Journal of Medicinal Plants Research Vol. 5(11), pp. 2405-2412, 4 June, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

Full Length Research Paper

Fucoxanthin extraction and fatty acid analysis of *Sargassum binderi* and *S. duplicatum*

Dedi Noviendri[1,2], Irwandi Jaswir[1]*, Hamzah Mohd. Salleh[1], Muhammad Taher[3], Kazuo Miyashita[4] and Nazaruddin Ramli[5]

[1]Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia Gombak, 53100 Kuala Lumpur, Malaysia.
[2]Ministry of Marine Affairs and Fisheries, Petamburan VI, 10260, Jakarta, Indonesia.
[3]Faculty of Pharmacy, International Islamic University Malaysia, Kuantan Campus, Kuantan, Malaysia.
[4]Graduate School of Fisheries Sciences, Hokkaido University, Hakodate 041 8611, Japan.
[5]The School of Chemical Sciences and Food Technology, faculty of Science and Technology, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia.

Accepted 28 March 2011

Fucoxanthin has been successfully extracted and purified from two species of Malaysian brown seaweeds, namely *S. binderi* and *S. duplicatum.* The purity of the fucoxanthin is >99% as indicated by HPLC analysis. Fucoxanthin content, total lipid and fatty acid composition of the seaweeds showed that both samples contained a considerable amount of fucoxanthin and total lipid. The amount of fucoxanthin and total lipid contents of *S. duplicatum* (1.01 ± 0.10 and 21.3 ± 0.10 mg/g dry-weight, respectively) was significantly higher than those of *S. binderi* (0.73 ± 0.39 and16.6 ± 4.10, respectively). Both types of seaweeds also contained a considerable amount of unsaturated fatty acids. However, in terms of docosahexanoic acid, eicosapentanoic acid, arachidonic acid, linoleic acid and alpha-linolenic acid contents, *S. duplicatum* was found to be higher (0.76, 2.55, 13.64, 5.81 and 5.35%, respectively) than *S. binderi* (0.70, 1.82, 9.13, 6.37 and 4.39%, respectively). For saturated fatty acids, palmitic (C16:0) was found to be the major fatty acid in both samples studied.

Key words: *Sargassum binderi* and *S. duplicatum*, fucoxanthin; fatty acids, total lipids.

INTRODUCTION

In the Indian Ocean region countries like Indonesia, Malaysia, Singapore, Thailand, Korea, etc., seaweeds are used in salad, jelly, soup, etc (Dhargalkar and Pereira, 2005). Seaweeds or marine macroalgae are potential renewable resource in the marine environment. Seaweeds provide for an excellent source of bioactive compounds, such as carotenoid, dietary fibre, protein, vitamins (Holt, 2008), essential fatty acids, and minerals (Bhaskar and Miyashita, 2005; Sugarawa et al., 2002).

Interest in seaweed lipid has been on the rise owing to the recognition of important bioactive molecules like conjugated fatty acids, pigments (especially fucoxanthin),that have profound physiological effects in the treatment of tumors and other cancer related problems (Hosokawa et al., 2004; Kohno et al., 2002; Kohno et al., 2004). Furthermore, pigments in seaweeds have important nutraceutical properties, including antioxidant and biological response modifying qualities (Holt, 2008). In addition, polyunsaturated fatty acids (PUFAs) are reported to share more than 30% of total fatty acids in diatom or brown algae (Nomura et al., 1997).

In the present study, we extracted, purified and investigated the fatty acid content from two brown seaweeds, *S. binderi* and *S. duplicatum,* obtained from Malaysia. To the best of our knowledge, there has been no report on extraction and purification of fucoxanthin,

*Corresponding author. E-mail: irwandi@iium.edu.my. Tel: +603 6196 4549. Fax: +603 6196 4442.

and investigation of the fatty acid content from Malaysian brown seaweeds.

MATERIALS AND METHODS

Materials

Brown seaweeds used in this study were *S. binderi* and *S. duplicatum.* They were collected fresh from the Straits of Malacca near Port Dickson, Malaysia in August 2009. Each seaweed sample was washed thoroughly with freshwater to remove salt and sand attached to the surface (Soo-Jin et al., 2008; Soo-Jin and You-Jin, 2009), then stored frozen at -80°C and thawed before using it for analysis. The solvents used for high performance liquid chromatography (HPLC) analysis were of HPLC grade. All other solvents and chemicals used in the study were of analytical grade. Standard fucoxanthin (>99% purity established by HPLC) was prepared from wakame (*Undaria pinnatifida*) lipids as described (Hosokawa et al., 1999) previously.

Total lipid contents

The methods of Terasaki et al., (2009) were adopted to determine total lipid (TL). All the extractions were carried out under dim light, and air in the extraction vessel was replaced with nitrogen to prevent any possible degradation of carotenoids or lipids. TL in the samples was extracted overnight with methanol (1:10 w/v), filtered to collect the filtrate and the residue was subjected to one more overnight extraction (1:10 w/v) with methanol to collect the filtrate again. The filtrates were pooled and solvent removed under vacuum at 30 ± 1°C using a rotary flash evaporator (Eyela N1000; Tokyo Rikkakikai Ltd., Tokyo, Japan). The last traces of the solvents and water remaining were removed under high vacuum. TL in the form of viscous green residue was weighed, re-dissolved in methanol and stored at -35°C until further analysis. TL was used for further analysis of fucoxanthin and fatty acid composition.

