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FATTY ACID PROFILE AND NUTRITION VALUES OF THE MICROALGA (*THALASSIOSIRA PSEUDONANA*) USED IN WHITE SHRIMP CULTURE

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ABSTRACT

The microalga *Thalassiosira pseudonana* is an important feed item in the aquaculture industry, especially for white leg shrimp production. This study examined the fatty acid composition and other chemical components of *T. pseudonana* cultivated as biomass for the production of shrimp seedlings with the aim to evaluate its nutritional values. For the investigation, the microalga was grown under optimal conditions and harvested during log and early stationary phase after 6–8 days of cultivation. Chemical analysis showed that the main organic components of the biomass were lipid (20.8 % of dry cell weight), protein (13.2 %), and carbohydrate (10.0 %). GC-MS analysis showed that the lipid fraction consisted of 42.5 % SFAs (hexadecanoic acid 38.1 % of total fatty acid), 15.0 % MUFAs, and 42.4 % PUFAs (EPA 16.4 % and DHA 1.7 %). Moreover, the *T. pseudonana* biomass was found to contain a large variety of minerals and micronutrients (mg/kg), such as I (55.10), Fe (222.74), Cu (1.96), Mn (3.11), Mg (2.52), Ca (0.97), and Co (< 0.10), while boron was undetectable. Importantly, the contents of heavy metals, such as Cd, Pb, As, and Hg were lower than required levels for aquaculture feed by Vietnamese standards. Thus, *T. pseudonana* is a quality feed with high nutritional values for aquaculture.

Keywords: fatty acid profile, lipid, microalga *Thalassiosira pseudonana*.

1. INTRODUCTION

Microalgae are rich in nutrition as protein, lipid, carbohydrate, vitamins, mineral salts, pigments and natural bioactive compound. For long time, algal biomass can be used as material for functional food, aquaculture feed, for medicine, pharmaceutics, biodiesel, bio-oil, biofertilizer, biological carbon sequestration and sewage treatment. Microalgae are considered as valuable primary food sources and utilized in aquaculture. Marine microalgae such as *Thalassiosira* genus have received increasing interest as a suitable nutritional diets used in aquaculture due to its suitable size (2-6 μm), easy digestibility, rich nutritional value and precious polyunsaturated fatty acids (PUFAs) contents, such as docosahexaenoic acid (DHA,

22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3). These PUFAs belonging to n-3 are essential for growth and development of animals and human [1, 2]. The feeding of DHA and EPA improves growth and feeding efficiency, while EPA alone is less effective in preventing high mortality and poor growth. Both EPA and DHA are particularly found in taxa belonging to the super group of Chromalveolata such as diatoms, dinoflagellates and prymnesiophytes [3, 4]. *Thalassiosira pseudonana* Cleve 1873 is a species of marine centric diatoms and rich in PUFAs and grows well in mass cultures, either indoors or outdoors. Microalga *T. pseudonana* is an important feed item in the aquaculture industry nowadays and its biomass is essential for shrimp seeding production at Vietnam. However, the information of fatty acids compositions and nutrition values of this microalga is still lacking. In this study we aim to fulfill this gap. In this paper, the fatty acids composition and nutrient of *T. pseudonana* was examined. Obtained results in this present supply scientific basic for using this microalga as feed for aquatic animals.

2. MATERIALS AND METHODS

2.1. Microalga cultures and sample preparation

Microalga *Thalassiosira pseudonana* Cleve 1873 with size range 4.9 ± 0.2 - 6.5 ± 0.2 µm were selected from algal culture collection of Plankton Laboratory of C.P Joint Stock Company which was located at Bac Hoa Village, Ngu Thuy Bac Commune, Le Thuy District, Quang Binh Province. All experiments of *T. pseudonana* were cultured by composite tank of 1 m^3. The optimal culture conditions for *T. pseudonana* were the salinity of 30 - 31 ‰; pH 7.5 - 8.5; at temperature 25 – 27 ^oC; light intensity of 5.5 klux with light: dark cycle as 2:12 h; alkalinity 150 - 180 ppm; media of AGP 20 %; initial cell density 0.2 × 10^6 cells/ml; 24 hour aeration mode. Microalga was harvested at the exponential and early stationary phase after 6 - 8 days of cultivation by centrifugation at 3,000 rpm at – 4 ^oC for 20 min and was frozen at - 80 ^oC until for biochemical analysis.

