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IDENTIFICATION OF NUTRIENT COMPOSITION OF SOME ISOLATED MICROALGAE FROM MANGROVES OF XUAN THUY NATIONAL PARK

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ABSTRACT

Microalgae species, such as *Amphiprora alata*, *Chaetoceros muelleri*, *Nannochloropsis oculata*, *Navicula tuscula* and *Chlorella vulgaris* from mangrove area of Xuan Thuy National Park are usually used as food sources for fishes, shrimps and bivalves and very environment friendly. The samples of these microalgae were enriched, isolated and purified in F/2 medium with salinity of 20%. The purified colonies were cultured in media with different salinity in 14 days to find out the most optimal medium for the best growth as well as the stage yielding maximum living mass. These microalgae were then cultured in their optimal media at salinity of 25% until they reached their maximum living mass stage. They were cultivated and analyzed of their fatty acid, protein carbonhydrate components and percentages. The research has identified 24 fatty acids in 5 phytoplankton species. Unsaturated fatty acids components of *Chaetoceros muelleri*, *Nannochloropsis oculata* are the highest (76,35% and 71,17%), and fatty acid components of are medium *Chlorella vulgaris* and *Amphiprora alata* (59,24% and 52,21%), meanwhile, *Navicula tuscula* has 29.56% of unsaturated fatty acids. *Amphiprora alata* and *Chlorella vulgaris* have protein content of 8.1g per 100g dry weight and 4.44g per 100g dry weight accordingly. Carbohydrate content of *Nannochloropsis oculata* and *Navicula tuscula* are 11.8g per 100g dry weight and 5.47g per 100g dry weight, respectively.

Keywords: Microalgae, Mangroves, Nannochloropsis oculata, Navicula tuscula, fatty acid, protein, carbohydrate.

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INTRODUCTION

Mangroves of Xuan Thuy National Park (NP) plays a very important role due to their functions and services. Within the mangrove ecosystem, microorganisms, especially microalgae, are very important because they are essential in material and energy metabolism as well as have rapid growth creating huge biomass. They produce several chemical components, such as proteins, lipids, fatty acids, carbohydrates and pigments as well as silica, carbonate calcium and pectin which are part of bone composition (Ohse et al., 2015). According to Muller-Feuga et al. (2003), overall world production of these microalga-consuming species reached 12 × 106t in 1999, i.e. 28% of world aquacultural production (FAO: Shatz, 2000). Highly unsaturated fatty acids like EPA (eicosapentaenoic acid), DHA (docosahexaenoic acid) and AA (arachi- donic

acid) are essential in the development, growth, and physiological activities of aquaculture organisms. Microalgae are important main sources of EPA and DHA. (Ronquillo et al., 2012). The main microalga-consuming aquaculture groups include filtering mollusks, shrimps and small larva fish. Hatcheries in which larval and juvenile production depends on cultured microalgae are assuming an increasingly important role; and the survival and growth of various marine fish larvae are improved by the addition of microalgae.

In marine hatcheries, *Amphiprora alata*, *Chaetoceros muelleri*, *Chlorella vulgaris*, *Nannochloropsis oculata* and *Navicula tuscula* are common microalgae used as food source for shrimp and mollusk (Shirota, 1996; Truong Ngoc An, 1993; Ben-Amotz et al., 1987; Parrish et al., 1998) as well as to maintain water quality (Le Xuan Tuan et al., 2005, 2008). There are differences in lipid composition of microalgae cultures including fatty acid profiles between different species and within the same species (Malakootian et al., 2016). The studies on fatty acids of some strains of microalgae have been implemented (Ronquillo et al., 2012, Liang et al., 2004, Hoa et al., 2010). Still, not much studies on the nutrient content included fatty acid composition of microalgae have been carried out in mangrove area of Vietnam. Thus, present study aims to identify nutrient content of microalgae from mangroves of Xuan Thuy NP in order to contribute in improving quality of alga-based food source cultivated for coastal aquaculture.

