Vietnam Journal of Marine Science and Technology; Vol. 21, No. 2; 2021: 311–318
DOI: https://doi.org/10.15625/1859-3097/15942
http://www.vjs.ac.vn/index.php/jmst

Content and composition of lipid classes, fatty acid from *Sargassum* seaweed collected at Con Dao and Van Phong bay

Thu Hue Pham[1,2], Van Tuyen Anh Nguyen[3], Thi Hoang Yen Kieu[3], Le My Anh Nguyen, Hai Nam Hoang[3], Quoc Long Pham[3], Duc Tien Dam[4,5], Thi Thanh Van Tran[6], Tat Thanh Le[1,3,*]

[1]*Graduate University of Science and Technology, VAST, Vietnam*
[2]*Vietnam Naval Academy, Nha Trang, Vietnam*
[3]*Institute of Nature Products Chemistry, VAST, Vietnam*
[4]*Institute of Marine Environment and Resources, VAST, Vietnam*
[5]*Haiphong University of Medicine and Pharmacy*
[6]*Nha Trang Institute of Technology Research and Application, VAST, Vietnam*
[*]E-mail: thanh.biotech@gmail.com

Received: 26 November 2020; Accepted: 1 February 2021

Abstract
This study studied the content and composition of the total lipid, lipid classes and fatty acids in 13 brown seaweed *Sargassum* species collected from Con Dao and Van Phong, Vietnam. The total lipid has a low content and varies among species from 0.10–1.70% of the fresh weight. From 13 species, seven lipid classes including polar lipid (Pol), free fatty acids (FFA), sterol (ST), hydrocarbon and wax (HW), triacylglycerol (TG), diacylglycerol (DG), and monoalkydiacylglycerol (MADG). Using the GC-FID technique, we have identified 29 fatty acids classified into 3 groups of saturated fatty acid, monounsaturated fatty acids, polyunsaturated fatty acids with an average content of 44.93%, 24.57% and 27.44%, respectively. Among those, many value fatty acids have been detected with high content such as C18:3n-3, C20:4n-6, 20:5n-3, and 22:6n-3. The lipid of 13 brown seaweed *Sargassum* species also fully contains omega-3,6,9 fatty acids with the content of 9.28%, 16.28% and 16.63%, respectively.

Keywords: Omega-3,6,9, *Sargassum*, Con Dao.

Citation: Thu Hue Pham, Van Tuyen Anh Nguyen, Thi Hoang Yen Kieu, Le My Anh Nguyen, Hai Nam Hoang, Quoc Long Pham, Duc Tien Dam, Thi Thanh Van Tran, Tat Thanh Le, 2021. Content and composition of lipid classes, fatty acid from Sargassum seaweed collected at Con Dao and Van Phong bay. *Vietnam Journal of Marine Science and Technology*, *21*(2), 311–318.

INTRODUCTION

The genus *Sargassum* (Phaeophyta) species are found primarily in tropical and subtropical shallow marine waters [1]. Due to their high concentration in polysaccharides, natural richness in minerals, polyunsaturated fatty acids, and vitamins with their bioactivities are known to be a good source of healthy food [2].

In Vietnam, there are more than 1,000 seaweed species, in which 143 species belong to the Phaeophyta phylum, including 35 species of *Sargassum* genus with 22 species in the North and 13 species in the South [3]. Their bioactive compounds that are most extensively studied include sulfated polysaccharides, phlorotannins, and diterpenes. These compounds have been reported to possess anti-viral, anti-tumor, and anti-cancer properties [2]. However, studies on lipids and fatty acids on *Sargassum* species are rarely conducted in Vietnam.

The lipid content and fatty acid composition of seaweed are related to environmental factors, the difference between species, and collected place [4–6]. This study investigates and compares the fatty acid composition of thirteen seaweed samples belonging to the genus *Sargassum* from Con Dao and Van Phong Bay, Vietnam.

MATERIALS AND METHOD

Material

Thirteen seaweeds belong to the *Sargassum* genus were collected from Con Dao and Van Phong, Vietnam, and identified by Dr. Dam Duc Tien in 2017 (table 1). The collected samples were cleaned and stored under standard conditions at -20^{o}C.

