ORIGINAL ARTICLE

Fatty acids composition of 10 microalgal species

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Abstract

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Fatty acids composition of 10 species of microalgae was determined at the exponential phase and the stationary phase. The microalgae consist of two species of diatoms, Bacillariophyceae, (*Nitzschia* cf. *ovalis*, *Thalassiosira* sp.) five species of green microalgae, Prasinophyceae (*Tetraselmis* sp.) and Chlorophyceae, (*Dictyosphaerium pulchellum*, *Stichococcus* sp., *Chlorella* sp., *Scenedesmus falcatus*) and three species of blue green microalgae, Cyanophyceae (*Anacystis* sp., *Synechococcus* sp., *Synechocystis* sp.).

Medium for culture diatoms and green microalgae was F/2, and BG-11 media was used for Cyanophyceae. The microalgae were cultured beneath light intensity 143 μEm^{-2}s^{-1}, light: dark illustration 12:12 hrs., temperature 28ºC, and salinities 8-30 psu. The microalgae were harvested for analyzing fatty acid by centrivugal machine at 3500 rpm. for 5 min. at temperature 20ºC and stored at -80ºC prior to analysis.

Fatty acids composition of microalgae differed from species to species. The majority fatty acids composition of diatoms at the exponential phase and the stationary phase were C16:1n-7 (17.12-31.47% and 28.22-42.02%), C16:0 (13.25-19.61% and 18.83-20.67%), C20:5 n-3 (16.65-26.67% and 11.32-23.68%) respectively. The principle fatty acids composition of green microalgae, Prasinophyceae, *Tetraselmis* sp. were C18:3n-3 (16.17-16.67%), C16:0 (15.33-17.45%), C18:1n-9 (12.25-15.43%), C18:2n-6 (9.66-19.97%). The fatty acids composition of green microalgae, Chlorophyceae, were C18:3 n-3 (20.02-26.49% and 15.35-30.63%), C16:0 (5.76-17.61% and 11.41-20.03%), C18:2n-6 (4.67-17.54% and 7.48-20.61%) respectively. The major amounts of fatty acids content of blue green microalgae were C16:1n-7 (9.28-34.91% and 34.48-35.04%), C14:0 (13.34-25.96% and 26.69-28.24%), C16:0 (5.89-29.15% and 5.70-16.81%) except for *Anacystis* sp.which had a high amount of C18:3 n-3 (23.18-27.98%) but low amount of C14:0 (3.66-4.98%).

Bacillariophyceae contained the highest amount of highly unsaturated fatty acids (HUFAs) at both growth phases. Prasinophyceae had a small amount while in Chlorophyceae and Cyanophyceae they were

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not detected. *Nitzschia* cf. *ovalis* and *Thalassiosira* sp. had amount of C20:4n-6 (0.08-4.40%),C20:5 n-3 (11.32-26.67%) and C22:6 n-3 (0.80-4.20%) respectively. *Tetraselmis* sp. had amounts of C20:4n-6 and C20:5 n-3 ranging from 0.99-1.13% and 4.18-4.70% respectively. In conclusion, *Nitzschia* cf. *ovalis* and *Thalassiosira* sp. would serve as good nutritional sources of HUFAs for aquaculture animals.

Key words : microalgae, fatty acids, Bacillariophyceae, Chlorophyceae, Cyanophyceae

บทคัดย่อ

จารุนันท์ ประทุมยศ ปิยะวรรณ ศรีวิลาศ และ ธิดารัตน์ น้อยรักษา

องค์ประกอบชนิดและปริมาณกรดไขมันในสาหร่ายขนาดเล็ก 10 ชนิด

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ศึกษาชนิดและปริมาณกรดไขมันในสาหร่ายขนาดเล็ก 10 ชนิด ประกอบด้วย สาหร่ายในกลุ่มไดอะตอม Bacillariophyceae 2 ชนิด (*Nitzschia* cf. *ovalis*, *Thalassiosir*a sp.) สาหร่ายสีเขียว 5 ชนิด Prasinophyceae (*Tetraselmis* sp.) และ Chlorophyceae (*Dictyosphaerium pulchellum*, *Stichococcus* sp., *Chlorella* sp., *Scenedesmus falcatus*) และสาหร่ายสีเขียวแกมน้ำเงิน 3 ชนิด (*Anacystis* sp., *Synechococcus* sp., *Synechocystis* sp,) ในระยะการเจริญเติบโตรวดเร็วและในระยะการเจริญเติบโตคงที่

