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STUDY ON USING FATTY ACID DATA IN THE BOTANICAL CHEMOTAXONOMY FOR VIETNAMESE SEAWEED SPECIES

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SUMMARY

Vietnam's sea is assessed to be very diverse and rich in seaweed species. It is about 1000 different species of seaweed, of which more than 800 species have been identified, classify into genus, families, classes, phylums, and continuously updated from the 1950s to the present. Previous studies by Vietnamese and international scientists have shown that lipids from seaweed contain many valuable active ingredients such as acids C20: 4n-6 (AA), C20:5n-3 (EPA), C22:6n-3 (DHA), prostaglandin E2… In this study, fatty acids were converted to methyl esters and identified by gas chromatography using flame ionization detector (GC-FID) with column Cap Mao Equity 5 (Merck, L×ID 30m×0.25 mm, df 0.25 µm). From the total lipid of 50 of Vietnamese seaweed, we have identified 30 fatty acids, in which, C16:0, C18:1n-9, C20:4n-6 (AA) fatty acids have the high content, and C20:5n-3 (EPA), C22:6n-3 (DHA), C22:5n-3 (DPA) fatty acids have the high bioactivities. By the method of PCA main component analysis, from the dataset of fatty acids, we have identified 8 main fatty acids with high correlation and used to represent the distribution of seaweed species on the two-way plane. Three phylums were classified by different fatty acid groups with the high reliability. In the detail, the distribution of the phylum Phaeophyta depends on the content of 3 fatty acids including C16:1n-7, C18:1n-9 và C20:4n-6, the phylum Rhodophyta depends on C15:0, C16:0, C18:0 fatty acids, and the phylum Chlorophyta depends on C18:1n-7, C18:3n-6 fatty acids. This method can may help provides more chemical data in the taxonomy of Vietnamese seaweed species.

Keywords: Chemotaxonomy, Chlorophyta, fatty acids, Phaeophyta, Principal Component Analysis, Rhodophyta, seaweed

INTRODUCTION

The biosynthesis of fatty acids is characteristic for each species and is predetermined in the organism's genome. In addition, in the seaweed species, the biosynthesis of fatty acids depends on the temperature, geographical conditions, habitat... (Berge, Barnathan, 2005). Some studies have suggested that the composition and content of fatty acids are used as fingerprints or biomarkers for chemical classification (chemotaxonomy) for complex groups of organisms such as bacteria, fungi, microalgae and macroalgae (Berge, Barnathan, 2005; Dunstan *et al.*, 2005; Volkman *et al.*, 1998).

Up to now, the research data on the fatty acid composition of seaweed obtained is either scattered in many different works or performed by many different analytical methods, that makes it impossible to compare the information to classify of seaweed species. In recent studies, by

modern analytical techniques such as GC-FID, we have carried out researches on the composition and content of fatty acids from seaweeds in the same regimen for research samples. Hence, we have accumulated a reliable data on the composition and content of fatty acids to use for the botanical chemotaxonomy with Vietnamese seaweed species belonging to the phylum of Rhodophyta, Ochrophyta and Chlorophyta. The results are processed through multi-factor statistical analysis by the principal component analysis (PCA) method on JMP software. This method provides more information to classify seaweed species which have many difficulties in classifying due to the similarity in the morphology.

MATERIALS AND METHODS

Research obbjects

Study subjects were 50 seaweed samples collected in June and July in 2017, of which: 28 samples collected in the coastal waters of Khanh Hoa province (Van Phong Bay, Nha Phu Lagoon, Hon Tre Island, Grand Bank, Binh Ba Island), in which red seaweed - Rhodophyta had 12 samples, brown seaweed - Phaeophyta 11 samples and green seaweed - Chlorophyta 5 samples; 22 oceanic samples collected from Big Truong Sa Island, Thuyen Chai Island - Truong Sa Archipelago in Khanh Hoa province and Con Dao in Ba Ria - Vung Tau province, of which Rhodophyta had 6 samples, Phaeophyta 12 samples and Chlorophyta 4 samples.