Fucoxanthin analysis by HPLC analitic

The methods of Terasaki et al. (2009) were adopted for fucoxanthin content determination by HPLC. All HPLC analyses were carried out using a Hitachi L-7000 HPLC system (Hitachi, Tokyo, Japan) equipped with a pump (L-7000), auto-sampler (L-7200) and a photo diode-array spectrophotometric detector (Hitachi L-7455). Fucoxanthin content in seaweed TL was determined by reversed-phase HPLC (RP-HPLC) with methanol-acetonitrile (7:3 v/v) as the mobile phase at a flow rate of 1.0 ml/min (Maeda et al., 2005; Maeda et al., 2006; Maeda et al., 2007). All RP-HPLC analyses were carried out at 28°C using a RP column (Develosil-ODS, UG-5, 5.0 µm particle size, 250 mm x 4.6 mm i.d.; Nomura Chem, Co., Seto, Aichi, Japan) (Maeda et al., 2006; Maeda et al., 2007) protected with a guard column (10 x 4.0 mm i.d.) having the same stationary phase. Briefly, an aliquot of TL was dissolved in the mobile phase, filtered with a 0.22 µm membrane filter, and an aliquot of the filtered sample was submitted to HPLC analysis. The detection wavelength was set at 450 nm for detecting fucoxanthin (Yan et al., 1999; Mori et al., 2004; Maeda et al., 2006; Cheng-Ling et al., 2009; Nakazawa et al., 2009). A standard curve prepared using authentic standard was used for quantification of fucoxanthin content in seaweed samples.

Fucoxanthin content in seaweed samples were expressed as $mg.g^{-1}$ dry weight of seaweed sample. The amount of fucoxanthin was quantified from the peak area using a standard curve with purified fucoxanthin (Maeda et al., 2006).

Extraction and purification of fucoxanthin

Extraction of fucoxanthin

A slight modification of the method described by Haugen et al. (1992) was adopted for the extraction and purification of fucoxanthin. Cold acetone-methanol (7:3 v/v) was added to a 1 L flask containing dried and ground brown seaweed (Haugan and Liaaen-Jensen, 1989). These mixtures were homogenized on ice for 10-15 min, and then mixtures filtered through a filter paper. The steps were repeated at least three times. The acetone: methanol extracts were pooled and left at room temperature, under N_2 and in the dark until the extract becomes colorless. The extract was evaporated to dryness at 30 to 35°C on a rotary evaporator, and the residue was dissolved in methanol. The reconstituted residue was partitioned in a separation funnel between n-hexane and 90% (v/v) aqueous methanol for three times. The hexane phase was discarded. Fucoxanthin from the aqueous phase was moved to diethyl ether. The diethyl ether phase was evaporated to dryness on a rotary evaporator. The residue was re-dissolved in minimum amount of benzene for the purification step (Haugan et al., 1992; Haugan and Jensen, 1994).

Purification of fucoxanthin

The benzene containing residue was loaded to a silica column (Silica 60G, Merck, 0.040 - 0.063 mm). Elution was initially performed with n-hexane (100%) to remove chlorophyll and carotenoids other than fucoxanthin (Sangeetha et al., 2009). Elution was continued with n-hexane:acetone (6:4; v/v) to recover fucoxanthin (Hosokawa et al., 1999). Finally, residual fucoxanthin was eluted with acetone solvent. The acetone and hexane: acetone (6:4; v/v) fractions containing fucoxanthin were together evaporated to dryness by a rotary evaporator.

Further purification of fucoxanthin

The residue from the combined acetone and hexane: acetone (6:4; v/v) evaporation step was re-dissolved in methanol. The concentration of fucoxanthin was checked by HPLC preparative (ODS double column, 1 ml/min at 450 nm, methanol and acetonitrile were the mobile phase). All procedures were carried out under dim yellow light to minimize degradation and isomerization of fucoxanthin by light irradiation (Sugarawa et al., 2002).

Fatty acid contents

Fatty acid (FA) analysis was accomplished by injecting FA methyl esters (FAMEs) into a gas chromatography (GC) system (Shimadzu GC-14B; Shimadzu Seisakusho, Kyoto, Japan) equipped with a flame-ionization detector (FID) and a capillary column (Omegawax-320; 30 m x 0.32 mm i.d.; Supelco, Bellefonte, PA). The carrier gas was helium at a flow rate of 50 Kpa (Bhaskar et al., 2004). The detector, injector and column temperature were 260, 250 and 200°C respectively. Briefly, to an aliquot of TL, 1 ml n-hexane and 0.2 ml 2N NaOH in methanol were added, vortexed and incubated at 50°C for 30 min. Post incubation, 0.2 ml 2N HCl in methanol solution was added, gently mixed to recover the upper n-hexane layer containing FAMEs. FA content in seaweed samples was expressed as weight percentage of total FAs (Terasaki et al., 2009).

Statistical analysis

Mean and standard deviations were computed using Microsoft

Table 1. Total lipid, fucoxanthin content and purity of fucoxanthin after silica gel open column chromatography (B1), and after HPLC-ODS double column (B2).

Species	Total lipid (mg/g dry wt)	Fucoxanthin (mg/g dry wt)	Purity of fucoxanthin (%)	
			B1	B2
S. binderi	16.60 ± 4.10	0.73 ± 0.39	90.7	98.2
S. duplicatum	21.30 ± 0.10	1.01 ± 0.10	90.1	99.8

About 1 g dry weight of sample was used in the analysis. The experiment has been done triplicate (n = 3).