Methods

Lipid analysis

The total lipid was extracted according to the modified method of Bligh and Dyer (1959) [7]. 100 g seaweed was chopped down, extracted with 200 mL MeOH and 100 mL $CHCl_3$, then put into the ultrasonic bath for 60 minutes, at 37 kHz. Then, solid-phase was subjected for the second extraction using 100 mL $CHCl_3$ and ultrasonic assisting for 1 hour. The two extractions were combined, then added 80 mL H_2O, and shaken; the lipid organic phase was separated on the funnel, and the total lipid was obtained after evaporation.

Lipid classes analysis

Thin-layer chromatography (TLC) for the main lipid classes was carried out on the 6 cm x 6 cm plates covered with silica gel (Sorbfil, Krasnodar, Russia). The mobile phase was n-hexane/di-ethyl ether/acetic acid 85:25:5 (v/v/v). Each sample was tested in triplicate on TLC. Then the 10% H_2SO_4/MeOH reagent was sprayed to cover plates. Then the layer was heated at 210^{o}C for 20 mins. Lipid classes were identified by comparison with standards. The chromatograms were scanned by Epson Perfection 2400 PHOTO (Nagano, Japan) in a grayscale mode with standard conditions. The content of the lipid class was determined based on the area and color intensity using the image analysis program Sorbfil TLC Videodensitometer, Krasnodar, Russia [8].

Fatty acids analysis

FAMEs were prepared following the ISO/DIS 5509:1998, Germany [9]. The lipid was treated with the mixture of 2% H_2SO_4 in CH_3OH and incubated at 80^{o}C for 2 hours. Then the mixture of 2 mL n-hexane and 1 mL H_2O was added and vortexed for 3 minutes at room temperature. After that, the hexane phase containing FAMEs was recovered, and the crude FAMEs was purified by TLC using hexane/diethyl ether 95:5 (v/v) as mobile phase and analyzed by gas chromatography Shimadzu GC-2010 (Kyoto, Japan). This instrument was equipped with a flame ionization detector (FID) on an Equity 5 capillary GC column (Merck, length × internal diameter = 30 m × 0.25 mm, film thickness = 0.25 µm).

RESULTS

Total lipid

The total lipid contents of 13 seaweed samples are ranged from 0.10–1.70% of the fresh weight (table 1). In detail, only 29KT sample has a total lipid content of more than 1%, while others have those less than 1%. This result is similar with previous reports such as from 0.11–0.99% in Vietnamese brown seaweeds [10], from 0.1–0.6% in Vietnamese red seaweeds [11, 12], from 0.6–1.0% in Indian seaweeds [13].

Table 1. Total lipid content (% of fresh weight)

Name of species	Lable	Place	Time	Total lipid (% FW)
Sargassum binderi Sond	6KT	Con Dao	07/2017	0.90
Sargassum echinocarpum J. Ag.	9KT	Con Dao	07/2017	0.70
Sargassum kuetzing Setchell	10KT	Con Dao	07/2017	0.32
Sargassum polycystum C. A	20KT	Con Dao	07/2017	0.43
Sargassum duplicatum J.Ag.	25KT	Con Dao	07/2017	0.31
Sargassum heterocystum Mont	26KT	Con Dao	07/2017	0.41
Sargassum mcclurei Seychell	28KT	Con Dao	07/2017	0.48
Sargassum paniculatum J. Ag.	29KT	Con Dao	07/2017	1.70
Sargassum feldmanii Pham-Hoangho	30KT	Con Dao	07/2017	0.10
Sargassum angustyfolium Grunow	6B	Van Phong	08/2017	0.62
Sargassum incassum Grunow	7B	Van Phong	08/2017	0.24
Sargassum longifructum Tseng et Lu	8B	Van Phong	08/2017	0.27
Sargassum crassifolium J. Ag.	11B	Van Phong	08/2017	0.40