เพาะเลี้ยงสาหร่ายขนาดเล็กซึ่งเป็นสาหร่ายในกลุ่มไดอะตอมและสาหร่ายสีเขียวด้วยสูตรอาหาร F/2 และสาหร่ายสีเขียวแกมน้ำเงินด้วยสูตรอาหาร BG-11 ที่อุณหภูมิ 28°C ความเข้มแสง 143 $\mu Em^{-2}s^{-1}$ ระยะเวลาให้แสงและไม่ให้แสง 12:12 ชั่วโมง ความเค็ม 8-30 psu เก็บเซลล์เพื่อวิเคราะห์กรดไขมันโดยการปั่นแยกให้ตกตะกอนที่อุณหภูมิ 20°C ความเร็ว 3500 รอบ/วินาที เป็นเวลา 5 นาที เก็บรักษาตัวอย่างในอุณหภูมิ -80°C จนกระทั่งวิเคราะห์กรดไขมัน

ผลการวิเคราะห์พบว่า องค์ประกอบกรดไขมันในสาหร่ายขนาดเล็กแตกต่างกัน องค์ประกอบกรดไขมันในไดอะตอม (Bacillariophyceae) ที่มีมากในระยะการเจริญเติบโตรวดเร็วและระยะการเจริญเติบโตคงที่ ได้แก่ C16:1n-7 (17.12-31.47% และ 28.22-42.02 %), C16:0 (13.25-19.61%และ 18.83-20.67 %), C20:5 n-3 (16.65-26.67% และ 11.32-23.68%) ตามลำดับ องค์ประกอบกรดไขมันในสาหร่ายขนาดเล็กสีเขียว (Prasinophyceae) ที่มีมากในระยะการเจริญเติบโตรวดเร็วและระยะการเจริญเติบโตคงที่ ได้แก่ C18:3n-3 (16.17-16.67%), C16:0 (15.33-17.45%), C18:1n-9 (12.25-15.43%), C18:2n-6 (9.66-19.97%) ตามลำดับ องค์ประกอบกรดไขมันในสาหร่ายขนาดเล็กสีเขียว (Chlorophyceae) ที่มีมากในระยะการเจริญเติบโตรวดเร็วและระยะการเจริญเติบโตคงที่ ได้แก่ C18:3 n-3 (20.02-26.49% และ 15.35-30.63%), C16:0 (5.76-17.61% และ 11.41-20.03%), C18:2n-6 (4.67-17.54% และ 7.48-20.61%) ตามลำดับ องค์ประกอบกรดไขมันในสาหร่ายขนาดเล็กสีเขียวแกมน้ำเงิน (Cyanophyceae) ที่มีมากในระยะการเจริญเติบโตรวดเร็วและระยะการเจริญเติบโตคงที่ ได้แก่ C16:1n-7 (9.28-34.91% และ 34.48-35.04%), C14:0 (13.34-25.96% และ 26.69-28.24%), C16:0 (5.89-29.15% และ 5.7-16.81%) ยกเว้น *Anacystis* sp. ที่มีองค์ประกอบกรดไขมัน C18:3 n-3 (23.18-27.98%) สูง แต่มีปริมาณกรดไขมัน C14:0 ต่ำ (3.66-4.98%) ตามลำดับ

ไดอะตอมมีปริมาณกรดไขมันที่จำเป็นสำหรับสัตว์น้ำในกลุ่ม HUFAs มากที่สุด สาหร่ายขนาดเล็กสีเขียวชนิด *Tetraselmis* sp. มีปริมาณน้อย ส่วนสาหร่ายขนาดเล็กสีเขียวชนิดอื่นและสาหร่ายขนาดเล็กสีเขียวแกมน้ำเงินไม่มีกรดไขมันในกลุ่ม HUFAs เลย ไดอะตอมชนิด *Nitzschia* cf. *ovalis* และ *Thalassiosira* sp. มีกรดไขมันที่จำเป็นสำหรับสัตว์น้ำ C20:4n-6, C20:5 n-3 และ C22:6 n-3 มากที่สุด (0.08-4.40%, 11.32-26.67% และ 0.80-4.20%) ตามลำดับ *Tetraselmis* sp. มีกรดไขมันที่จำเป็นสำหรับสัตว์น้ำ C20:4n-6 และ C20:5 n-3 อยู่ในช่วง 0.99-1.13% และ 4.18-4.70% ตามลำดับ สรุปได้ว่าไดอะตอม *Nitzschia* cf. *ovalis* และ *Thalassiosira* sp. สามารถเป็นแหล่งกรดไขมันในกลุ่ม HUFAs ของสัตว์น้ำได้ดี