2.2. Lipid extraction

Analysis of total lipid content using a Soxhlet apparatus was determined as described in the report of Bligh and Dyer [5] with some modification for suitable for laboratory condition at Vietnam. The cells were harvested by centrifugation at 3,000 rpm for 10 min, with the supernatant discarded. Harvested cells were washed with culture medium (15 mL) and extracted into 100 mL chloroform ⁄ methanol (2:1, v⁄v) at room temperature. The lipid extract was dried over anhydrous Na_2SO_4; then, the solvent was removed by evaporation. Afterward, the total lipid was weighed.

2.3. Fatty acids analysis

The fatty acid composition and content of the samples was determined via gas chromatography. The samples were run on a gas chromatography HP-6890 system (Hewlett-Packard, Palo Alto, CA) equipped with a Mass selective detector Agilent 5973. Separation was performed on a 0.25 µm × 0.25 mm × 30 m fused silica capillary column (film 0.32 µm) HP-5MS with helium as carrier gas (flow rate - 3.5 ml/min). The column temperature was programmed at an initial temperature of 80 ^oC for 1 min; raised to 150 ^oC at 4 ^oC/min; raised to 260 ^oC at 10 ^oC/min, maintained for 10 min. Library of mass spectrophotometer WILEY275.l and NIST98.L was carried out as described by Dang Diem Hong *et al*. [6]. The fatty acids were

identified by comparing the retention times with those of standard fatty acids and quantified by comparing their peak area with that of the internal standard.

2.4. Determination of chlorophyll content

Chlorophyll content was analyzed as described in the report of Lichtenthale *et al.* [7] using 80 % acetone as the following:

Step 1: 5 mL of algae broth solution was filtered using filter paper of GR/C. The filter paper contained algae biomass was grinded with porcelain mortar and pestle added grass sands (to break down the cell wall) until getting the smooth mix.

- Step 2: Adding 10 ml of 80 % acetone were grounded in a mortar and pestle. Transfer this mix to tubes enveloped by silver foil.

- Step 3: Repeat the second step for washing porcelain mortar and pestle. Keep the samples at least for 2 hours in the cold.

- Step 4: The mixture was filtered using filter paper after extraction or centrifuged at 10,000 rpm at 4 ^{o}C for 10 min to get the supernatant. Then pour this solution to 10 mL using 80 % acetone solution and put it on the other penicillin's bottle.

Note that all procedure of the extraction was carried out in the dark and cold.

Use the supernatant and measured the absorbance (OD) in wavelength at 664 nm, 663 nm and 470nm using spectrophotometer. Write the OD_{646nm}, OD_{663nm} and OD_{470nm}.

- Step 5: Determination of chlorophyll and carotenoid content using OD values obtained were carried out as following:

The concentration of photosynthetic pigments was calculated using the following equations [9]:

$$C_a = 12.21\ A_{663} - 2.81\ A_{646}\ (\mu g/ml)$$

$$C_b = 20.13\ A_{646} - 5.03\ A_{663}\ (\mu g/ml)$$

$$C_{x+c} = (1000\ A_{470} - 3.27\ C_a - 104\ C_b)/198\ (\mu g/ml).$$

in which: C_a is chlorophyll a content, C_b is chlorophyll b content; C_{x+c} is carotenoid content; A_{663} is OD at 663 nm; A_{646} is OD at 663 nm; A_{470} is OD at 470 nm.

2.5. Chemical analysis

Total protein content was determined by total nitrogen multiplication with coefficient of 6.25. Total nitrogen (%) and phosphor (%), fiber (%), carbohydrate, ash and moist were determined by the analysis method of AOAC 2000.

The content of macro elements in *T. pseudonana* such as Na, K, Mg, and Ca were analyzed using an atomic absorption spectrophotometer developed by Jarrell-Ash (AA-IEWT, Kyoto, Japan). The content of microelements in *T. pseudonana*, such as Fe, Mn, Co, Zn, Cu, Mo, Bo, Pb, Cd, Cr, Sr, As, Hg, was analyzed through the use of atomic absorption spectrophotometers. The content of I and B was analyzed by the color comparison method using ultraviolet visible, UV-160 IPC (Shimadzu, Kyoto, Japan). The organic and inorganic contents of microalga were analyzed as previously described by Hong and Hien *et al.*[8] and Horwitz *et al.* [9].