Lipid class content and composition

Seven lipid classes have been determined in 13 species belonging to the *Sargassum* genus (table 2), in which five classes, including Pol, FFA, ST, TG, HW, are found in all species, while diacylglycerol (DG) and monoalkydiacylglycerol (MADG) have appeared in several species. In particular, the FFA, TG, and Pol classes possess high content with an average of 25.52%, 22.95% and 22.42%, respectively. The HW and ST classes have an average content of 11.14% and 10.27%. Finally, the DG and MADG classes showed in 4 or 5 samples with a low content of 0.97% and 3.69%, respectively. The content of the unknown class was 2.79% and appeared in 4 samples. This result has demonstrated that the number of lipid classes from the *Sargassum* species in this research is higher than those in other *Sargassum* species reported by Pham et al., [10].

Table 2. The content and composition of lipid classes

Samples	Content and composition (% total lipid)							
	Pol	ST	DG	FFA	TG	MADG	Unknow	HW
6KT	22.8	11.6	nd	16.0	31.6	11.5	nd	6.5
9KT	16.2	9.9	nd	11.0	41.0	12.7	nd	9.4
10KT	33.3	14.5	nd	7.2	31.2	9.1	nd	4.7
20KT	29.2	9.0	nd	38.1	14.1	nd	9.2	0.5
25KT	17.6	11.2	2.2	37.5	16.0	nd	14.4	1.1
26KT	23.6	10.9	2.0	27.0	18.9	nd	nd	17.6
28KT	15.0	9.0	nd	34.2	19.8	nd	6.9	15.1
29KT	16.4	7.5	nd	40.8	15.5	nd	5.8	14.0
30KT	19.6	10.2	2.2	27.4	20.4	nd	nd	20.2
6B	22.9	10.8	1.9	27.7	16.7	nd	nd	20.1
7B	22.0	9.0	nd	28.6	21.8	nd	nd	18.6
8B	33.5	15.6	nd	36.3	7.5	nd	nd	7.1
11B	19.4	4.3	4.3	-	43.8	14.7	nd	9.9
Average	22.42	10.27	0.97	25.52	22.95	3.69	2.79	11.14

Notes: nd: Not detected, Pol: Polar lipids, ST: Sterol, DG: Diacyglycerol, FFA: Free fatty acids, TG: Triacyglycerol, MADG: Monoalkydiacylglycerol, HW: Hydrocarbons and wax.

The fatty acid content and composition

There are 29 fatty acids identified from 14 to 22 carbon atoms (C14 to C22), in which six popular FAs in seaweeds are C14:0, C16:0, C18:0, C18:1n-7, C18:1n-9 and C18:3n-3 appeared in 13 samples. The fatty acids composition of 13 *Sargassum* species is more diverse than that of *Sargassum* species collected from Northern Vietnam with 17 fatty acids [10] but less than that of the *Hypnea* and *Gracilaria* genus in Vietnam with 53 fatty acids found in seven *Hypnea* samples and 56 fatty acids in 48 *Gracilaria* samples [11].

Saturated fatty acids (SFA)

In the *Sargassum* genus, the saturated fatty acid (SFAs) content is averaged at 44.93% (%TL), reaches the highest point at 73.63% (9KT), and the lowest point at 19.62% (20KT). C16:0 is the most popular saturated fatty acid in the seaweed with the high content reached the highest point at 62.12% in the 9KT sample and the lowest at 14.96% in the 20KT. Although the C19:0 fatty acid is rare to be identified in seaweeds, it is found in the 6B sample with a content of 0.98%. The fatty acid contents of the *Sargassum* seaweeds in this study are lower than those of the *Sargasssum* species collected from Thua Thien Hue and Quang Ninh coast reported by Pham et al., [10].

Monounsaturated fatty acids (MUFA)

The MUFA content is from 15.50–28.24%. C18:1n-9 and C18:1n-7 have been identified in all species with the average content of 15.67% and 1.67%, respectively, which are important fatty acids belonging to the MUFA group relating to the phytochemicals in plants [11]. Interestingly, a very long-chain fatty acid has been found with the formula of C22:1n-9, which is rare to appear in seaweeds [10, 14].