สถาบันวิทยาศาสตร์ทางทะเลบางแสน มหาวิทยาลัยบูรพา อำเภอเมือง จังหวัดชลบุรี 20131

Microalgae belong to a highly diverse group of photoautotrophic organisms which are important for aquatic animal feeding. They play important roles of primary producers in mariculture as food for consumers such as rotifer, copepod, daphnia, brine shrimp etc. which are fed to late larval and juvenile fish and crustaceans. (Volkman *et al*., 1989). Moreover, they occupy the base of the food chain in the open sea (Ariyadej *et al*.,2004). Based on the taxonomical schemes of the numbers of algal divisions, they range from 4 to 13 with as many as 24 classes and about 26,000 species (Bold and Wynne, 1985) The checklist of algae in Thailand, compiled from 53 publications, lists 161 genera, 1,001 species, 287 varieties and 63 forms (Ariyadej *et al*., 2004 cited Wongrat, 1999). Since the diversity of microalgae is large, a number of microalgae have been intensively studied because of high nutritional quality, in term of lipid class and fatty acids composition, optimal for the animals being reared (Brown *et al*., 1989; 1997; 2002; Volkman *et al*., 1980; Volkman *et al*., 1991 cited Marlowe *et al*., 1984; Reitan *et al*., 1997).

At present, a number of microalgae are being cultured commercially as industrial production, as food for captive aquatic animals and intensive cultured aquatic animals. Although microalgae are diverse in the open sea and the estuary area, the nutritive value, especially fatty acid, of microalgae has been little examined (Chomrung, 2000; Brown *et al*., 1997). Therefore, Bangsaen Institute of Marine Science, Burapha University (BIMS), has collected microalgae as BIMS microalgae culture collection. The present paper examined the fatty acids composition of 10 microalgal species of 4 classes. All strains belong to Bangsaen Institute of Marine Science (BIMS) microalgal culture collection and have never been studied for nutritional quality.

Materials and Methods

1. Microalgae cultures and sample preparation

Ten microalgae species with size range 2-20 ?m were selected from algal culture collection of Bangsaen Institute of Marine Science (BIMS), Burapha University. The conditions and medium used for culture microalgae are shown in Table 1. At the middle exponential phase and the middle stationary phase, microalgae were harvested by centrifugal machine at -20ºC, 3500 rpm. for 5 min. Prior to analysis, they were frozen at -80ºC.

Table 1. The conditions and medium used for culture microalgae.

Class/Scientific name	Media	Temp. (ºC)	Salinity (psu.)	Light intensity ($\mu E\ m^{-2}s^{-1}$)	Light: Dark Illustration (hrs.)
Bacillariophyceae					
- *Nitzschia* cf. *ovalis* BIMS-PP0004	F/2**	28	30	143	12:12
- *Thalassiosira* sp. BIMS-PP0014	F/2**	28	30	143	12:12
Prasinophyceae					
- *Tetraselmis* sp. BIMS-PP0017	F/2**	28	30	143	12:12
Chlorophyceae					
- *Dictyosphaerium pulchellum* BIMS-PP0033	F/2**	28	15	143	12:12
- *Stichococcus* sp. BIMS-PP0045	F/2**	28	15	143	12:12
- *Chlorella* sp. BIMS-PP0081	F/2**	28	15	143	12:12
- *Scenedesmus falcatus* BIMS-PP0082	F/2**	28	8	143	12:12
Cyanophyceae					
- *Anacystis* sp. BIMS-PP0028	BG-11*	28	30	143	12:12
- *Synechococcus* sp. BIMS-PP0044	BG-11*	28	15	143	12:12
- *Synechocystis* sp. BIMS-PP0069	BG-11*	28	30	143	12:12