MATERIALS AND METHODS

The experiments were conducted in Laboratory at Department of Algae Technology, Institute of Microorganisms and Biotechnology, Faculty of Biological Sciences, Hanoi University of Education. The studied microalgae included *Amphiprora alata, Chaetoceros muelleri, Chlorella vulgaris, Nannochloropsis oculata,* and *Navicula tuscula.* The experimental design comprised randomised samples with three repetitions.

Material, enrichment and isolation

Microalgae samples were collected, enriched and briefly morpholocial identified using Olympus photomicroscope. After that, samples were then subdivied. 100 µl of sample were driped on petri dish containing culture medium, after culturing for 5–7 days at room temperature with light intensity of 10,000 luxat light/dark cycle of 10-14 hours, colonies developed on the petri dish. These colonies were then separeated and examined using Olympus stereo microscope. Purified colonies were then cultured into slant and kept at 4°C for further step of experiment.

Growth analysis of microalgae

Pure microalgae strains of *Amphiprora alata, Chaetoceros muelleri, Chlorella vulgaris, Nannochloropsis oculata* and *Navicula tuscula* were cultured in different media and salinity (0, 5, 10, 15, 20, 25, 30 and 35 ‰) as follows:

Amphiprora alata: in media ASW, F/2, ESM; *Chaetoceros muelleri:* in media ASW, F/2, ESM, F/2 without silica; *Nannochloropsis oculata:* in media F/2, ASW, F/2 without silica, Walne; *Navicula tuscula:* in media ASW, ESM, F/2; and *Chlorella vulgaris:* in media BBM, BG11, C.

All microalgae culture bottle of 8 little were in conduction of air pumped for 24/24 and light/dark cycle of 10 hours-light intensity of 10,000 lux. Each experiment typically lasted 14 days. At 2 days intervals, each culture was removed, cells counted using Neubauer calculation chamber, then, growth curves were drawn to identify optimal condition of media and salinity for their growth. Using growth curve, the stage of maximal biomass was determined in days from 8 to 9 for all species.

Identification of fatty acids of microalgae

The pure microalgae strains of *Amphiprora alata, Chaetoceros muelleri, Chlorella vulgaris, Nannochloropsis oculata,* and *Navicula tuscula* at the media and salinity condition as follows:

Amphiprora alata: in media ASW and salinity of 25‰; *Chaetoceros muelleri:* in media ASW and salinity of 25‰; *Nannochloropsis oculata:* in media F/2 and salinity of 25‰; *Navicula tuscula:* in media ESM and salinity of 25‰; and *Chlorella vulgaris:* in media BBM and salinity of 25‰.

At stage of maximal biomass, in day 8, cultured microalgae were extracted by folowing procedure. Firtly, samples of cultured microalgaes were centrifugated at 10000 rpm for 15 minutes and at 2°C, then, added into extraction solution (metanol/chloroform (1:1) v/v) and rotary evoporated to have the cells followed by lipid extraction.

Fatty acid methyl esters were obtained by dissolving the cells in mixture of metanol and sulfuric acid (with ratio of 95/5) and boiled at 80°C for 4 hours; and then 2 ml of water were added. The esterified fatty acids were extracted again with 2 ml n-hexan. The mixture of methyl esters of fatty acids profiles were determined by Gas Chromatography (Finnigan Trace GC, colum BPX70 (50M)) at Department of Marine Biology, Institute of Natural Products Chemistry, Vietnam Academy of Science and Technology. Fatty acids profile data were obtained by comparing retention time in relation to that of standard solution.

Protein content identification of microalgae

As identification of fatty acids of microalgae, cultured microalgaes were collected at stage of maximal biomass and then were dried. Dried microalgae samples were hydrolized in 1N of NaOH solution for 1 hour. After that, the hydrolysis solution was diluted 5 times and centrifugated at speed of 4000 rpm for 15 minutes. Sample solution was analysed using Bradford method with Bovine Serum Albumin (BSA) as standard solution at 595nm wavelength (Ben-Amotz et. al., 1987; Nguyen Van Mui, 2001).