Samples were classified and identified scientific name by Associate Professor, Dr. Dam Duc Tien, and kept the specimen at the Institute of Natural Products Chemistry (Vietnam Academy of Science and Technology).

Sampling and determining scientific names

Seaweed sampling was based on protocol described in the publication of "Investigation of Marine Environment and Resources - Section Biology and Environmental Chemistry" (Do Cong Thung, 2014) and the document of English and his colleagues (English *et al*, 1997) using SCUBA diving equipment and OLYMPUS digital underwater camera.

The analysis was performed in the laboratory of the Department of Marine Ecology and Plant Resources (Institute of Marine Resources and Environment). The classification was mainly based on the criteria of the morphology and the structure (by sliced specimens under Leica microscope with a magnification of 1350 times). The classification of seaweed was followed the general principle of botanical classification based on the books of Pham Hoang Ho (1969), Nguyen Huu Dinh (1993) (Pham Hoang Ho, 1969; Nguyen Huu Dinh *et al.*, 1993).

Extraction of total lipids and determination of the content of fatty acids

Total lipids were extracted according to the method of Bligh and Dyer, 1959. The composition and content of fatty acids in seaweed samples were analyzed on gas chromatography GC-FID on Capillary Equity 5 (Merck, L × ID 30m × 0.25 mm, df 0.25 µm). Helium was carrier gas with the speed of 20 mL/min. Temperature of the oven operates was at 160°C, raised 2°C/min to 240°C then kept for 20 minutes. The identification of fatty acids was based on the equivalent retention time value Equivalent Chain Length with the standard system of fatty acids C16:0 and C18:0 according to ISO/FDIS 5509:1988, Germany.

Principal Component Analysis

Eight fatty acids with significant differences between three phylums of Phaeophyta, Rhodophyta, Chlorophyta were analysed using the Principle component Analysis (PCA) method (Mattheaus, Otgonbayar, 2016). The data was evaluated using the JMP software (JMP, SAS Institute Inc.).

RESULTS AND DISCUSSION

Selecting the main fatty acids

From the results of the composition and fatty acid content of 50 seaweed samples, 30 fatty

acids were identified, in which C16:0 fatty acid had the highest content of 14.96 - 67.12% (the average of 37.02%), following by C18:1n-9 with the content of 6.50 - 29.19% (the average of 15.72%), others with the average content lower than 7%. Some samples contained long chain and super long chain polyunsaturated fatty acids with valuable bioactivities including C20:5n-3 (eicosapentaenoic acid - EPA), C22:6n-3 (docosahexaenoic acid - DHA), C22:5n-3 (docasapentaenoic acid - DPA) (Berge, Barnathan, 2005).

Preliminary selection of main fatty acids from 30 fatty acids were performed by removing fatty acids with the lower content (<1% of total fatty acid) and the frequency of appearance below 50% of all samples. Sixteen fatty acids were removed including C12:0, C17:0, C18:4n-3, C18:1n-11, C19:0, C20:4n-3, C20:3n-9, C20:3n-6, C20:2n-6, C20:3n-3, C22:6n-3, C22:4n-6, C22:5n-3, C22:2n-6, C22:1n-9, C22:0. The 14 remaining fatty acids with the total content from 71.4 – 95.93% of all fatty acids were determined their correlation by the PCA method and selected the main fatty acids (Table 1).

Table 1. Correlation matrix of 14 fatty acids.