**** Guillard, 1975; *Vonshak,1986**

2. Lipid extraction

Total lipids were extracted from 1 g of wet sample according to Bligh and Dyer, (1959). All samples were dissolved in chloroform (contained BHT 0.1 ppm.) : methanol (contained BHT 0.1 ppm.) in a ratio of 2:1, pooled the solvent and dried under nitrogen gas. A known amount of heneicosanoic acid (C21:0) was used as internal standard. Fatty acids methyl esters were prepared by acid- catalyzed transmethylation of total lipids (10 ml. of 2 % sulphuric acid in methanol and oven at 80ºC for 4 hour. Added 5 ml. of 5% sodium chloride, 5 ml. of hexane and 40 ml. of 2% potassium bicarbonate and then filtered through anhydrous sodium sulphate and dried under nitrogen gas (Cristies, 1982).

3. Fatty acids analysis

Separation and identification of fatty acids were carried out. They were analysed in Hewlett Packard, HP 5890 series II equipped with a FAME WAX, USA fused silica capillary column (30 m x0.25 mm. i.d., 0.25 µm film thickness), using helium as carrier gas at 1.3 ml/min. Samples were injected 1 ml at the following condition. The column temperature was 120ºC during 0.5 min, the thermal gradient to 195ºC at a rate of 18ºC $min.^{-1}$, maintained for 5 min., 195 to 205ºC at a rate of 3ºC $min.^{-1}$, maintained for 7 min., 205 to 220ºC at a rate of 8ºC $min.^{-1}$ and maintained for 10 min. Injector and flame ionization detector temperature were 250ºC and 250ºC, respectively. Fatty acids methyl esters were identified by comparison with known standard mixtures (PUFA no.3 cat.47085-U, Supelco, USA) and quantified by area percent of total fatty acids.

4. Data control and data recovery

The reference materials, PUFA No.3 (Menhaden oil) were identified and calculated to compare the results of known reference materials with our method. The external standard (cis-5,8, 11,14,17- eicosapentaenoic acid) was analyzed to find out the percent recovery, which was 89.19% (n=3).

Results

The fatty acid compositions of ten species of microalgae at the exponential phase and the stationary phase are shown in Table 2 and 3. The major fatty acids of Bacillariophyceae, *Nitzschia* cf. *ovalis*, and *Thalassiosira* sp. at the exponential phase were C16:1n-7 (range 17.12-31.47% total fatty acids) at the stationary phase (range 28.22-42.02% total fatty acids), C16:0 (range 13.25-19.61% and 18.83-20.67%), C20:5 n-3 (range 16.65-26.67% and 11.32-23.68%). Diatoms contained HUFAs at two growth phases as arachidonic acid (C20:4n-6) ranged 0.12-4.40 and 0.08-2.25%, Eicosapentaenoic acid (C20:5 n-3) ranged 16.65-26.67% and 11.32-23.68% and Docosahexaenoic acid (C22:6 n-3) ranged 1.33-4.20 and 0.80-3.96% (Table 2 and 3).

The Prasinophyceae (*Tetraselmis* sp.) had amounts of C16:0, C18:1n-9,C18:3 n-3 as principle fatty acids. *Tetraselmis* sp had the high amount of C18:3 n-3 (range 16.17-16.67%), C16:0 (range 15.33-17.45), C18:1n-9 (range 12.25-15.43%). Moreover, it contained small amounts of HUFAs, C20:4n-6 and C20:5 n-3 at two growth phases as 0.99-1.13% and 4.70-4.18% (Table 2-3).

Chlorophyceae (*Dictyosphaerium pulchelum*, *Stichococcus* sp., *Chlorella* sp., *Scenedesmus falcatus* had high amount of fatty acids at both growth phases as C18:3 n-3 (range 20.02-26.49% and 15.35-30.63%), C16:0 (range 5.76-17.61% and 11.41-20.03%), C18:2n-6 (range 4.67-17.54% and 7.48-20.61%) (Table 2 and 3).

Cyanophyceae (*Synechococcus* sp., *Synechocystis* sp.) had high amounts of fatty acids at two growth phases as C16:1n-7 (range 16.72-34.91% and 34.48-35.04%), C14:0 (range 13.34-25.96% and 26.69-28.24%), C16:0 (range 5.89-13.94% and 5.7-16.81%) except for *Anacystis* sp. which had a high amount of C18:3 n-3 (range 23.18-27.98%) but low amount of C14:0 (range 3.66-4.98%) (Table 2 and 3).