Polyunsaturated fatty acids (PUFAs)

The PUFA content is very different among species, ranging from 3.4–53.18% with an average of 27.44%. The 20KT and 26KT samples, having PUFAs content at 53.18% and 40.26%, respectively, are promised for applying in the food and the medicine. Ten samples have PUFAs xcontents varying from 10.00% (9KT) to 35.92% (25KT), with the 6B sample having the lowest content of 3.4%. The studied samples have remarkedly high PUFAs content compared to the Northern *Sargassum* species from 1.28–23.97% [10], the green seaweeds from 14.8–29.2% [15] and from 12.2–39.0% [16]. The benefits of PUFA are well documented for preventing of several types of cancer and various biological activities [17]. It has been confirmed that seaweeds contain higher levels of PUFAs than vegetables [18].

Among PUFAs, the C20 long-chain fatty acids, including C20:3n-3, C20:4n-6 (Arachidonic acid - AA), and C20:5n-3 (Eicosapentaenoic acid - EPA) are the most considered because those fatty acids play critical roles in the immune and inflammatory responses [19]. Numerous bioactivate lipid mediators have been derived from 20-carbon that produced prostaglandins in the inflammation [17, 20]. Notably, AA has a high content in 10/13 samples (> 10%) and reaches 27.12% in the 20KT sample. Additionally, the super long carbon-chain C22:6n-3 (DHA) fatty acid is found in 7 samples with the content ranged from 0.15–1.05%.

Fatty acids of the omega-3 group

All samples contain omega-3 fatty acids with an average content of 9.28% ranging from 2.9–19.39%. Table 2 showed the omega-3 highest content at 19.39% (20KT) and the lowest content of 2.9% in 9KT. There are several important fatty acids including 18:3n-3, 18:4n-3, C20:3n-3, C20:4n-3, C20:5n-3 (EPA), and C22:6n-3 (DHA).

Fatty acids of the omega-6 group

The omega-6 fatty acids have been observed in all samples with an average content of 16.28% varying from 0.43–28.67%. The highest content has shown in 29KT (28.79%), followed by 20KT (28.67%), rich in AA C20:4n-6 with 24.70% and 27.12%, respectively. Arcording to a previous study, Pham et al., (2017) has reported that both AA are very poor in *Sargassum* species harvested from Co To (Quang Ninh, Vietnam) and Lang Co (Thua Thien Hue, Vietnam), with the content ranging from 1.56% to 13.26% [10].

Table 3. Fatty acid content and composition of samples (% of total fatty acid)

Samples	6KT	9KT	10KT	20KT	25KT	26KT	28KT	29KT	30KT	6B	7B	8B	11B
12:0	nd	nd	0.30	nd	nd	nd	nd	nd	nd	1.36	nd	nd	nd
14:0	10.10	3.88	5.63	3.37	5.34	4.21	3.72	2.86	4.84	7.53	6.87	6.67	3.81
15:0	nd	1.03	0.48	0.22	0.40	0.39	0.62	0.43	0.52	2.47	0.49	0.51	0.45
16:0	40.98	67.12	30.32	14.96	30.33	24.61	30.27	35.62	37.48	52.63	34.64	36.81	42.63
17:0	0.18	0.18	0.35	0.24	0.24	0.24	0.24	nd	nd	0.49	0.32	0.29	nd
18:0	1.02	1.29	0.66	0.17	0.52	0.64	2.18	1.07	1.37	1.76	0.81	0.99	1.14
19:0	nd	nd	nd	nd	nd	nd	nd	nd	nd	0.98	nd	nd	nd
20:0	2.16	nd	0.21	0.52	0.74	0.55	0.94	0.17	1.14	nd	0.10	0.78	0.60
22:0	0.32	0.15	0.13	0.14	0.12	nd	0.34	0.27	ND	0.51	nd	nd	nd
Σ*SFA*	54.75	73.63	38.09	19.62	37.67	30.64	38.31	40.41	45.35	67.71	43.23	46.05	48.64
16:1n-7	4.96	2.82	10.26	6.88	7.18	7.55	6.68	6.47	5.40	nd	7.99	6.46	5.15
16:1n-5	1.86	nd	0.23	0.64	0.37	0.47	0.44	0.46	0.98	4.78	0.58	0.19	0.46
18:1n-11	nd	nd	nd	nd	nd	nd	nd	5.29	nd	nd	nd	nd	nd
18:1n-9	6.46	10.58	18.16	16.79	16.56	17.80	19.67	11.42	16.88	16.36	18.99	16.72	17.37
18:1n-7	6.21	2.09	1.30	0.31	0.65	1.19	1.23	1.25	0.71	4.40	0.66	1.27	0.48
22:1n-9	nd	nd	nd	nd	nd	nd	0.22	nd	nd	nd	nd	nd	nd
Σ*MUFA*	19.04	15.05	29.96	24.62	24.76	27.01	28.24	24.88	23.97	25.55	28.22	24.64	23.46
18:2n-6	0.31	0.10	1.65	0.21	0.12	3.01	nd	1.69	2.28	nd	1.27	1.61	2.68
20:2n-6	0.36	0.11	0.81	0.19	0.17	0.21	0.68	0.48	0.68	nd	0.11	0.12	nd