	15:0	**16:1n-7**	**16:1 n-5**	**16:0**	**18:3 n-6**	**18:2 n-6**	**18:3 n-3**	**18:1 n-9**	**18:1 n-7**	**18:0**	**20:4 n-6**	**20:5 n-3**	**20:0**
14:0	0.26	0.12	0.10	-0.12	-0.14	-0.15	0.11	0.24	-0.05	0.07	-0.04	-0.28	-0.14
15:0	1.00	-0.13	0.00	**0.55**	**-0.51**	-0.45	-0.43	-0.25	0.07	**0.64**	-0.35	-0.24	-0.07
16:1n-7		1.00	-0.18	-0.24	-0.33	-0.08	-0.22	0.25	-0.40	-0.07	**0.52**	0.01	0.24
16:1n-5			1.00	-0.05	-0.06	0.02	-0.11	0.29	-0.04	-0.13	-0.31	0.33	-0.05
16:0				1.00	-0.27	-0.34	-0.46	-0.46	0.32	0.47	-0.47	-0.37	-0.02
18:3n-6					1.00	0.25	0.43	-0.17	0.41	-0.11	0.04	0.12	0.02
18:2n-6						1.00	0.26	0.25	-0.36	-0.28	0.06	0.28	-0.06
18:3n-3							1.00	0.00	0.18	-0.34	0.09	-0.19	-0.02
18:1n-9								1.00	**-0.65**	-0.06	0.12	0.04	-0.15
18:1n-7									1.00	0.13	-0.37	-0.23	0.11
18:0										1.00	-0.38	-0.43	0.01
20:4n-6											1.00	0.10	-0.16
20:5n-3												1.00	-0.02
20:0													1.00

The results in Table 1 showed the negative correlation between C18:1n-9 and C18:1n-7 fatty acids (r =-0.65). The C15:0 fatty acid had a positive correlation with C18:0 (r =0.64) and C16:0 (r =0.55) and a negative correlation with C18:3n-6 (r=-0.51). In addition, there was a positive correlation between C16:1n-7 and C20:4n-6 (r=0.52). Other correlations were weak (absolute value of r <0.5). Six fatty acids C22:0, C14:0, C16:1n-5, C20:5n-3, C18:2n-6 and C18:3n-3 were weak correlated with all remaining fatty acids (with highest absolute values r of 0.24, 0.28, 0.33, 0.43, 0.45 and 0.46, respectively). Thus these fatty acids were removed in the PCA analysis.

Finally, 8 main fatty acids were selected including C15:0, C16:1n-7, C16:0, C18:3n-6, C18:1n-9, C18:1n-7, C18:0, C20:4n-6 (Table 2). The similar method had been also used by Le Tat Thanh *el al.*, 2015, in which using 12 fatty acids in the PCA analysis had provided more chemical signal to classify 69 red seaweed samples into families (Le Tat Thanh *el al.*, 2015).

Distribution of seaweed species on the 2-dimensional plane

By the PCA combined with JMP software using 8 main fatty acids as above, the diagram of distribution seaweed species in the 2-dimensional plane of the component 1 (PC1) versus component 2 (PC2) was shown in Figure 1.

Analysis results showed that, on the two-dimensional coordinate system of PCA, the PC1 and PC2 were counted for 58.8% of the total variation, in which PC1 was 39.1% and PC2 was 19.7%, respectively. Along with that, 50 seaweed samples were distributed into 3 separate regions corresponding to 3 phylums of red seaweed, brown seaweed, green seaweed on a 2-dimensional plane (Figure 1).

Table 2. Content of 8 main fatty acids *(% of total fatty acids).*

N0	Fatty acids / Species	Coastal/Oceanic	15:0	16:1 n-7	16:0	18:3 n-6	18:1 n-9	18:1 n-7	18:0	20:4 n-6
Red seaweed phylum- Rhodophyta										
Hypneaceae family										
1	*Hypnea panosa* J. Ag.	Coastal	2.55	4.73	45.82		14.26	2.44	3.93	1.97
2	*Hypnea panosa* J. Ag.	Coastal	1.49	7.18	43.92	0.22	12.50	2.50	1.32	5.05
3	*Hypnea valentiae* (Tunr.)	Coastal	0.43	3.85	29.20	1.68	24.81	0.57	3.34	7.30
4	*Hypnea panosa* J. Ag.	Coastal	2.51	4.65	45.03		14.02	2.40	3.86	1.93
5	*Hypnea nidulans* Setchell	Oceanic	0.62	6.64	30.11	0.53	19.57	1.23	2.17	12.71
6	*Hypnea fragelliformis* Grev.	Oceanic	2.21	2.86	42.57		11.90	3.61	2.35	0.83
Gracilariaceae family										
7	*Gracilaria salicornia (C. Ag.)*	Coastal	2.34	4.14	49.92		12.32	4.18	1.67	
8	*Hydropuntia edulis* (S.G.Gmelin.)	Oceanic	0.73	8.00	31.53	0.10	20.57		2.58	2.07
9	*Gracilaria tenuistipitata* Chang & Xia.	Oceanic	0.75	5.05	32.36	0.27	29.19	1.66	1.47	1.42
10	*Hydropuntia ramulosa* (C.F. Chang & B.M. Xia)	Oceanic	0.42	4.59	39.37	0.60	18.75	0.62	0.91	2.70
11	*Gracilaria arcuata* Zan.	Coastal		6.19	28.99	0.74	27.04	1.23	1.04	11.36
Phyllophoraceae family										
12	*Ahnfeltiopsis divaricata* (Holmes) Masuda	Oceanic	1.16	3.62	55.40	0.44	13.41	5.36	2.83	0.77
Galaxauraceae family										