High percentages of PUFAs were found in all microalgae used in the experiment except for *Synechococcus* sp., *Synechocystis* sp. PUFAs of

Table 2. Fatty acids composition (percent of total fatty acids)of 10 microalgal species at the exponential phase.

Fatty acid	***Nitzschia* cf. *ovalis***	***Thalassiosira* sp.**	***Tetraselmis* sp.**	***Dictyosphaerium pulchellum***	***Stichococcus* sp.**	***Chlorella* sp.**	***Scenedesmus* sp.**	***Anacystis* sp.**	***Synechococcus* sp.**	***Synechocystis* sp.**
Identity	BIMS-PP0004	BIMS-PP0014	BIMS-PP0017	BIMS-PP0033	BIMS-PP0045	BIMS-PP0081	BIMS-PP0082	BIMS-PP0028	BIMS-PP0044	BIMS-PP0069
Saturated fatty acids (SFA)										
14:0	2.67	4.59	1.31	2.38	2.12	1.58	1.12	3.66	25.96	13.34
16:0	13.25	19.61	15.33	12.56	17.61	13.08	5.76	27.60	13.94	5.89
18:0	0.45	0.35	0.43	0.77	0.54	1.23	0.33	1.40	0.58	1.17
Total SFAs	16.37	24.55	17.07	15.71	20.27	15.89	7.21	32.66	40.48	20.40
Monounsaturated fatty acids (MUFA)										
16:1n-7	17.12	31.47	3.23	2.11	-	-	1.06	9.28	34.91	16.72
18:1n-9	0.59	0.41	12.25	2.51	2.87	6.68	2.65	4.83	1.70	1.22
18:1n-7	0.33	0.27	2.26	-	1.63	1.21	0.22	4.04	-	-
20:1n-9	-18.04	-	1.12	-	0.15	0.12	0.49	-	-	-
Total MUFAs		32.15	18.86	4.62	4.65	8.01	4.42	18.15	36.61	17.94
Polyunsaturated fatty acids (PUFA)										
16:2n-4	2.45	7.45	4.51	3.06	0.19	-	1.24	-	-	1.96
16:3n-4	7.20	2.84	0.35	0.69	-	9.56	0.92	0.35	0.15	-
18:2n-6	0.43	2.0	9.66	7.76	14.78	17.54	4.67	1.60	1.46	-
18:3n-3	0.37	1.10	16.17	26.49	25.71	20.02	20.79	23.18	-	-
18:4n-3	-	2.15	1.80	1.84	-	-	2.52	-	-	-
20:4n-6	4.40	0.12	0.99	-	-	-	-	-	-	-
20:4n-3	-	0.16	0.16	-	-	-	-	-	-	-
20:5n-3	26.67	16.65	4.70	-	-	-	-	-	-	-
22:5n-3	0.28	-	-	-	-	-	-	-	-	-
22:6n-3	4.20	1.33	-	-	-	-	-	-	-	-
Total PUFAs	46.0	33.8	38.38	39.84	40.68	47.12	30.14	25.13	1.61	1.96
Total HUFAs	35.55	18.26	5.85	-	-	-	-	-	-	-

Table 3. Fatty acids composition (percent of total fatty acids) of BIMS algal culture collection 10 species at the stationary phase.