Excel software.

RESULTS AND DISCUSSION

Total lipid and fucoxanthin

The quantitative data on total lipid and fucoxanthin of two brown seaweeds are presented in Table 1. Results showed that both samples contained a considerable amount of fucoxanthin and total lipid. The fucoxanthin and total lipid contents of *S. duplicatum* (1.01 ± 0.10 and 21.3 ± 0.10 mg/g dry-weight, respectively) were significantly higher than those of *S. binderi* (0.73 ± 0.39 and 16.6 ± 4.10, respectively). These results are relatively the same with *S. fugiforme* (1.1 ± 0.6 and 27.5 ± 11.9 mg/g dry-weight, respectively) as reported by Terasaki et al. (2009). The percentage of total lipid contents from *S. duplicatum* and *S. binderi* are between 1.7 to 2.1%. These results are nearly the same with the results obtained from two brown seaweeds, such as *L. digita* (2.4%) and *Padina tetrastromatica* (1.7%) (Dhargalkar and Pereira, 2005), but the percentage of lipids in *S. vulgare* was very low (0.45%) (Plaza et al., 2008) (Table 1).

Furthermore, the fucoxanthin contents of *Sargassaceae*, such as *S. thunbergii*, *S. fusiforme* and *S. confusum* (1.8 ± 1.0; 1.1 ± 0.6; and 1.6 ± 0.8, respectively) from Hakodate, Japan (Terasaki et al., 2009) are significantly comparable with fucoxanthin contents of *S. duplicatum* and *S. binderi* from Malaysia determined in this study. Nevertheless, the fucoxanthin contents of *S. duplicatum* and *S. binderi* were lower than S. *horneri* from Hakodate, Japan (3.7 ± 1.6 mg/g dry-weight). However, the fucoxanthin contents of two brown seaweeds in the present study were higher than four brown seaweeds: *Scytosiphon lomentaria*, *Leathesia difformis*, *Sphaerotrichia divaricata* and *Desmarestia viridis* (0.5 ± 0.1; 0.3 ± 0.1; 0.2 ± 0.1 and 0.1 ± 0.1 mg/g dry weight, respectively) from Hakodate, Japan (Terasaki et al., 2009).

The combined acetone and n-hexane/acetone (6:4, v/v) factions from the silica column chromatography contained the desired fucoxanthin. The *S. duplicatum* and *S. binderi* fucoxanthin in these combined fractions showed purity > 90%. The purity of fucoxanthin from the two Malaysian brown seaweeds was nearly the same (Table 1). Maeda et al., (2007) reported that *wakame* lipid extraction with acetone followed by salicic acid column chromatography using n-hexane/acetone (7:3, v/v) as the mobile phase was successful for fucoxanthin separation. When this separation step was repeated three times, the recovered fucoxanthin showed purity > 78% on HPLC. In the present study, fucoxanthin has been successfully extracted and purified from two species of Malaysian brown seaweeds, namely *S. binderi* and *S. duplicatum,* with the purity of fucoxanthin obtained up to 99.8% (Table 1). In addition, the HPLC chromatograms of purified fucoxanthin from *S. duplicatum* and *S. binderi* showed only one major peak with a retention time of about 7.8 min (Figure 1).

Fatty acid contents

In this study, fourteen fatty acids were identified in extracts of Malaysian brown seweeds. The fatty acid compositions of the brown seaweeds are shown in Table 2 whereas Figure 2 shows a typical chromatogram of the FAMEs of *S. duplicatum*. In all samples studied, the single most abundant fatty acid was C16:0 (palmitic acid), which in *S. binderi* and *S. duplicatum* accounted for 25.09 and 24.88% of all fatty acids, respectively. These results were comparable to other brown seaweeds, such as *Hormosira banksii*, *Ralfsia* sp., *Dictyota dichomota* (Johns et al., 1979), *Stilophora rhizodes, Entonema parasiticum, Pylaiella littoralis, Corynophlaea umbellate*, *Cystoseria crinita*, (Dembitsky et al., 1990), *Saccorhiza polyscides*, *H. elongate*, and *L. ochroleuca* (Sánchez-Machado et al., 2004), *S. marginatum* (Bhaskar et al., 2004), and *Laminaria* sp. (Dawczyski et al., 2007) where the single most abundant fatty acid was palmitic acid. The predominant fatty acids found in the brown seaweeds in the present study were palmitic (24.88 to 25.09%), stearidonic (3.48 to 12.87%), arachidonic (9.13 to 13.64%), linoleic (5.81 to 6.37%), α-linolenic (4.38 to 5.35%) acids were also found in all brown seaweeds. Arachidic (0.22 to 0.28%) and lauric (0.03 to 0.52%) acids were found at very low levels.