2.6. Data control and data recovery

The research data obtained were submitted to a variance analysis and Tukey's test to identify differences between the mean values of each treatment.

3. RESULTS AND DISCUSSION

3.1. The nutrition values of *T. pseudonana* biomass

The nutrition values of *T. pseudonana* biomass are show in Table 1.

*Table 1.*The nutrition values of microalga *T. pseudonana.*

Nutrition values	% of dry cell weight (DCW)
Lipid	20.8 ± 0.24
Protein	13.2 ± 0.01
Fibre	0.64 ± 0.00
Total nitrogen	0.04 ± 0.00
Total phosphor	0.06 ± 0.00
Ash	77.6 ± 0.80
Carbohydrate	10.0 ± 0.12
Chlorophyll a	1.01 ± 0.13
Carotenoids	0.15 ± 0.01

The result in Table 1 shows that the protein, fiber, total nitrogen, total phosphor, ash, carbohydrate, chlorophyll a, carotenoids and lipid components was reach up 13.20 ± 0.01 %, 0.64 ± 0.00 %, 0.04 ± 0.00 %, 0.06 ± 0.00 %, 77.55 ± 0.80 %, 9.96 ± 0.12 %, 1.01 ± 0.13 %, 0.15 ± 0.01 %, 20.79 ± 0.24 % of DCW, respectively. Microalgae were found to varyin their proportions of protein (6 - 52 %), carbohydrate (5 - 23 %) and lipid (7 - 23 %) [10]. Mata *et al.* [11] reported total lipid content per dry mass (TLDM) values of 20.6 %. Ohse *et al.* [12] reported a TLDM value of 24.67 % was higher than that in this present (20.70 % of DCW). Differences in microalgal chemical composition including lipid content and fatty acid profiles may be caused by different species. In addition, even in the same species when the culture conditions are different then its biochemical components also differ [13].

3.2. Fatty acids composition of the lipid fraction of *T. pseudonana*

The fatty acid compositions of microalga *T. pseudonana* at the exponential phase are showed in Table 2.

Many reports confirmed that survival and growth rate of aquatic animals are related with the fatty acids content of their feeds [14, 15, 16].

The result in Table 2 shows that the major fatty acids of *T. pseudonana* were C16:0 (38.06 % of total fatty acid - TFA), C16:1n - 7 (6.15 %), C16:1n - 9 (4.74 %), C16:2n - 4 (12.85 %), C16:3n - 3 (8.87 %), C18:1n - 7 (1.37 %), C18:1n - 9 (2.06 %), C20:1n - 7 (0.68 %), C20:5n - 3

(16.42 %) and C22:6n - 3 (1.65 %). The fatty acid content of this microalga was similar in report of Pratoomyot *et al.* [16].

Table 2. Fatty acids composition of microalga *T. pseudonana.*

Fatty acid	Science name	Fatty acid composition (compared to TFA)
Saturated fatty acids (SFA)		
14:0	Tetradecanoic acid	1.06 ± 0.01
15:0	Pentadecanoic acid	1.07 ± 0.01
16:0	Hexadecanoic acid	38.1 ± 0.03
17:0	Heptadecanoic acid	0.72 ± 0.01
18:0	Octadecanoic acid	1.31 ± 0.02
19:0	Nonadecanoic acid	0.31 ± 0.01
Total SFAs		42.5 ± 0.08
Monounsaturated fatty acids (MUFA)		
16:1n – 9	Hexadecenoic acid	4.74 ± 0.01
16:1n – 7	9-Hexadecenoic acid	6.15 ± 0.01
18:1n – 9	Octadecenoic acid	2.06 ± 0.03
18:1n – 7	Octadecenoic acid	1.37 ± 0.01
20:1n – 7	13-Eicosenoic acid	0.68 ± 0.01
Total MUFAs		14.99 ± 0.06
Polyunsaturated fatty acids (PUFA)		
16:3n – 3	Hexadecatrienoic acid	8.87 ± 0.01
16:2n – 4	Hexadecadienoic acid	12.85 ± 0.02
18:3n – 6	Octadecatrienoic acid	0.61 ± 0.01
18:2n – 6	Octadecenoic acid	1.16 ± 0.03
20:4n – 6	Arachidonic acid (AA)	0.1 ± 0.00
20:5n – 3	5.8.11.14.17–Eicosapentaenoic acid (EPA)	16.42 ± 0.06
22:4n – 6	Docosatetraenoic acid	0.73 ± 0.02
22:6n – 3	Docosahexaenoic acid (DHA)	1.65 ± 0.01
Total PUFAs		42.44 ± 0.16
Total HUFAs		18.94 ± 0.09
Others		0.02 ± 0.01