22:2n-6	nd	4.07	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
18:3n-6	0.41	0.11	0.55	0.95	0.70	0.42	0.53	0.65	nd	nd	0.47	0.49	0.62
18:3n-3	4.28	1.44	7.77	5.34	5.57	6.09	6.06	0.16	3.80	1.13	6.35	6.58	5.14
20:3n-9	0.54	0.24	1.07	2.19	1.47	1.54	0.79	1.27	0.84	nd	0.64	0.64	0.97
20:3n-6	0.25	nd	0.75	0.21	0.92	1.20	0.99	1.27	0.72	0.43	0.49	0.50	0.97
20:3n-3	0.45	nd	nd	0.25	nd	nd	0.52	0.53	nd	1.83	nd	nd	nd
18:4n-3	nd	nd	0.26	7.71	4.78	0.25	2.46	0.77	0.33	nd	0.60	0.36	0.59
20:4n-3	0.48	0.21	0.29	2.93	1.52	1.85	0.25	nd	1.11	nd	1.05	1.41	1.12
20:4n-6	3.64	2.26	15.86	27.12	15.34	19.96	12.77	24.70	12.83	nd	12.91	13.95	12.83
20:5n-3	2.46	1.31	1.92	6.09	5.17	4.69	2.65	3.19	2.10	nd	1.40	1.80	2.56
22:5n-3	0.53	nd	nd	nd	nd	nd	0.54	nd	0.31	nd	Nd	nd	nd
22:6n-3	nd	0.15	0.11	nd	0.16	1.05	0.61	nd	nd	nd	0.14	0.30	nd
Other	12.03	0.86	0.91	2.58	1.64	2.10	4.58	0.00	5.68	3.34	3.12	1.55	0.41
Σ*PUFA*	13.72	10.00	31.05	53.18	35.92	40.26	28.86	34.71	24.99	3.40	25.42	27.76	27.49
Omega-3	7.73	2.90	10.07	19.39	15.69	12.07	12.85	4.65	6.54	2.96	8.49	9.04	8.30
Omega-6	4.97	6.66	19.62	28.67	17.25	24.80	14.97	28.79	16.50	0.43	15.24	16.67	17.10
Omega-9	7.00	10.82	19.23	18.98	18.04	19.34	20.68	12.69	17.73	16.36	19.63	17.36	18.33
PUFA/SFA	0.25	0.14	0.82	2.71	0.95	1.31	0.75	0.86	0.55	0.05	0.59	0.60	0.57
n-3/n-6	1.56	0.44	0.51	0.68	0.91	0.49	0.86	0.16	0.40	6.84	0.56	0.54	0.49

Notes: nd: not detected, SFA: Saturated Fatty Acids, MUFA: Monounsaturated Fatty Acids, PUFA: Polyunsaturated Fatty Acids.

