00	7.46
$C_{20:5}$	--	20.60	15.99	2.77
$C_{22:0}$	11.36	--	--	--

0.20% on wet weight basis. Glycolipids (MGDG, DGDG and SQDG) were 1.38% and 1.96% of dry sample-1 and sample-2, respectively. On the other hand, in the samples digested with abalone enzyme, glycolipids were 2.45% and 2.75% of dry sample-1 and sample-2, respectively. The lipid extraction assisted with herbivorous digestive enzymes is an effective method for obtaining whole complex lipids. MGDG, DGDG and SQDG were nearly 15%, 15% and 68%, respectively in complex lipid in both samples (Fig. 1). SQDG typically constitutes about 5% among the total acyl lipids in higher plant leaves. In certain algae, SQDG is a major lipid component and levels of up to 29% of total lipids have been reported (Padley *et al*., 1994).

Fatty acid compositions of simple lipids, MGDG, DGDG and SQDG of *S. horneri* are shown in Table 2. Saturated fatty acids 16:0 and 22:0 and monounsaturated fatty acid 18:1 were the major fatty acids for simple lipids. The findings agree with that of Cohen *et al*. (1989) who found

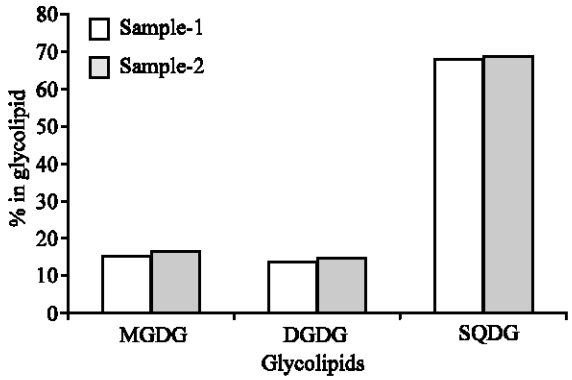


Fig. 1: Percentage of MGDG, DGDG and SQDG in glycolipids of *S. horneri*

nearly similar results for the red algas *Prophyridium cruentum* except 20:5. The major fatty acids of MGDG were 16:0, 18:1, 20:1, 20:4 and 20:5 in *S. horneri*. The findings coincide with that of Arao and Yamada (1989) who found nearly similar results for *S. ringgoldianum*. On the other hand, Murakami *et al*. (2003) found 18:0, 18:1 and 18:3 as the major fatty acids in MGDG of a green vegetable, spinach (*Spinacia oleracea* L.). The major fatty acids of DGDG were 16:0, 20:0, 20:4 and 20:5 for *S. horneri*. The results were also similar to Arao and Yamada (1989) except 18:3. On the contrary, Murakami *et al*. (2003) reported that 16:0 was the major fatty acid in DGDG of spinach *Spinacia oleracea*. The major fatty acids of SQDG were 16:0, 18:0, 18:1, 18:2 and 20:4. Similarly, the results agree with the findings of Cohen *et al*. (1989). But Murakami *et al*. (2003) reported that 16:0 and 18:3 were the major fatty acids in SQDG of spinach *Spinacia oleracea*. From the results, algae are good sources for the glycolipids containing highly unsaturated fatty acids, compared to terrestrial plants. It may be concluded that *S. horneri* is a potential source of valuable glycolipids. Further experimentation should be conducted with the samples of all seasons through a year for getting biochemical variation in a year.

Acknowledgments

This work was partially supported by the grant of 2001, Academic Frontier Promotion Project, Hokkaido Asai Gakuen University. The authors also wish to acknowledge the contribution of Chisato Sekikawa and Seigo Ono in offering the valuable suggestion during the course of this work.

References

Arao, T. and M. Yamada, 1989. Positional distribution of fatty acids in galactolipids of algae. Phytochemistry, 28: 805-810.

Becker, E.W., 1994. Microalgae: biotechnology and microbiology. In Baddiley J., N.H. Carey, I.J. Higgins and W.G. Potter (Eds.). Cambridge studies in biotechnology, vol. 10. Cambridge University Press, Cambridge.

Bligh, E.G. and W.J. Dyer, 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37: 911-917.

Borowitzka, M.A., 1988. Fat, oils and hydrocarbons. In Borowitzka, L.J. (Eds.). Microalgae biotechnology. Cambridge University Press, Cambridge pp: 257-287.

Callegari, J.P., 1989. Feu vert pour les microalgues. Biotutur., 76: 25-40.

Caughey, G.E., E. Mantzioris, R.A. Gibson, L.G. Cleland and M.J. James, 1996. The effect of human tumor necrosis factor and interleukin 1 production of diets enriched in n-3 fatty acids from vegetable oil or fish oil. Am. J. Clin. Nutr., 63:116-122.

Cohen, Z., A. Vonshak and A. Richmond, 1988. Effects of environmental conditions of fatty acid composition of red algae *Porphyridium cruentum*: correlation to growth rate. J. Phycol., 24: 328-332.

Henrikson, R., 1989. New medical research with Spirulina. In Ronose Enterprises, Inc. (Ed.) Earth Food Spirulina. Laguna Beach California, pp: 61-73.

Murakami, C., T. Kumagai, T. Hada, U. Kanekazu, S. Nakazawa, S. Kamisuki, N. Maeda, X. Xu, H. Yoshida, F. Sugawara, K. Sakaguchi and Y. Mizushina, 2003. Effects of glycolipids from spinach on mammalian DNA polymerases. Bioche. Pharma., 65: 259-267.

Nizamuddin, M., 1962. Classification and distribution of the Fucales. Bot. Mar., 4:191-203.

Padley, F.B., F.D. Gunstone and J.L. Harwood. 1994. Occurrence and characteristics of oils and fats. In the lipid handbook. Gunstone, F.D., J.L. Harwood and F.B. Padley. (Eds.) Chapman and Hall, Cambridge, England, pp: 47-223.

Richmond, A., 1990. Large scale microalgal culture and applications. In Round, M. and S. Chapman. (Eds.) Progress in phycological research, Vol. 7. Biopress Ltd., pp: 269-330.

Ricketts, T.R., 1966. On the chemical composition of some unicellular algae. Phytochem., 5: 67-76.

Rodriguez, H. and M.G. Guerrero, 1992. Products and uses of cyanobacteria (blue-green algae). In Villa, T.G. and J. Abalde. (Eds.) Profiles on biotechnology. Universidad de Santiago de Compostela, Santiago, Chile, pp: 247-260.

Tokida, J. and T. Masaki, 1959. Fac. Fish., Hokkaido University, 10: 173-195.

Vagas, M.A., H. Rodriguez, J. Moreno, H. Olivares, A Del Campo, J. Rivas and M.G. Guerrero, 1988. Biochemical composition and fatty acid content of filamentous N_2-fixing cyanobacteria. J. Phycol., 34: 812-817.