Carbohydrate content identification of micro algae

As identification of protein content of microalgae, cultured microalgaes were collected at stage of maximal biomass and then were dried. According to method proposed by Ben-Amotz et al. (1987), dried microalgae samples were hydrolized in 2.5N HCl solution for 1 hour. After that, the hydrolysis solution was diluted 20 times and centrifugated at speed of 8000 rpm for 15 minutes. Sample solution was analysed by phenol-acid sulfuric method using 5% phenol and 96% H_2SO_4 with glucose as standard at 490nm wavelength.

RESULTS AND DISCUSSION

Fatty acid component of *Amphiprora alata*

Fatty acid is a carboxylic acid with a long aliphatic chain. Most naturally occurring fatty acids have an unbranched chain of an even number of carbon atoms, from 4 to 28. Fatty acids are surfactants one water soluble endand one oil soluble end. Fatty acid is either saturated or unsaturated. Fatty acids are essential components of cell membrane of living organisms. The results of fatty acid analysis for microalgae *Amphiprora alata* are shown in table 1.

Table 1 shows that fatty acid content of *Amphiprora alata* is very diverse, from 12C to 24C chains. Among these, 9 saturated fatty acid make up to 44.43% and 12 unsaturated fatty acid to 52.21%. Percentage of polyunsaturated fatty acid (PUFA) is 30%. These fatty acids are important in forming cell membrane and have high pharmaceutical

values. Especially, *Amphiprora alata* contains valuable unsaturated fatty acid such as C 22:6n-3 (DHA) (5.25%) playing important role in brain and retina development and tissue regeneration; C 20:5n-3 (EPA) (9.12%) being essential element in progastagladin synthesis; and C 20:4n-6 (AA) (7.96%) being crucial fatty acid in bond tissue development and recover. This shows that *Amphiprora alata* has very high quality nutrient and can be used in aquaculture food production and human functional food production.

Table 1. Fatty acid content of *Amphiprora alata*

No.	Fatty acid	Scientific name	Common name	Percentage (% total fatty acid)
1	C 12:0	Dodecanoic acid	Lauric	0.63
2	C 14:0	Tetradecanoic acid	Myristic	13.26
3	C 15:0	Pentadecanoic acid	Convolvulinolic	1.10
4	C 15:1n-5	Pentadecenoic acid	Hormelic	0.34
5	C 16:0	Hexadecanoic acid	Palmitic	14.31
6	C 16:1n-7	9-hexadecenoic acid	Palmitoleic	13.15
7	C 16:1n-9	7-hexadecenoic acid	Ambrettolic	4.47
8	C 17:0	Heptadecanoic acid	Margric	5.01
9	C 17:1n-7	Heptadecenoic acid	--	0.82
10	C 18:0	Octadecanoic acid	Stearic	4.15
11	C 18:1n-7	11-octadecenoic acid	Asclepic	4.65
12	C 19:0	Nonadecanoic acid	Isoarachidic	0.92
13	C 18:5n-3	Octadecapentaenoic acid	--	0.58
14	C 18: 4n-3	Octadecatetraenoic ***acid***	--	0.59
15	C 20:0	Eicosanoic acid	Arachidic	2.21
16	C 20:1n-9	11-eicosaenoic acid	Gondoic	1.45
17	C 20:4n-6	5,8,11,14-eicosatetraenoic acid	Arachidonic acid (AA)	7.97
18	C 20:5n-3	5,8,11,14,17-eicosapentaenoic acid	Eicosapentaenoic acid (EPA)	9.12
19	C 22:5n-6	Docosatetraenoic acid	Docosatetraenoic acid (DPA)	3.65
20	C 22:6n-3	4,7,10,13,16,19-docosahexaenoic	Docosahexaenoic acid (DHA)	5.25
21	C 24:0	Tetracosanoic acid	Lignoceric	2.83
Total saturated fatty acid (9)				44.43
Total unsaturated fatty acid (12)				52.21

Note: symbol (--) indicates fatty acids that do not present (either unidentified or insignificant amount detected) or are not yet named

Fatty acid component of *Chaetoceros muelleri*

The results of fatty acid analysis for microalgae *Chaetoceros muelleri* are shown in table 2.