13	*Galaxaura fastigiata* Dcne	Coastal		3.70	37.53	0.55	11.38	0.51	0.22	14.64
14	*Galaxaura* sp1.	Coastal		6.52	33.91	0.96	11.95	0.90	0.39	3.55
15	*Galaxaura* sp2.	Coastal	0.61	4.60	56.50	0.26	9.54	9.89	2.51	1.21
				Rhodomelaceae family						
16	*Acanthophora spicifera*	Coastal	2.59	4.79	46.46		10.75	2.47	3.98	5.70
17	*Laurencia obtusa* (Hudson) Lamouroux	Coastal	2.53	4.69	45.40		14.13	2.42	3.89	2.30
				Schizymeniaceae family						
18	*Titanophora pulchra* Dawson	Coastal	0.63	4.72	55.71	0.07	10.22	2.61		1.27
				Brown seaweed phylum - Phaeophyta						
				Dictyotaceae family						
19	*Stypopodium flabelliforme*	Coastal	0.65	3.55	26.29	0.52	18.43	0.64	0.72	1.14
20	*Spatoglossum vietnamense* Pham-Hoang Ho 1969	Coastal	0.46	7.79	27.63	0.15	17.94	0.35	0.51	11.59
21	*Dictyopteris jamaicensis* W.R. Taylor	Coastal	0.41	2.68	28.09	0.05	24.30	0.81	0.97	4.57
22	*Spatoglossum pacificum* Yendo	Oceanic	0.41	6.20	29.32	0.63	27.12	1.20	1.02	10.77
23	*Dictyota indica* Sonder	Oceanic	0.74	5.01	32.14	0.27	29.00	1.65	1.46	1.41
24	*Padina* sp.	Oceanic	0.20	3.46	30.49	2.06	19.90	3.80	0.62	1.84
25	*Padina australis* Hauck	Coastal	0.66	3.60	33.10	0.53	18.66	0.64	0.73	1.15
				Sargassaceae family						
26	*Sargassum binderi* Sonder	Oceanic		4.96	40.98	0.41	6.46	6.21	1.02	3.64
27	*Sargassum echinocarpum* J. Ag.	Oceanic	1.03	2.82	67.12	0.11	10.58	2.09	1.29	2.26
28	*Sargassum ilicifolium* (Turner) C. Ag.	Oceanic	0.48	10.26	30.32	0.55	18.16	1.30	0.66	15.86
29	*Turbinaria ornata (Turner) J.Ag.*	Oceanic		5.22	42.77	0.78	6.73	6.04	2.16	2.84
30	*Sargassum ilicifolium* (Turner) C. Agardh	Oceanic	0.22	6.88	14.96	0.95	16.79	0.31	0.17	27.12
31	*Sargassum heterocystum* Mont.	Oceanic	0.39	7.55	24.61	0.42	17.80	1.19	0.64	19.96
32	*Sargassum mcclurei* Setchell	Oceanic	0.62	6.68	30.27	0.53	19.67	1.23	2.18	12.77