The two Malaysian brown seaweeds in this study also contained the essential fatty acids (EFAs) omega 6 and omega 3, namely C18:2n-6 (linoleic acid, LA), and C18:

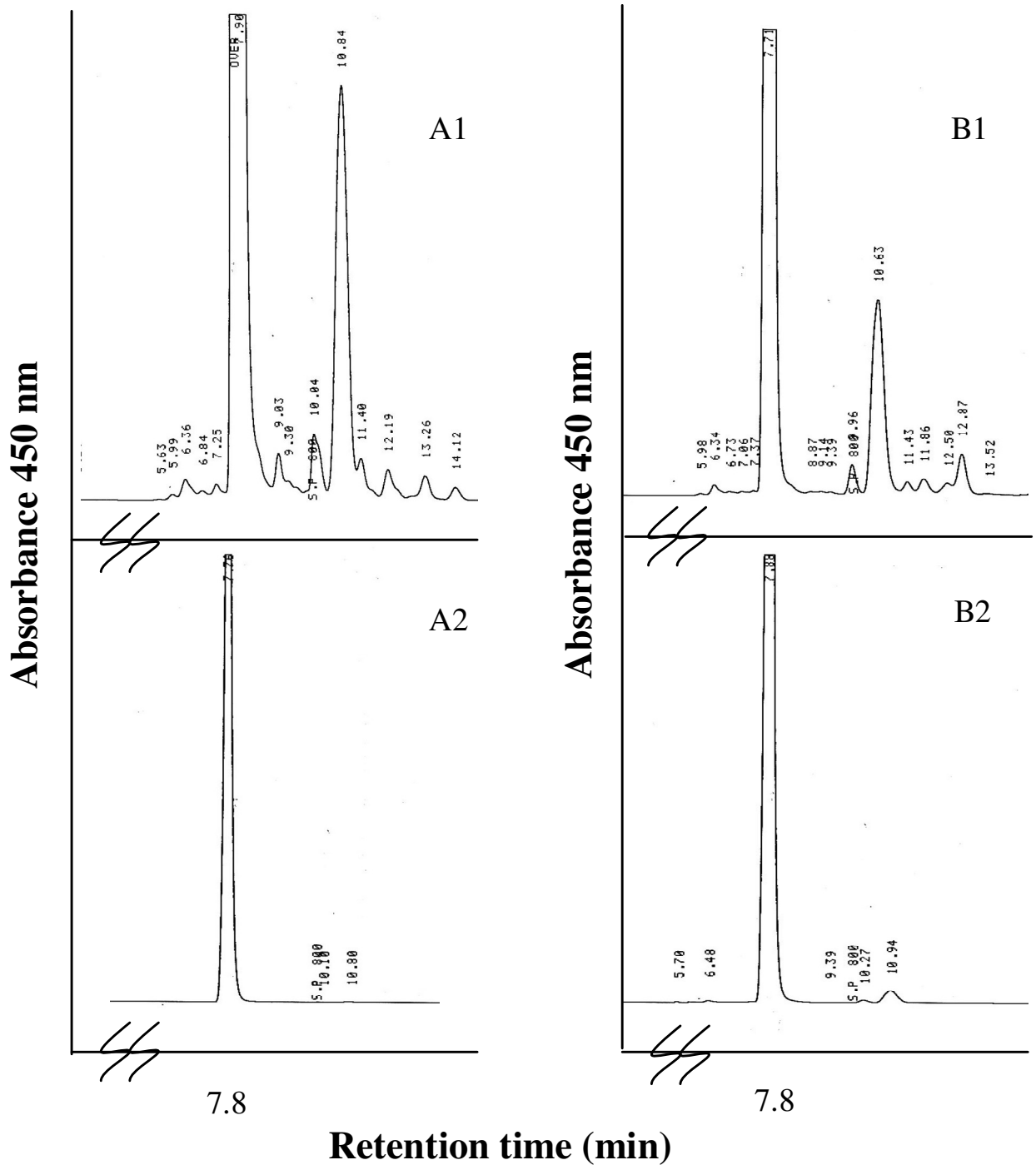

Figure 1. HPLC chromatograms of fucoxanthin extracted from *S. duplicatum*: after SiO_2 resin open column chromatography (A1), and after ODS-double column (A2), and *S. binderi*: after SiO_2 resin open column chromatography (B1), and after ODS-double column (B2).

3n-3 (alpha-linolenic acid, ALA). Then, non essential fatty acid omega 6 and omega 3, namely C20:4n-6 (arachidonic acid, AA) and C20:5n-3 (eicosapentaenoic acid, EPA) have also been reported in macroalgae from southern Yemen (Banainmoon, 1992) and brown seaweeds from Spain (Sánchez-Machado et al., 2004). LA, ALA and their long-chain derivatives are important components of animal and plant cell membranes (Simopoulus, 2002).

Essential fatty acids are required for maintenance of optimal human health, but they can not be synthesized in the body and must be obtained from dietary sources

Table 2. Metabolic conversion of EFAs to their active metabolites in humans adults (Pawlosky et al., 2001; Singh, 2005).

The omega 6 polyunsaturated	The omega 3 polyunsaturated
Linoleic acid (LA) (C18:2n-6)	Alpha-linolenic acid (ALA) (C18:3n-3)
↓ Δ-6 desaturase	↓ Δ-6 desaturase
Gamma-linolenic acid (GLA) (C18:3n-6)	Eicosapentaenoic acid (EPA) (C20:5n-3)
↓ Δ-5 desaturase	↓ Δ-5 desaturase
Arachidonic acid (AA) (C20:4n-6)	Decosapentaenoic acid (DPA) (C22:5n-3)
	↓ Δ-4 desaturase
	Decosahexaenoic acid (DHA) (C22:6n-3)