Fatty acids of the omega-9 group

The omega-9 fatty acids have been found in all samples with an average value of 16.63%. The 28KT sample has the highest content of 20.68%, while the 6KT has 7.00%. These results are similar to a previous publication [10, 11]. The main fatty acid is 18:1n-9, with the content ranged from 6.46% to 19.67%.

PUFA/SFA index

In table 2, 10/13 samples (excepted 6KT, 9KT and 6B) have the PUFA/SFA index being higher than 0.4, which is recommended good for health by The World Health Organization (WHO). In particular, two samples including 20KT and 26KT have the high index of 2.71 and 1.31, while others are between 0.55 (30KT) and 0.95 (25KT).

n3/n6 index

All species have the n-3/n-6 index ranging from 0.16 (29KT) to 6.88 (6B). The WHO currently recommended the n-3/n-6 ratio to be higher than 0.1 in diet to prevent inflammation, cardiovascular and nervous system disorders [21].

From the results of the total lipid and fatty acids of 13 brown seaweed Sargassum species, we have reconigzed that three species, including S. polycystim (20KT), S. heterocyst (26KT), and S. paniculatum (29KT), have a high value on the lipid composition. In particular, both of them belong to the 3rd top of the PUFA content, omega-6 content, and PUFA/SFA index. The PUFA content of the three samples is 4.6 to 7.1 times higher than those of the species collected from Quang Ninh to Thua Thien Hue, Vietname [10]. Also, three samples contain AA and EPA with high content ranging from 19.96–27.12% and from 3.19–6.09%, respectively. Thus, they could be merit to further study on the chemicals and bioactivities for the application on the food and the medicine.

CONCLUSIONS

In summary, we have determined the total lipid and content and composition of the lipid class of 13 species collected from Con Dao and Van Phong in Vietnam. The total lipid content of *Sargassum* seaweeds is ranged from 0.1–1.70% of the fresh weight. The PUFA/SFA and n-3/n-6 index are suitable with the WHO recommendation. Thus lipids of these species belonging to the *Sargassum* genus are good for health. Five lipid classes, including Pol, ST, FFA, TG, HW, have been identified in almost all species, while two lipid classes, including DG and MADG, are present in 4 to 5 samples. Twenty-nine fatty acids have been detected, in which SFA has an average content of 44.93%; the MUFA has an average content of 24.57%; PUFA has a moderate content of 27.44%. Among unsaturated fatty acids, various valuable fatty acids belong to omega-3,6,9, such as C18:3n-3, C20:4n-6, 20:5n-3, and 22:6n-3.

REFERENCES

[1] De Wreede, R. E., 1976. The phenology of three species of *Sargassum* (Sargassaceae, Phaeophyta) in Hawaii. *Phycologia*, *15*(2), 175–183. doi: 10.2216/i0031-8884-15-2-175.1

[2] Gupta, S., and Abu-Ghannam, N., 2011. Bioactive potential and possible health effects of edible brown seaweeds. *Trends in Food Science & Technology, 22*(6), 315–326. https://doi.org/10.1016/j.tifs.2011.03.011

[3] https://www.algaebase.org/browse/taxonomy/?tc=accept&id=4360; accessed July 30, 2020.

[4] Chen, Z., Xu, Y., Liu, T., Zhang, L., Liu, H., and Guan, H., 2016. Comparative studies on the characteristic fatty acid profiles of four different Chinese medicinal *Sargassum* seaweeds by GC-MS and chemometrics. *Marine drugs*, *14*(4), 68. https://doi.org/10.3390/md14040068

[5] Gerasimenko, N., and Logvinov, S., 2016. Seasonal composition of lipids, fatty acids pigments in the brown alga *Sargassum pallidum*: The potential for health. *Open Journal of Marine Science*, *6*(04), 498–523. doi: 10.4236/ojms.2016.64041

[6] Cohen, Z., Vonshak, A., and Richmond, A., 1988. Effect of environmental conditions on fatty acid composition of the red alga Porphyridium cruentum:

Correlation to growth rate 1. *Journal of phycology*, *24*(3), 328–332. doi: 10.1111/j.1529-8817.1988.tb04474.x