Table 2 shows that fatty acid content of *Chaetoceros muelleri* is fairly diverse, including 12C to 24C chains. Among these, there are 7 saturated fatty acid making up to 20.33% and 12 unsaturated fatty acid making up to 76.35%. Saturated fatty acid counts for highest percentage (9.52%) of C17:0, and unsaturated fatty acid count for highest percentage (24.76%) of C20:5n-3 (EPA). Percentage of polyunsaturated fatty acids of *Chaetoceros muelleri* is 36.63% and EPA and AA of them is of 7.84%). Meanwhile, *Chaetoceros muelleri* collected form the

Brazil contained high levels of the 18:0 (14.38%), followed by the 14:0 (1.89%) and the 20:3 (9.69%) (Ohse et al., 2015). It can be seen that *Chaetoceros muelleri* provides nutritious food source for aquaculture of mollusks and crustaceans.

Table 2. Fatty acid content of *Chaetoceros muelleri*

No.	Fatty acid	Scientific name	Common name	Percentage (% total fatty acid)
1	C 14:0	Tetradecanoic acid	Myristic	1.91
2	C 14:1n-5	Tetrade cenoic acid	Myristoleic	18.09
3	C 15:0	Pentadecanoic acid	Convolvulinolic	0.74
4	C 15:1n-5	Pentadecenoic acid	Hormelic	0.096
5	C 16:0	Hexadecanoic acid	Palmitic	5.53
6	C 16:1n-7	9-hexadecenoic acid	Palmitoleic	15.23
7	C 16:1n-9	7-hexadecenoic acid	Ambrettolic	2.20
8	C 17:0	Heptadecanoic acid	Margric	9.52
9	C 18:0	Octadecanoic acid	Stearic	1.46
10	C 18:1n-7	11-octadecenoic acid	Asclepic	3.74
11	C 18:2n-6-t	9,12-octadecadienoic acid	Linoleic	2.70
12	C 18:3n-6	6,9,12-octadecatrienoic acid	γ - Linolenic acid (GLA)	1.12
13	C 18:4n-3	Octadecatetraenoic ***acid***	--	0.22
14	C 20:0	Eicosanoic acid	Arachidic	1.05
15	C 20:1n-7	13-eicosaenoic acid	Paullinic	0.26
16	C 20:1n-9	11-eicosaenoic acid	Gondoic	0.10
17	C 20:4n-6	5,8,11,14-eicosatetraenoic acid	Arachidonic acid (AA)	7.84
18	C 20:5n-3	5,8,11,14,17-Eicosapentaenoic acid	Eicosapentaenoic acid (EPA)	24.76
19	C 24:0	Tetracosanoic acid	Lignoceric	0.12
Total saturated fatty acid (7)				20.33
Total unsaturated fatty acid (12)				76.35

Note: symbol (--) indicates fatty acids that do not present (either unidentified or insignificant amount detected) or are not yet named

Fatty acid content of *Chlorella vulgaris*

The results of fatty acid analysis for microalgae *Chlorella vulgaris* are shown in table 3.

As shown in table 3, the fatty acid content of microalgae *Chlorella vulgaris* is not as diverse as previously analyzed microalgae. There are 11 fatty acids in *Chlorella vulgaris* comprising of 5 saturated fatty acids (39.55%) and 6 unsaturated fatty acids (59.24%). *Chlorella vulgaris* does not contain AA, EPA, DHA, DPA but two double-bond unsaturated fatty acids which are C18:2n-6-t (LA) and C18:3n-3 make up to 25.88%. These are two important fatty acids for growth, development of organisms that cannot be synthesized directly by human and animals. These two fatty acids play crucial roles in brain development. LA content is

indicator to evaluate bio-value of fat. *Chlorella vulgaris* collected form the Brazil contained high levels of the 18:3d (22.17%), followed by the 16:0 (21.17%), 18:0 (15.47%), and the 18:1c (13.46%) (Ohse et al., 2015). Hence, identifying exact culture time to get the highest content of fatty acids for *Chlorella vulgaris* and combining *Chlorella vulgaris* with other microalgae will help to produce high nutritious food sources for aquaculture.