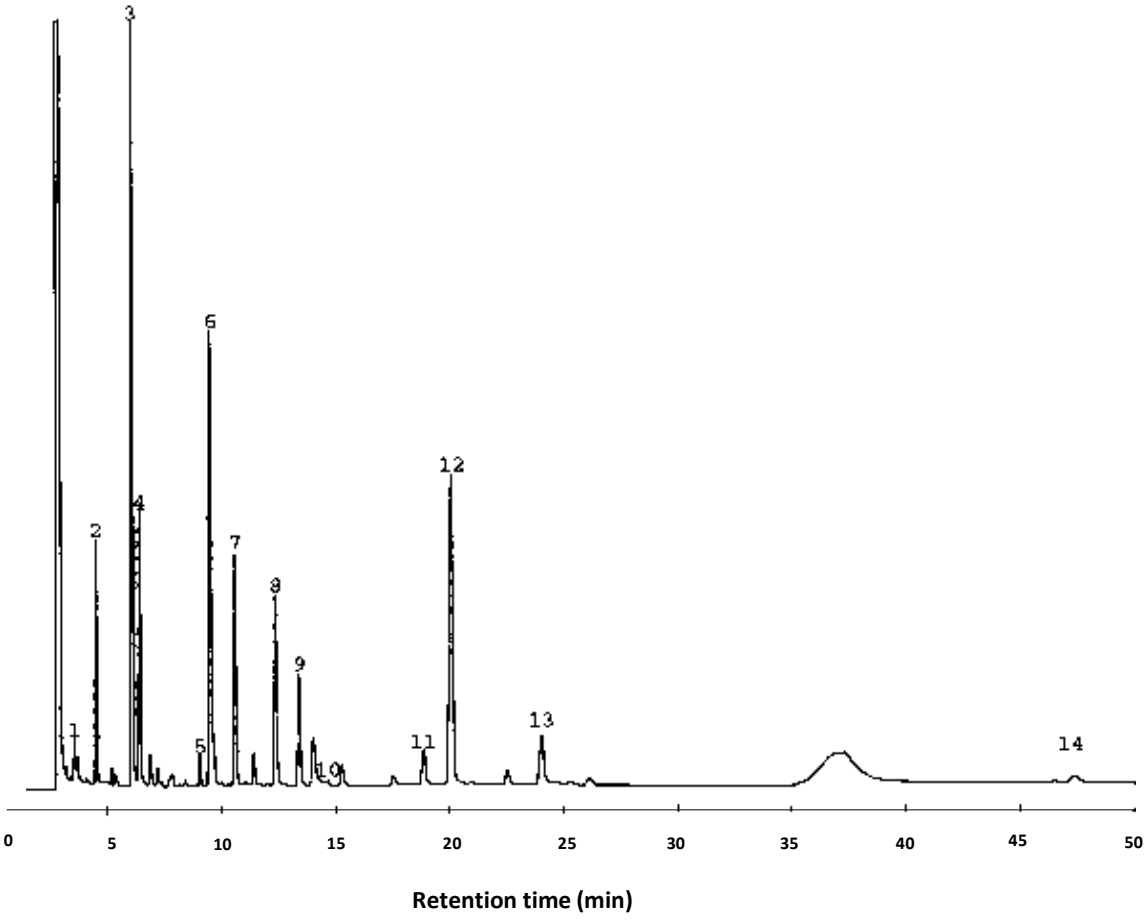

Figure 2. A typical chromatogram of the FAMEs of *S. duplicatum*.

(Singh, 2005; PennState, 2006). The body uses essential fatty acids (EFAs) for the formation of healthy cell membranes, the proper development and functioning of the brain and nervous system, and for the production of hormone-like substances called eicosanoids (thromboxanes, leukotrienes, prostaglandins) (Bruno, 2005). A fatty acid is considered essential if the body is unable to synthesize it and the only way it can be obtained is through the diet. In addition, it is considered essential if a deficiency will cause a disease (Lee, 1997).

EFAs are also called polyunsaturated fatty acids (PUFAs (Singh, 2005). There are two groups of PUFAs, omega 6 (n-6) and omega 3 (n-3). The parent omega 6 fatty acid is linoleic acid (LA) and the parent omega 3 fatty acid is alpha-linolenic acid (ALA) (Clandinin et al., 1994). PUFAs synthesis from parent essential fatty acids is shown in Figure 3. In humans, both EFAs (omega 3 and omega 6 are 18 carbon atoms fatty acids) are