Fatty acid	***Nitzschia* cf. *ovalis***	***Thalassiosira* sp.**	***Tetraselmis* sp.**	***Dictyosphaerium pulchellum***	***Stichococcus* sp.**	***Chlorella* sp.**	***Scenedesmus* sp.**	***Anacystis* sp.**	***Synechococcus* sp.**	***Synechocystis* sp.**
Identity	BIMS-PP0004	BIMS-PP0014	BIMS-PP0017	BIMS-PP0033	BIMS-PP0045	BIMS-PP0081	BIMS-PP0082	BIMS-PP0028	BIMS-PP0044	BIMS-PP0069
14:0	3.15	6.37	1.22	2.45	1.54	1.62	1.03	4.98	26.69	28.24
16:0	18.83	20.67	17.45	11.41	20.03	16.46	17.30	29.15	16.81	5.70
18:0	0.24	0.27	0.30	0.61	0.68	3.27	1.73	1.25	0.45	0.39
Total SFAs	22.22	27.31	18.97	14.47	22.25	21.35	20.06	35.38	43.95	34.33
Monounsaturated fatty acids (MUFA)										
16:1n-7	28.22	42.02	3.27	1.89	-	-	1.90	11.35	35.04	34.48
18:1n-9	0.17	0.39	15.43	1.40	1.54	13.49	23.10	5.30	0.35	0.81
18:1n-7	0.48	0.31	2.48	-	1.50	1.15	0.47	2.54	1.50	-
20:1n-9	-	0.10	1.34	-	0.08	-	0.26	-	-	-
Total MUFAs	28.87	42.82	22.52	3.29	3.12	14.64	25.73	19.19	36.89	35.29
Polyunsaturated fatty acids (PUFA)										
16:2n-4	1.04	2.50	2.52	4.26	-	-	0.10	-	-	0.15
16:3n-4	4.42	2.63	0.39	0.61	7.81	7.41	1.72	0.35	0.16	-
18:2n-6	0.21	1.82	5.95	7.48	17.66	20.61	8.28	1.71	0.91	0.36
18:3n-3	0.36	1.52	16.97	30.63	28.25	15.35	16.78	27.98	-	-
18:4n-3	0.07	2.51	2.24	2.30	-	-	2.68	-	-	-
20:4n-6	2.25	0.08	1.13	-	-	-	-	-	-	-
20:4n-3	0.31	0.14	0.13	-	-	-	-	-	-	-
20:5n-3	23.68	11.32	4.18	-	-	-	0.19	-	-	-
22:5n-3	0.36	-	-	-	-	-	-	-	-	-
22:6n-3	3.96	0.80	-	-	-	-	-	-	-	-
Total PUFAs	36.66	23.32	33.51	45.28	53.72	43.37	29.56	30.04	1.07	0.51
Total HUFAs	30.56	12.34	5.43	-	-	-	-	-	-	-

Nitzschia cf. *ovalis*, and *Thalassiosira* sp. at the exponential phase and at the stationary phase ranged 33.8-46.0% and 23.32-36.66% of total fatty acids. *Tetraselmis* sp. (38.38-33.51%), *Dictyosphaerium pulchelum*, *Stichococcus* sp., *Chlorella* sp., *Scenedesmus falcatus* (30.14-39.84 and 29.56-53.72%), *Anacystis* sp. (25.13-30.04%) respectively (Table 2 and 3).

Highest percentage of HUFAs were found in Bacillariophyceae while Prasinophyceae was found in small amount at two growth phases. The HUFAs of Bacillariophyceae at the exponential phase and the stationary phase were 18.26-35.55% and 12.34-30.56% of total fatty acids. In the Prasinophyceae the content of HUFAs was 5.85-5.43% while in others HUFAs were not found (Table 2 and 3).

Discussion

The major fatty acids of Bacillariophyceae (diatoms) in this study were C16:1n-7, C16:0, and C20:5 n-3. The results are similar to those reported for diatoms, *Nitzschia* spp., *Thalassiosira* spp., *Chaetoceros* spp. (Brown *et al*., 1997; Thompson *et al*., 1992, 1996; Dunstan *et al*., 1994) except C14:0 content of diatoms in this study was lower than previously reported (Dunstan *et al*., 1994). *Nitzschia* cf. *ovalis* in this study had the medium amount of AA, C20:4n-6 (2.25-4.4%) which was comparable to *N. closterium* CS-5 (Dunstan *et al*., 1994) but lower than *Nitzschia* spp. (Renaud *et al*., 1999). In this study, *Thalassiosira* sp. had the percentages of AA (0.08-0.12%) which were in the same range of *T. stellaris* CS-16 but lower than *T. nitzschioides* CS-146 and *T. heteromorpha* CS-132-8 (Dunstan *et al*., 1994) while *T. pseudonana* were deficient in AA. (Thompson *et al*., 1992, 1996). Both EPA (C20:5 n-3) and DHA (C22:6n-3) content of *Nitzschia* cf. *ovalis* were in the same range of those previously reported for *N. closterium* CS-5 (Dunstan *et al*., 1994) but higher than those reported for *Nitzschia* spp. (Renaud *et al*., 1999). *Thalassiosira* sp. were in the same amount of EPA and DHA of *T. heteromorpha* CS-132-8 but lower than those *T. nitzschioides* CS-146 (Dunstan *et al*., 1994.).