33	*Sargassum paniculatum* J. Ag.	Oceanic	0.43	6.47	35.62	0.65	11.42	1.25	1.07	24.70
34	*Sargassum feldmanii* Pham-Hoang Ho 1969	Oceanic	0.52	5.40	37.48		16.88	0.71	1.37	12.83
35	*Sargassum angustifolium* (Tunr.) Grunow	Coastal	2.47		52.63		16.36	4.40	1.76	
36	*Sargassum incassum* Grunow	Coastal	0.49	7.99	34.64	0.47	18.99	0.66	0.81	12.91
37	*Sargassum longifructum* Tseng & Blu	Coastal	0.51	6.46	36.81	0.49	16.72	1.27	0.99	13.95
38	*Sargassum crassifolium* J. Ag.	Coastal	0.45	5.15	42.63	0.62	17.37	0.48	1.14	12.83
39	*Turbinaria ornata* (Turn.) J. Ag.	Coastal	0.75	8.22	32.40		21.13	0.28	2.65	2.13
	Scytosiphonaceae family									
40	*Chnoospora implexa* (Hering) J. Ag.	Coastal	0.88	4.64	39.72	0.95	16.80	2.55	4.11	6.38
41	*Pseudochnoospora implexa* (J. Ag.) Santiañez	Coastal	0.57	6.14	38.73	0.22	16.98	1.13	2.01	11.74
	Green seaweed phylum - Chlorophyta									
Ulvaceae family										
42	*Ulva compressa* Linnaeus	Oceanic	0.27	4.01	41.14	0.14	7.73	8.54	2.05	1.10
43	*Ulva fasciata* Delile	Coastal	0.53	2.63	27.32		12.58	5.13	0.84	
	Caulerpaceae family									
44	*Caulerpa racemosa* (Forsk.) J. Ag.	Coastal	0.27	4.03	41.43	1.30	7.79	8.60	2.06	1.10
45	*Caulerpa sertularioides* (Gmelin) Hown	Coastal	0.11	2.98	24.93	1.60	19.97	1.33	0.48	2.72
	Halimedaceae family									
46	*Halimeda discoidea* Decaisne	Oceanic		5.09	42.22	1.03	6.50	6.34	1.03	3.67
47	*Halimeda incrassata* Lamouroux	Oceanic	0.23	4.96	26.14	2.07	8.93	6.45	0.89	11.68
48	*Halimeda discoidea* Decaisne	Coastal		4.71	33.01	0.49	10.32	3.74	1.15	2.29
	Cladophoraceae family									
49	*Chaetomorpha capillaris* (Kuetz.) Borg.	Oceanic	0.20	2.59	26.99		12.42	5.06	0.82	
	Valoniaceae family									
50	*Valonia fastigiata* Harv.	Coastal		2.64	29.58	5.73	9.09	9.60	0.78	8.15