[7] Bligh, E. G., and Dyer, W. J., 1959. A rapid method of total lipid extraction and purification. *Canadian journal of biochemistry and physiology*, *37*(8), 911–917. https://doi.org/10.1139/o59-099

[8] Phattanawasin, P., Sotanaphun, U., Sriphong, L., Kanchanaphibool, I., and Piyapolrungroj, N., 2011. A comparison of image analysis software for quantitative TLC of ceftriaxone sodium. *Science, Engineering and Health Studies*, *5*(1), 7–13. https://doi.org/10.14456/sustj.2011.1

[9] ISO/DIS 5509:2000, Animal and vegetable fats and oils - Preparation of methyl esters of fatty acids.

[10] Pham, T. H., Nguyen, V. T. A., Nguyen, T. C. B., Dam, D. T., Le, T. T., and Pham, Q. L., 2017. Research on the Content of Lipid Classes and Fatty Acids from Sargassum Seaweed. In *Proceedings of ASAM conferences in Vietnam*. pp. 525–531.

[11] Le Tat Thanh, 2015. Screening, isolation and identification of fatty acids constituents, arachidonic acid and prostaglandin from red Seaweeds. *PhD thesis in Chemistry, Graduate University of Science and Technology, VAST*, 143 p.

[12] Le, T. T., Nguyen, V. T. A., and Nguyen, T. H., 2013. Survey lipid content and composition of fatty acids from Hypnea seaweed. *Vietnam Journal of Chemistry*, *51*, 49–53.

[13] Saini, R. K., and Keum, Y. S., 2018. Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance—A review. *Life sciences*, *203*, 255–267. https://doi.org/10.1016/j.lfs.2018.04.049

[14] Khotimchenko, S. V., Vaskovsky, V. E., and Titlyanova, T. V., 2002. Fatty acids of marine algae from the pacific coast of North California. *Botanica Marina*, *45*(1), 17–22. doi: 10.1515/BOT.2002.003

[15] Bhaskar, N., Kinami, T., Miyashita, K., Park, S. B., Endo, Y., and Fujimoto, K., 2004. Occurrence of conjugated polyenoic fatty acids in seaweeds from the Indian Ocean. *Zeitschrift für Naturforschung C*, *59*(5-6), 310–314. https://doi.org/10.1515/znc-2004-5-602

[16] Darcy-Vrillon, B., 1993. Nutritional aspects of the developing use of marine macroalgae for the human food industry. *International Journal of Food Sciences and Nutrition*, *44*, S23–S35.

[17] Zárate, R., el Jaber-Vazdekis, N., Tejera, N., Pérez, J. A., and Rodríguez, C., 2017. Significance of long chain polyunsaturated fatty acids in human health. *Clinical and translational medicine*, *6*(1), 1–19. https://doi.org/10.1186/s40169-017-0153-6

[18] Kumari, P., Reddy, C. R. K., and Jha, B., 2011. Comparative evaluation and selection of a method for lipid and fatty acid extraction from macroalgae. *Analytical biochemistry*, *415*(2), 134–144. https://doi.org/10.1016/j.ab.2011.04.010

[19] Radzikowska, U., Rinaldi, A. O., Çelebi Sözener, Z., Karaguzel, D., Wojcik, M., Cypryk, K., Akdis, M., Akdis, C. A., and Sokolowska, M., 2019. The influence of dietary fatty acids on immune responses. *Nutrients*, *11*(12), 2990.

[20] Cardoso, C., Ripol, A., Afonso, C., Freire, M., Varela, J., Quental-Ferreira, H., Pousão-Ferreira, P., and Bandarra, N., 2017. Fatty acid profiles of the main lipid classes of green seaweeds from fish pond aquaculture. *Food science & nutrition*, *5*(6), 1186–1194. doi: 10.1002/fsn3.511

[21] Simopoulos, A. P., 2002. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomedicine & Pharmacotherapy*, *56*(8), 365–379. doi: 10.1016/S0753-3322(02)00253-6