Fatty acid content of *Nannochloropsis oculata*

Fatty acid content of *Nannochloropsis oculata* is quite rich (Table 4).

Table 3. Fatty acid content of *Chlorella vulgaris*

No.	Fatty acid	Scientific name	Common name	Percentage (% total fatty acid)
1	C 14:0	Tetradecanoic acid	Myristic	1.53
2	C 16:0	Hexadecanoic acid	Palmitic	27.43
3	C 16:1n-7	9-hexadecenoic acid	Palmitoleic	5.15
4	C 16:1n-9	7-hexadecenoic acid	Ambrettolic	1.2
5	C 17:0	Heptadecanoic acid	Margric	2.69
6	C 17:1n-7	Heptadecenoic acid	--	6.15
7	C 18:0	Octadecanoic acid	Stearic	2.91
8	C 18:1n-9	9-octadecenoic acid	Oleic	20.06
9	C 18:2n-6-t	9,12-octadecadienoic acid	Linoleic acid(LA)	8.42
10	C 18:3n-3	9,12,15-octadecatrienoic acid	Anpha-Linoleic acid(LNA)	17.46
11	C 20:0	Eicosanoic acid	Arachidic	4.98
Total saturated fatty acid (5)				39.55
Total unsaturated fatty acid (6)				59.24

Note: symbol (--) indicates fatty acids that do not present (either unidentified or insignificant amount detected) or are not yet named

Table 4 shows that fatty acid content of *Nannochloropsis oculata* is quite diverse. *N. oculata* has more than 30 fatty acids, among those are 6 saturated fatty acids (25.6%) and 18unsaturated fatty acids (71.7%). EPA (C20:5n-3) has the highest percentage (26.7%). This is the characteristic of the genus *Nannochloropsis*. Fatty acid palmitic (16:0) and fatty acid palmitoleic (16:1n-7) occupy 21.3% and 14.4% accordingly. Especially, acid linoleic (LA) and acid α-linoleic also have quite high percentages, 7.6% and 5.8%. The result shows that total of fatty acid group (n-3) occupy 36.3% of total fatty acid content. These are unsaturated fatty acids that are very important in enhancing active ability of brain cells. Moreover, total percentage of fatty acid group (n-6) which is important to child development is 8.7%. Ohse et al., 2015 showed that *Nannochloropsis oculata* in Brazile contained quite high percentage of fatty acids of 16:0 and 16:1 (33.17% and 30.96%), which are much higher than those of *Nannochloropsis oculata* in this study. Ronquillo et al. (2012) indicated that the best diet in culturing European oyster juveniles was the mixture of *Nannochloropsis oculata* and *Pavlova lutheri* by providing better growth rates and higher levels of PUFAs. In conclusion, microalgae *Nannochloropsis oculata* has very diverse and rich fatty acid content comprising high percentage of important fatty acids. This has very valuable application to aquaculture and daily use.