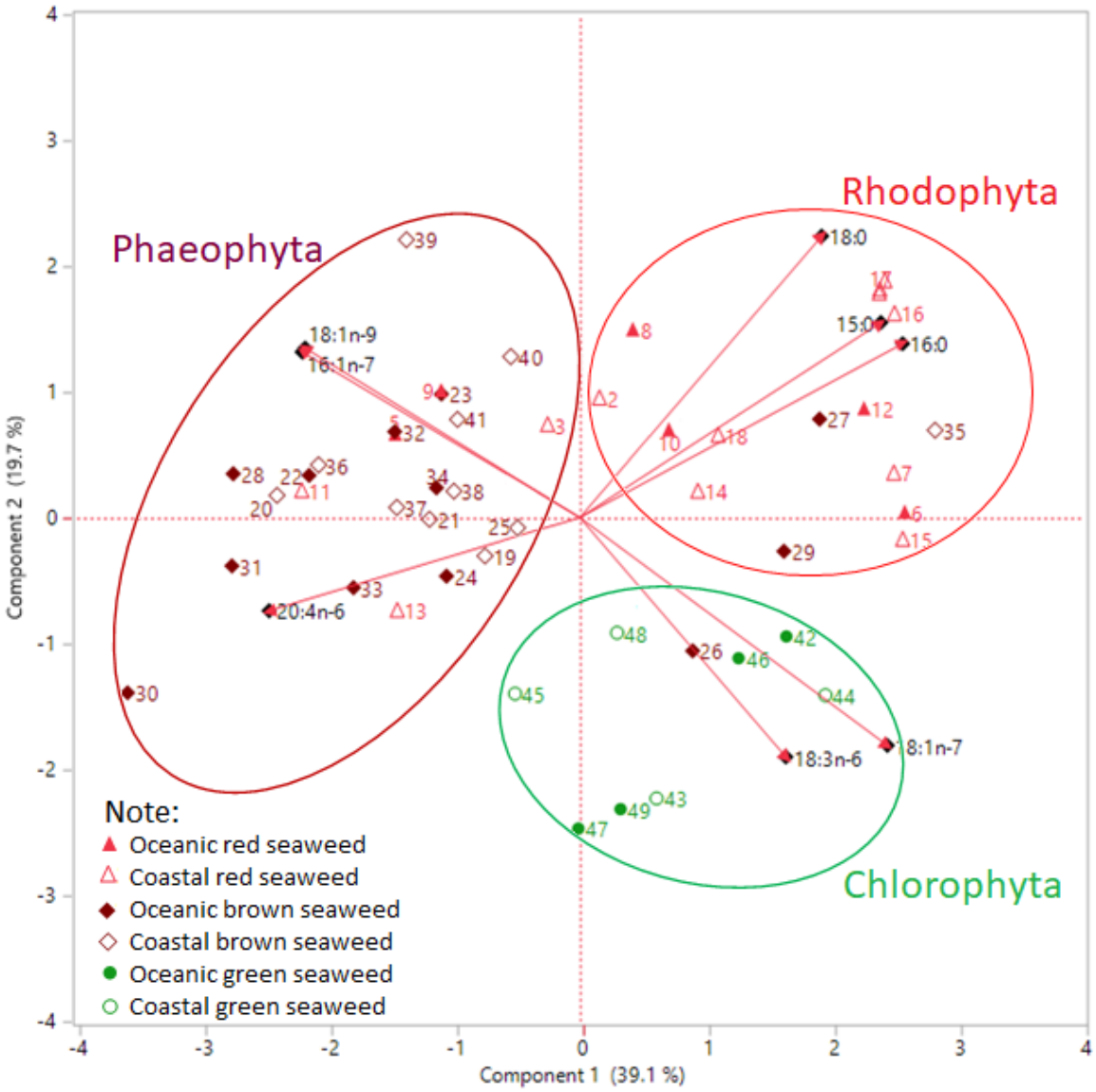

Figure 1. Scores bi-plot of PCA base on components of fatty acids contained in seaweed samples in Vietnam waters.

The first group was the brown seaweed (Phaeophyta), the distribution depended on the content domination of 3 fatty acids C16:1n-7, C18:1n-9, C20:4n-6. The second group was the red seaweed (Rhodophyta), the distribution depended on the dominant content of 3 fatty acids C15:0, C16:0 and C18:0. The third group was the green seaweed (Chlorophyta), the distribution predominantly depended on the content of 2 fatty acids C18:1n-7, and C18:3n-6.

Figure 1 also showed that several samples tended to distribute into other phylums, especially the red and brown seaweed phylums. In the detail, 5/18 red seaweed samples distributed in the distribution area of the brown seaweed phylum, while 3/23 brown seaweed samples displayed mixing in the red seaweed distribution and 1/23 brown seaweed samples distributed into the green seaweed area.

In addition, the oceanic samples of the brown seaweed phylum tended to be more dispersed than the coastal samples, thus many oceanic brown seaweed samples displayed at the edge of the distribution and 4 samples distributed mixing with red seaweed and green seaweed. Meanwhile, red seaweed and green seaweed phylums had no clear difference between the two sample groups collected coastal and oceanic waters.

Some samples of brown seaweed and red seaweed had mixed distribution because they belonged to the thallophyta and the biosynthesis of fatty acids depended on factors such as habitat, temperature, nutritional conditions, growth period and threats of other organisms… This agreed with some previously publications (Berge, Barnathan, 2005; Vaskovsky *et al.*, 1996).