The Prasinophyceae, *Tetraselmis* sp. had major amounts of fatty acids as C18:3n-3; C16:0, C18:1n-9, C18:2n-6, moderate amounts of EPA (4%) and small amount of AA (1%) at both growth phases which was comparable to previous reports for *T. suecica* (Volkman *et al*., 1989; Dunstan *et al*., 1992; Reitan *et al*., 1997; Renaud *et al*., 1999). However, this microalgae was deficient of DHA which was similar to *T. chui* and *T. suecica* (Brown *et al*., 1997) but contrasts to *Tetraselmis* spp. (Reitan *et al*., 1997; Renaud *et al*., 1999).

The HUFAs content of Chlorophyceae in this study were deficient. The fatty acid profiles of Chlorophyceae in this study were C18:3n-3, C16:0, C18:2n-6. The Chlorophyceae lacked highly unsaturated fatty acids (HUFAs). The fatty acids composition of Chlorlphyceae is rarely reported except for *Chlorella* spp. According to this result, fatty acid composition of marine *Chlorella* sp., cultured at 15 ppt, showed medium to high amounts of C18:3n-3, C18:2n-6, C16:0,C18:1n-9, which was similar to the fatty acid profile of freshwater *Chlorella* sp. (Watanabe *et al*., 1983; Orcutt and Patterson, 1974). However, *Chlorella* sp. in this study was deficient in HUFAs, which was similar to marine *Chlorella* sp. reported by Brown *et al*., 1997. However, *Chlorella* sp. in the present study contrasts to freshwater *Chlorella* sp. which had trace amount of C20:4n-6 and C20:5n-3 (Watanabe *et al*., 1983).

All Cyanophyceae in this study were deficient in HUFA. The fatty acids profiles of Cyanophyceae (blue-green microalgae) fell into two patterns. The priority majority of fatty acids of *Synechococcus* sp. and *Synchocystis* sp. were high in percentage of C16:1n-7, C14:0, C16:0, but *Anacystis* sp. was high and moderate amount of C16:0, C18:3n-3, C16-1n-7. Very few lipid class and fatty acids composition of blue-green microalgae have been examined and reported (Orcutt *et al*., 1986). High levels of fatty acid C16:0 and low levels of polyunsaturated fatty acids are common in bacteria and blue-green microalgae, especially unicellular blue-greens (Blumer *et al*., 1969; Piorreck *et al*., 1984; Orcutt *et al*., 1986 cited

Schnelder *et al*., 1970).

According to the results, most of the microalgae investigated in this study were similar in fatty acids profile, but differed in amount of fatty acids level, at the exponential phase and the stationary phase. The biochemical composition of cells in the exponential phase may differ from those in the stationary phase (Borowitzka, 1988). The fatty acids content of microalgae differed according to taxonomic group and the growth conditions (Brown, 2002; Brown, 2002 cited Harrison *et al*., 1990). In the present study, diatoms contained more fatty acids than those green microalgae and blue green microalgae at both the exponential phase and at the stationary phase because the structure of cell diatoms accumulate lipids but green microalgae and blue green microalgae do not accumulate lipids (Hoek, Main and Jahns, 1995; Wongrat, 1995). Moreover, almost microalgae in this study at the stationary phase had a high amounts of fatty acids than in the exponential phase. Because of the nutritional limitation, cell division gradually decreased and the cells began to store products. Microalgae species accumulated lipids (diatoms), the quantitative content of n-3 polyunsaturated fatty acids increased with increasing nutrient limitation. The species that did not accumulate lipids (*Tetraselmis* spp.) probably accumulated photosynthetic products in the form of carbohydrates (Brown, 2002).

Survival rate and growth rate of aquatic animals are related with the fatty acids content of their feeds. (Tamtin *et al*., 2004; Chomrung, 2000; Anaman *et al*., 2000; Sargent, McEvoy and Bell, 1997). Since microalgae can synthesize fatty acids but aquatic animals must consume directly from feeds because they lack Δ-5 desaturase activity to synthesize C18:2n-6 to C20:4n-6, which has an essential function in producing eicosanoids (Sargent, McEvoy and Bell,1997). In terms of the procession of all three HUFAs, C20:4:n-6, C20: 5n-3 and C22:6n-3, in this study, *Nitzschia* cf. *ovalis* and *Thalassiosira* sp., would serve as good nutritional sources of these HUFAs for aquaculture animals.

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