Table 4. Fatty acid content of *Nannochloropsis oculata*

No.	Fatty acid	Scientific name	Common name	Percentage (% total fatty acid)
1	C 12:0	Dodecanoic acid	Lauric	0.2
2	C 14:0	Tetradecanoic acid	Myristic	3.6
3	C 16:0	Hexadecanoic acid	Palmitic	21.3
4	C 16:1n-7	9-hexadecenoic acid	Palmitoleic	14.4
5	C 16:2n	--	--	1.2
6	C 16:3n-6	hexadecatrienoic acid	--	0.2
7	C 16:3n-3	7,10,13-hexadecatrienoic acid	--	3.7
8	C 18:0	Octadecanoic acid	Stearic	0.3
9	C 18:1n	Cis 9 oleic acid	--	7.6
10	C 18:2n-6	9,12-Octadecadienoic acid	Linoleic acid(LA)	7.6
11	C 18:3n-6	6,9,12-Octadecatrienoic acid	gamma -Linolenic Acid	0.3
12	C 18:3n-3	9,12,15-octadecatrienoic acid	Anpha-Linoleic Acid (LNA)	5.8
14	C 20:0	Eicosanoic acid	Arachidic	0.1
15	C 20:1n	11-eicosenoic acid	--	0,2
16	C 20:2n-9	8,11-cis-eicosadienoic acid	--	0,1
17	C 20:2n-6	11,14-ecosadienoic acid	Eicosadienoic Acid	0.1
18	C 20:3n-9	5,8,11-eicosatrienoic acid	Mead Acid	0.1
19	C 20:3n-6	8,11,14-eicosatrienoic acid	Dihomo-g -Linolenic Acid	0.2
20	C 20:4n-6	5,8,11,14-Eicosatetraenoic acid	Arachidonic Acid (AA)	3.0
23	C 20:5n-3	5,8,11,14,17-Eicosapentaenoic acid	Timnodonic Acid (EPA)	26.7
24	C 22:0	--	Behenic acid	0.1
25	C 22:1	13-docosenoic acid	--	0.1
27	C 22:4n-6	7,10-13-16-Ocosatetraenoic acid	Adrenic Acid	0.3
30	C 22:6n-3	4,7,10,13,16,19-Docosahexaenoic acid	Docosahexaenoic Acid (DHA)	0.1
31			other Acid	2.8
Total saturated fatty acid (6)				25.6
Total unsaturated fatty acid (18)				71.7

Note: symbol (--) indicates fatty acids that do not present (either unidentified or insignificant amount detected) or are not yet named.

Fatty acid content of *Navicula tuscula*

The results of fatty acid analysis for microalgae *Navicula tuscual* are shown in table 5.

Microalgae *Navicula tuscula* has 7 saturated fatty acids (69.761%) and 10 unsaturated fatty acid (29.597%). Content of unsaturated fatty acid of *Navicula tuscula* is lowest among all microalgae studied in this research (Table 5). Fatty acid having highest percentage is saturated acid C16:0 (52.57%). Besides, percentages of ω3 and ω6 fatty acid groups are also quite low but the composition of ω6 fatty acid group is very diverse (C 20:4n-6, C 22:4n-6, C 18:2n-6, C 18:3n-6). However, *Navicula tuscula* is large microalgae and appropriate to be used as food for parental bodies in aquaculture. The combination of *Navicula tuscula* with other microalgae to produce nutritious supplement

for aquaculture objects including both larvae and parental bodies are very useful. This is simlar to the resuls of fatty acid profiles of *Navicula tuscula* collected from Giao Thuy in 2010, which indicated high concentration of 16:0, 16:1n-7 and 14:0 (more than 60% of total fatty acids) (Hoa et al., 2010).

From results shown in tables 1–5, it is observed that the fatty acid contents of five studied microalgae are quite diverse. Most of microalgae have high percentage of unsaturated fatty acid except for the case of *Navicula tuscula* having higher percentage of saturated acid comparing to unsaturated one. This means studied microalgae have good nutritious quality. In general, nutritious value of fatty acid of diatoms is usually higher than that of green algae and the ratio of saturated acid and unsaturated acid varies for different microalgae. *Nannochloropsis oculata* is one-cell microalgae with diverse fatty acid content and can be good nutrition source for aquaculture. Therefore, a sophisticated combination of these microalgae as food supplied for different stages of development of aquaculture subjects such as snout otter clam, bivalves, crustaceans, etc. can yield high productivity and quality.

Protein contents of studied microalage

Protein content is one of the essential factors to evaluate nutritious value of microalgae. The results of protein content analysis are compiled in Fig. 1.