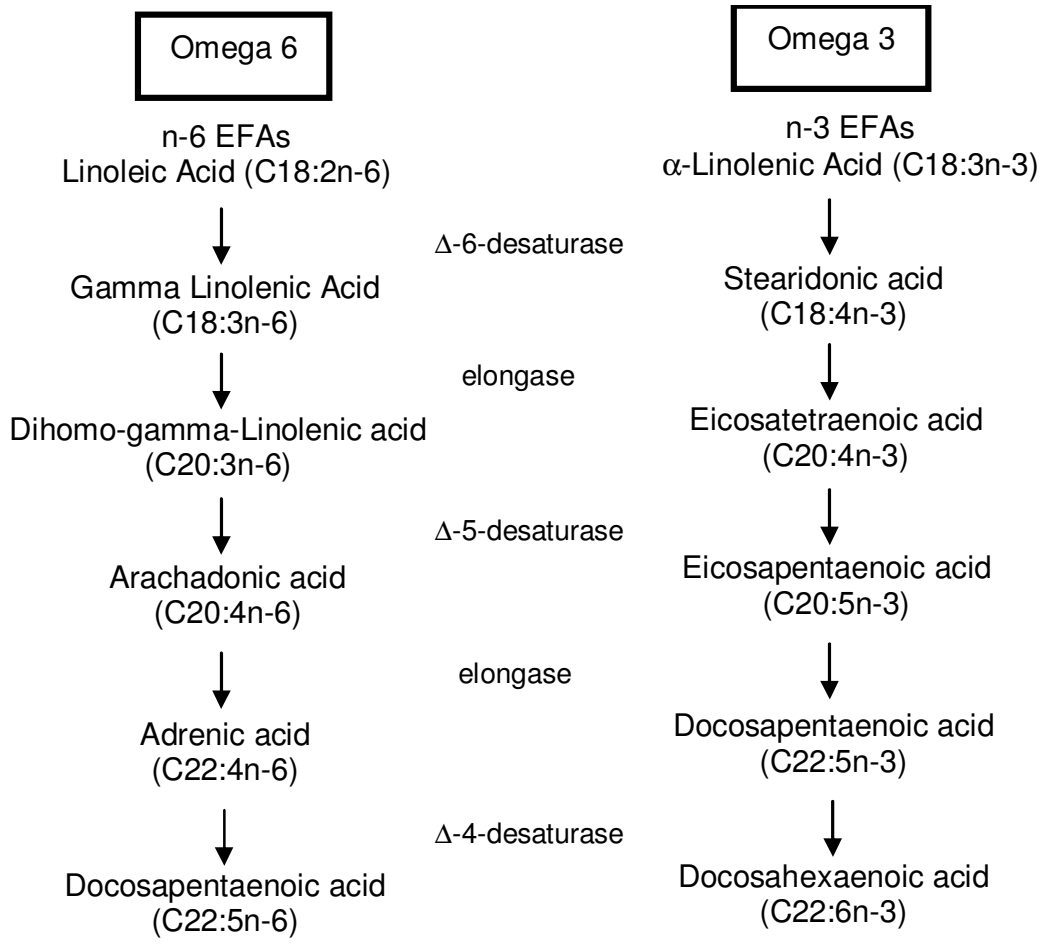

Figure 3. PUFA synthesis from parent essential fatty acids (Haag, 2003).

metabolized to long chain fatty acid be desaturation and adding extra double bonds to the carboxyl group end of the molecule (Singh, 2005). In this case, Linoleic acid is metabolized to arachidonic acid, while alpha-linolenic acid is metabolized to eicosapentaenoic acid and decosahexaenoic acid as active metabolic end products (Table 2) (Pawlosky et al., 2001; Singh, 2005).

Interestingly, *S. binderi* and *S. duplicatum* from the off-shore of Malaysia also contained a small amount of fatty acid C22:6n-3 (docosahexaenoic acid, DHA). In contrast, DHA was not found in the analysed brown seaweeds species of *Ralfsia* sp., *D. dichomota* (Johns et al., 1979), *Laminaria* sp. (Konbu), *U. pinnatifida* (Wakame), and *Hizkia fusiforme* (Hijiki) (Dawczynski et al., 2007). The contents of AA, EPA and DHA, of the two Malaysian brown seaweeds analysed, range from 9.13 to 13.64%, 1.82 to 2.55% and 0.70 to 0.76%, respectively. Arachidonic acid was the predominant fatty acid found in *Hormosira banksii*, *Ralfsia* sp., *D. dichomota* at 12.98, 6.83 and 11.46% content, respectively (Johns et al., 1979) (Table 3).

Generally, the PUFA contents of two brown seaweeds (*S. binderi* and *S. duplicatum*) were much higher (33 to 37%) than the SFAs contents (29 to 31%), and MUFA contents are lowest (13 to 15%). Furthermore, the concentration of omega 3 (n-3) PUFA of *S. binderi* (20%) in this study was found to be much greater than *S. duplicatum* (12%). In contrast, the concentration of omega 6 (n-6) PUFA of *S. binderi* (17%) is lower than *S. duplicatum* (21%) (Table 3). However, the PUFA/SFA ratio obtained from *S. binderi* (1.2%) is nearly the same with the value determined for *S. duplicatum* (1.1%). The PUFA/SFA ratio of 1:1 to 1:2 determined for the brown seaweeds in this study are good since many nutritional associations recommended a ratio of 1:1 for PUFA/SFA (Dashty et al., 2003). PUFA, especially the n-3 and n-6 PUFA, have been considered essential fatty acids and have been shown to have curative and preventive effect on cardiovascular diseases, neurodevelopment in infants, cancers and fat glycemic control (Corner, 1997).

Seaweed products represent an important source of long-chain PUFA (n-3; n-6) that is fundamental for the formation of important structural lipids and elements of cell membranes (Dawczyski et al., 2007). The seaweeds

Table 3. Fatty acids content (%) of *S. binderi and S. duplicatum*.