Especially, samples of green seaweed phylum were distributed quite concentratedly into a separate area without any samples mixing with the other two seaweed branches. This may be due to the reason that the samples of green seaweed mainly grow and develop on the surface of hard rock in the tidal flats, the composition and content of fatty acids are not affected by the environmental conditions and nutrients. Following recent studies, this study further indicated that fatty acid signatures could be employed as a valid chemotaxonomic tool to differentiate macroalgae at higher taxonomic levels such as family and orders (Gaubert *et al.,* 2019; Verma *et al.,* 2017; Kumari *et al.,* 2013).

CONCLUSION

In this study, we have determined the composition and content of fatty acids of 50 seaweed samples of 3 phylums of red seaweed, brown seaweed, green seaweed in which 2 fatty acids C16:0, C18:1n-9 had the highest content. Notable long-chain polyunsaturated fatty acids such as AA, EPA DHA, DPA were detected.

The PCA results initially showed that classification of 3 phylums of brown seaweed, red seaweed, green seaweed cound be depended on 8 main fatty acids: C15:0, C16:1n-7, C16:0, C18:3n -6, C18:1n-9, C18:1n-7, C18:0 and C20:4n-6, in which, the brown seaweed was characterized by the 3 predominant fatty acids C16:1n-7, C18:1n-9 and C20:4n-6, the red seaweed by the 3 fatty acids C15:0, C16:0, C18:0, and the green seaweed by the 2 fatty acids C18:1n-7 and C18:3n-6, respectively.

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NGHIÊN CỨU SỬ DỤNG DỮ LIỆU ACID BÉO TRONG PHÂN LOẠI HÓA HỌC THỰC VẬT ĐỐI VỚI CÁC LOÀI RONG BIỂN VIỆT NAM

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TÓM TẮT

Biển Việt Nam được đánh giá rất đa dạng, phong phú các loài rong biển. Hiện nay phát hiện hơn 1000 loài, trong đó hơn 800 loài đã được xác định tên khoa học và liên tục cập nhật từ những năm 1950 tới nay. Các nghiên cứu trước đây ở Việt Nam và quốc tế đã cho thấy, lipid từ rong biển có chứa nhiều hoạt chất quý như acid C20:4n-6 (AA), C20:5n-3 (EPA), C22:6n-3 (DHA), prostaglandin E_2… Trong nghiên cứu này, các acid béo từ các mẫu rong biển được chuyển hóa thành dạng metyl este và nhận dạng bằng kỹ thuật sắc ký khí sử dụng detector ion hóa ngọn lửa (GC-FID) với cột Capillary Equity 5 (Merck, L × ID 30m × 0.25 mm, df 0.25 µm). Từ lipid tổng 50 của mẫu rong biển Việt Nam chúng tôi đã nhận dạng được 30 acid béo. Kết quả phân tích cho thấy các acid béo có hàm lượng cao bao gồm C16:0, C18:1n-9, C20:4n-6 (AA). Bên cạnh đó là sự xuất hiện của các axit béo C20:5n-3 (EPA), C22:6n-3 (DHA), C22:5n-3 (DPA), đây là những acid béo có hoạt tính sinh học cao và có ý nghĩa quan trọng trong các hoạt động chuyển hóa của cơ thể. Bằng phương pháp phân tích cấu tử chính PCA, từ bộ dữ liệu các acid béo, chúng tôi đã xác định được 8 acid béo chính có độ tương quan cao và sử dụng để biểu diễn sự phân bố của các loài rong biển trên mặt phẳng hai chiều. Sự phân bố của các loài rong thuộc ngành rong nâu Phaeophyta phụ thuộc vào hàm lượng của 3 acid béo C16:1n-7, C18:1n-9 và C20:4n-6; các loài thuộc ngành rong đỏ Rhodophyta phụ thuộc vào C15:0, C16:0, C18:0 và các loài thuộc ngành rong lục Chlorophyta phụ thuộc vào C18:1n-7, C18:3n-6. Phương pháp này có thể giúp cung cấp thêm dữ liệu về hóa học trong quá trình phân loại các loài rong biển Việt Nam.

Từ khoá: Phân loại hóa học thực vật, Chlorophyta, Acid béo, Phaeophyta, Phân tích cấu tử chính, Rhodophyta, Rong biển