Table 5. Fatty acid content of *Navicula tuscula*

No.	Fatty acid	Scientific name	Common name	Percentage (% total fatty acid)
1	C 4:0	Butyric acid	--	1.17
2	C 10:0	Decanoic acid	--	0.33
3	C 14:0	Tetradecanoic acid	Myristic	9.69
4	C 14:1n-5	Tetrade cenoic acid	Myristoleic	0.80
5	C 15:1n-5	Pentadecenoic acid	Hormelic	0.70
6	C 16:0	Hexadecanoic acid	Palmitic	52.56
7	C 16:1n-7	9-hexadecenoic acid	Palmitoleic	13.67
8	C 17:0	Heptadecanoic acid	Margric	1.20
9	C 17:1n-7	Heptadecenoic acid	--	1.49
10	C 18:0	Octadecanoic acid	Stearic	3.77
11	C 18:1n-7	11-octadecenoic acid	Asclepic	8.62
12	C 18:2n-6	9,12-Octadecadienoic acid	Linoleic acid(LA)	1.27
13	C 18:3n-6	6,9,12-Octadecatrienoic Acid	gamma -linolenic acid	0.35
14	C 18:5n-3	Octadecapentaenoic acid	--	1.56
15	C 20:4n-6	5,8,11,14-eicosatetraenoic acid	Arachidonic acid (AA)	0.76
16	C 22:0	--	Behenic acid	1.04
17	C 22:4n-6	7,10-13-16-Ocosatetraenoic acid	Adrenic acid	0.34
Total saturated fatty acid (7)				69.76
Total unsaturated fatty acid (10)				29.56

Note: symbol (--) indicates fatty acids that do not present (either unidentified or insignificant amount detected) or are not yet named

Protein content is highest in *Amphiprora alata* species, followed by *Chaetoceros muelleri*, *Nannochloropsis oculata*, *Navicula tuscula* and lowest in *Chlorella vulgaris* (Fig. 2). It is also observed that microalgae with high fatty acid content has high protein content giving it high nutritious value. All studied microalgae have quite similar protein

content in the range of 5.08% to 8.01%. Protein is an important organic compound to all activities of living organisms such as catalysis, transport, protection, storage, harmonization, etc. Therefore, addition of protein in food source is very necessary. Moreover, to produce food source with enough content of amine acid used in aquaculture, a combination of all above-mentioned microalgae should be considered.

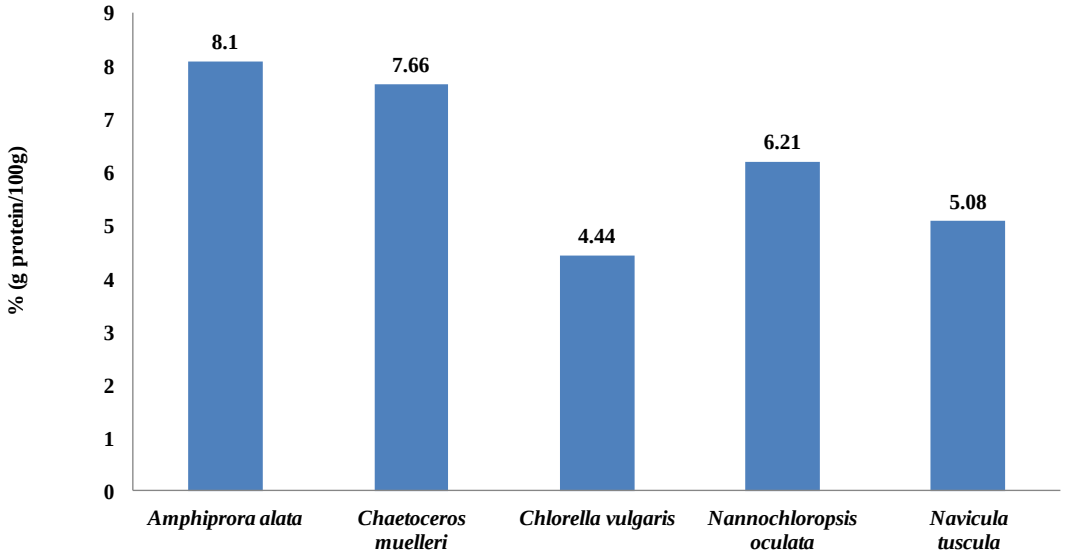

Figure 1. Protein content (g protein/100g dry weight) of studied microalgae

Carbohydrate contents of studied microalage

Carbohydrate (sugar, starch and fibre) is also named glucid. Carbohydrate plays important role in providing energy for organism bodies. Analysis results of carbohydrate content are shown in Fig. 2.