Peak	Retention time (minute)	Fatty acid content (%)	Species	
			S. binderi	***S. duplicatum***
1	3.65	Lauric; C12:0	0.03	0.52
2	4.49	Myristic; C14:0	4.94	3.09
3	6.04	Palmitic; C16:0	25.09	24.88
4	6.36	Palmitoleic; C16:1n-7	4.42	5.02
5	8.97	Stearic; C18:0	0.89	0.72
6	9.44	Oleic; C18:1n-9	9.23	10.37
7	10.53	Linoleic acid; C18:2n-6 (LA)	6.37	5.81
8	12.32	Alfa-linolenic acid; C18:3n-3 (ALA)	4.38	5.35
9	13.36	Stearidonic acid; C18:4n-3	12.87	3.48
10	14.57	Arachidic; C20:0	0.22	0.28
11	18.80	Homo-gamma-linolenic; C20:3n-6	1.64	1.51
12	20.02	Arachidonic acid; C20:4n-6 (AA)	9.13	13.64
13	23.96	Eicosapentaenoic acid; C20:5n-3 (EPA)	1.82	2.55
14	47.27	Docosahexaenoic acid; C22:6n-3 (DHA)	0.70	0.76
Total Saturated fatty acids (SFA)			31.17	29.49
Total monounsaturated fatty acids (MUFA)			13.65	15.39
Total polyunsaturated fatty acids (PUFA)			36.91	33.10
PUFA/SFA			1.18	1.12
Total n-3			19.77	12.14
Total n-6			17.14	20.96
n6/n3			0.87	1.73
Identified-total			81.73	77.98
Others (Un-identified)			18.27	22.02

studied in this work (*S. binderi* and *S. duplicatum)* have n6:n3 ratio between 0.9 and 1.7%. Sánchez-Machado et al. (2004) reported that *H. elongata* and *U. pinnatifida* have n6:n3 ratio 0.81 and 0.49%, respectively. The n6:n3 ratio is beneficial for health due to several sources of information suggesting that human beings evolved a diet in which the ratio of omega-6 to omega-3 essential fatty acids (EFA) was about 1, whereas in Western diets the ratio is 15/1 – 16.7/1. Excessive amounts of n-6 PUFA and a very high n6:n3 ratio will promote the pathogenesis of many diseases, including cardiovascular disease, cancer, and inflammatory and autoimmune disease, whereas increased levels of n-3 PUFA (a low n6:n3 ratio) exert suppressive effects (Simopoulus, 2002). The intake of food rich in n-3 PUFA can have a positive influence on the composition of blood lipids and can therefore be used for the prevention of arteriosclerosis (Murata et al., 2002).

Conclusions

Two Malaysian brown seaweeds, *S. duplicatum* and *S. binderi* were found to contain functional lipid component like fucoxanthin. Fucoxanthin has been successfully extracted and purified with the purity of fucoxanthin obtained up to 99.8%. Both types of seaweeds also contained a considerable amount of unsaturated fatty acids. Furthermore, the brown seaweeds contained the essential fatty acids omega 6 and omega 3, namely LA and ALA. Then, they also contained non essential fatty acid omega 6 and omega 3, namely AA, EPA and DHA.

ACKNOWLEDGEMENTS

This work was financially supported by a research grant (IIUM RMGS 09-05) from the Research Management Center, International Islamic University Malaysia. We thankfully acknowledge Associate Professor Masashi Hosokawa, Airanthi Ila and Masatoshi Nomura from the Laboratory of Biofunctional Material Chemistry, Hokkaido University, Hakodate, Hokkaido, Japan, for their excellent technical support.

REFERENCES

Banainmoon SA (1992). Fatty acids in marine macroalgae from southern Yemen (Hadramount) including occurrence of eicosatetraenoic (20:4) and eicosapentaenoic (20:5) acids. Bot. Marin., 35: 165-168.

Bhaskar N, Miyashita K (2005). Lipid composition of *Padina tetratomatica* (Dictyotales, Phaeophyta), a brown seaweed of the west coast of India. Indian J. Fish., 52: 263-268.

Bhaskar N, Hosokawa M, Miyashita K (2004). Growth inhibition of human pro-myelocytic leukemia (HL-60) cells by lipid extracts of marine alga *Sargassum marginatum* (Fucales, Phaeophyta) harvested off Goa (west coast of India) with special reference to fatty acid composition. Indian J. Mar. Sci., 33(4): 355-360.

Bruno G (2005). Essential & non essential fatty acids, Huntington Coll. Health Sci., pp. 1-4.

Clandinin MT, Jumpsen J, Suh M (1994). Relationship between fatty acid accretion, membrane composition and biologic functions. J. Pediatr., 125(5): S25-S32.

Corner WE (1997). The beneficial effects of omega-3 fatty acids:cardiovascular disease and neurodevelopment. Curr. Opin. Lipidol., 8: 1-3.

Dawczynski C, Schubert R, Jahreis G (2007). Amino acids, fatty acids, and dietary fibre in edible seaweed products. Food Chem., 103: 891-899.

Dhargalkar VK Pereira N (2005). Seaweed: promising plant of the millennium. Sci. Cult., 71: 60-66.

Dembitsky VM, Rozentsyet OA, Pechenkina EE (1990). Glycolipids, phospholipids and fatty acids of brown algae species. Phytochem., 29(11): 3417-3421.

Haag M (2003). Essential Fatty Acids and the Brain. Can. J. Psychiatr., 48(3): 193-203.

Haugan JA, Liaaen JS (1994). Algal carotenoids 54.* Carotenoids of brown algae. Biochemic. Syst. Ecol., 22(1): 31-41.

Haugan JA, Akermann T, Liaaen JS (1992). Isolation of fucoxanthin and piridinin. In: Packer L (ed) Carotenoids part A: Chemistry, separation, quatitation and antioxidants. Methods in Enzymology, vol.213. Academic Press Inc., Orlando, pp. 231-245.

Haugan JA, Liaaen JS (1989). Improved isolation procedure for fucoxanthin. Phytochemistry, 28(10): 2797-2798.

Holt S (2008). Seaweed for healing and weight loss. Nat. Benefit News, 2: 1-4.

Hosokawa M, Wanezaki S, Miyauchi K, Kurihara H, Kohno H, Kawabata J, Odashima S, Takahashi K (1999). Apoptosis-inducing effect of fucoxanthin on human leukemia cell line HL-60. Food Sci. Technol. Res., 5(3): 243-246.

Hosokawa M, Kudo M, Maeda H, Kohno H, Tanaka T, Miyashita K (2004). Fucoxanthin induces apoptosis and enhances the antiproliferative effect of the PPARγ ligand, troglitazone, on colon cancer cells. Biochim. Biophys. Acta, 1675: 113-119.

Johns RB, Nichols PD, Perry GJ (1979). Fatty acid composition of ten marine algae from Australia waters. Phytochemistry, 18: 799-802.

Kohno H, Suzuki R, Noguchi R, Hosokawa M, Miyashita K, Tanaka T (2002). Dietary conjugated linolenic acid inhibits azoxymethane-induced colonic aberrant crypt foci in rats. Jpn. J. Cancer Res., 93: 133-142.

Kohno H, Suzuki R, Yasui Y, Hosokawa M, Miyashita K, Tanaka T (2004). Pomegranate seed oil rich in conjugated linolenic acid suppresses chemically induced colon carcinogenesis in rats. Cancer Sci., 95: 481-486.

Lee D (1997). Essential fatty acids. Woodland Publishing, Inc. Pp. 1-39.

Maeda H, Hosokawa M, Sashima T, Funayama K, Miyashita K (2005). Fucoxanthin from edible seaweed, Undaria pinnatifida, shows antiobesity effect through UCP1 expression in white adipose tissues. Biochem. Biophys. Res. Comm., 332(2): 392-397.

Maeda H, Hosokawa M, Sashima T, Takahashi N, Kawada T, Miyashita K (2006). Fucoxanthin and its metabolite, fucoxanthinol, suppress adipocyte differentiation in 3T3-L1 cells. Int. J. Mol. Med., 18: 147-152.

Maeda H, Hosokawa M, Sashima T, Miyashita K (2007). Dietary combination of fucoxanthin and fish oil attenuates the weight gain of white adipose tissue and decreases blood glucose in obese/diabetic KK-A^y Mice. J. Agric. Food Chem., 55: 7701-7706.

Mori K, Ooi T, Hiraoka M, Oka N, Hamada H, Tamura M, Kusumi T (2004). Fucoxanthin and its metabolites in edible brown algae cultivated in deep seawater. Mar. Drugs, 2: 63-72.

Murata M, Sano Y, Ishihara K, Uchida M (2002). Dietary fish oil and *Undaria pinnatifida* (Wakame) synergistically decrease rat serum and liver triacylglycerol. J. Nutr., 132: 742-747.

Nomura T, Kikuchi M, Kubodera A, Kawakami Y (1997). Proton-donative antioxidant activity of fucoxanthin with 1,1-diphenyl-2-pycrylhydrazyl (DPPH). Biochem. Mol. Biol. Int., 42(2): 361-370.

Pawlosky RJ, Hibbelin JR, Novotny JA, Salem JJ (2001). Physiologycal compartmental analysis of linolenic acid metabolism in adult humans. J. Lipid Res., 42: 1257-1265.

Plaza M, Cifuentes A, Ibáñez (2008). In the search of new functional food ingredients from algae. Trends Food Sci. Technol., 19: 31-39.

Sánchez MDI, López CJ, López HJ, Paseiro LP (2004). Fatty acids, total lipid, protein and ash contents of processed edible seaweeds. Food Chem., 85: 439-444.

Sangeetha RK, Bhaskar N, Baskaran V (2009). Comparative effects of β-carotene and fucoxanthin on retinol deficiency induced oxidative stress in rats. Mol. Cell. Biochem., 331(1-2): 59-67.

Simopoulus AP (2002). The importance of the ratio of omega-6/omega-3 essential fatty acids. Biomed. Pharmacother., 56: 365-379.

Singh M (2005). Essential fatty acids, DHA and human brain. Indian J. Pediatr., 72: 239-242.

Soo-Jin H, Seok CK, Sung MK, Hahk SK, Jong PK, Soo HK, Ki WL, Man GC, You JJ (2008). Cytoprotective effect of fucoxanthin isolated from brown algae *Sargassum siliquastrum* against H_2O_2-induced cell damage. Eur. Food Res. Technol., 228: 145-151.

Soo-Jin H, You JJ (2009). Protective effect of fucoxanthin isolated from Sargassum siliquastrum on UV-B induced cell damage. J. Photochem. Photobiol. B. Biol., 95: 101-107.

Sugarawa T, Baskaran V, Tsuzuki W, Nagao A (2002). Brown algae fucoxanthin is hydrolyzed to fucoxanthinol during absorption by Caco-2 human intestinal cells and mica. J. Nutr., 132: 946-951

Terasaki M, Hirose A, Narayan B, Baba Y, Kawagoe C, Yasui H, Saga N, Hosokawa M, Miyashita K (2009). Evaluation of Recoverable functional lipid components of several brown seaweeds of Japan with special reference to fucoxanthin and fucosterol contents. J. Phycol., 45(4): 974-980.

Yan X, Chuda Y, Suzuki M, Nagata T (1999). Fucoxanthin as the major antioxidant in Hijikia fusiformis, a common edible seaweed. Biosci. Biotechnol. Bioeng., 63(3): 605-607.