Nannochloropsis oculata species has the highest carbohydrate content, followed by *Chaetoceros muelleri*, *Chlorella vulgaris*, *Amphiprora alata* and *Navicula tuscula.* The differences in carbohydrate content of studied microalgae are bigger than those in protein content (e.g. carbohydrate content in *Nannochloropsis oculata* is double that in *Navicula tuscula*). Compiling the results of protein contents and fatty acid contents shows that *Nannochloropsis oculata* has the highest nutritious value. However, it is not advisable to use only this species as food supplement. The better way is to combine with other species to produce the optimal food source.

Analysis results show that fatty acid content, protein content and carbohydrate content are quite diverse and typical for each microalgae species. The combination of all these microalgae will produce quite sufficient nutrient with high percentage of double-bond unsaturated fatty acid (DHA, EPA) as well as provide plentiful protein and carbohydrate amount for important metabolisms of organisms. Effective combination of these 5 microalgae with diversity in size will create high nutrient food sources for coastal quaculture.

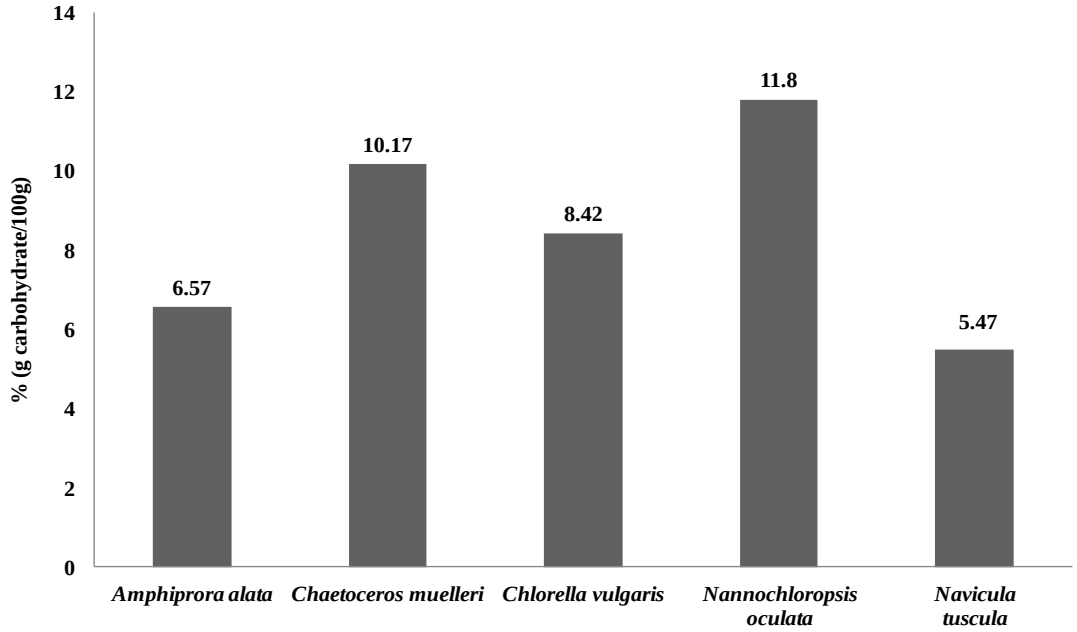

Figure 2. Carbohydrate content (g carbohydrate/100g dry weight) of microalgae

CONCLUSION

Of the total five studied microalgae rich in nutrient. *Nannochloropsis oculata* has the most diverse fatty acid content (24 types of fatty acids with 71.7% of unsaturated fatty acid). *Navicula tuscula* has the lowest fatty acid content (17 types of fatty acid with 29.6% of unsaturated fatty acid). *Amphiprora alata* is very richest in protein (8.1g/100g dry weight) and *Chlorella vulgaris* has the lowest protein content (4.44g/100g dry weight). *Nannochloropsis oculata* has the highest carbohydrate content (11.8g/100g dry weight) and *Navicula tuscula* has the lowest (5.47g/100g dry weight). From study results, it is suggested that pilot experiment should be conducted using microalage with different compositions in food portion of different aquaculture objects, in order to improve productivity and maximize the use of valuable nutritious sources from isolated microalgae in coastal mangrove.

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