The use of *T. pseudonana* biomass provides sufficient and essential fatty acids for aquaculture, especially for larvae of shrimp which was reported [14, 15]. *T. pseudonana* biomass

contained high total fatty acids offered nutritional feed for larval of white leg shrimp. According to report of Brown [2] indicated that the fatty acids of *T. pseudonana* pay the important role in helping for larval of white leg shrimp having high growth rates, faster transferring between stages and achieving good quality. In addition, the role of C14: 0 and C16: 0 fatty acids and their implications for the development of shrimp larvae were also highlighted in report of Brown [2]. The diets with higher percentages of the saturated fats were more beneficial for larvae growing rapidly, because energy is released more efficiently from saturated fats than unsaturated fats.

Thalassiosira pseudonana biomass cultured in our conditions had total SFAs (42.5 % included in C16: 0 - 38.1 % of TFA); MUFAs (15.0 %) and PUFAs (42.4 %) which was higher than that in report of Pratoomyot *et al.*[16] but lower than in paper published by Widiangsih *et al.* [17] and Tonon *et al.* [3]. However, it must be emphasized that the publication of Widiangsih *et al.* (2013) did not detect DHA in the microalgal fatty acid composition. In contrast, Lang *et al.* [18] could not determine EPA as well as DHA in a *T. weissflogii* strain isolated from a brackish habitat. The above mentioned differences may be due to differences in genetic characteristics of strain/species as well as differences in the timing of microalgal collection for analysis and in experimental conditions [19]. Based on above analysis, it could be concluded that *T. pseudonana* are suitable as a quality live feed for aquaculture. Microalga *T. pseudonana* could serve as good nutritional sources of PUFAs for aquaculture feed.

3.3. Minerals and micronutrients composition of microalga *T. pseudonana*

The minerals and micronutrients composition of microalga *T. pseudonana* at the early stationary phase are showed in Table 3.

Table 3. Minerals and micronutrients composition of microalga *T. pseudonana.*

Parameters	*T. pseudonana*	Parameters	*T. pseudonana*
K (mg/kg)	0.93	Cr (mg/kg)	0.89
Na (mg/kg)	27.80	Se (mg/kg)	< 0.10
Mg (mg/kg)	2.52	B (mg/kg)	KPH
Ca (mg/kg)	0.97	I (mg/kg)	55.10
Co (mg/kg)	< 0.10	Pb (mg/kg)	0.47
Mn (mg/kg)	3.11	Cd (mg/kg)	0.28
Fe (mg/kg)	222.74	As (mg/kg)	0.38
Cu (mg/kg)	1.96	Hg (mg/kg)	< 0.05
Zn (mg/kg)	4.75	Mo (mg/kg)	77.55

The data shown in Table 3 indicate that the *T. pseudonana* biomass contains a large variety of minerals and micronutrients (mg/kg) such as Na (27.80), K (0.93), I (55.10), Fe (222.74), Cu (1.96), Mn (3.11), Mg (2.52), Ca (0.97), Co (< 0.10) and undetectable B. Importantly, concentrations of heavy metals such as Cd, Pb, As and Hg met ranked among acceptable levels required for aquaculture feed with the content (mg/kg) of Pb (0.47), Cd (0.28), As (0.38) and Hg (< 0.05).

4. CONCLUSIONS

This study found that:

The microalgal species *T. pseudonana* under our experimental condition exhibited high lipid content (20.8 % of dry cell weight), high levels of saturated fatty acids (42.5 % of total fatty acids with hexadecanoic acid – 38.0 %) and polyunsaturated fatty acids (42.4 % with EPA - 16.4 % and DHA - 1.6 %), and low levels of monounsaturated fatty acids (15.0 %).

Besides lipid, it contained 13.2 and 10.0 % protein and carbohydrate, respectively.

Thalassiosira pseudonana biomass contained a large variety of minerals and micronutrients (mg/kg) such as I (55.10), Fe (222.74), Cu (1.96), Mn (3.11), Mg (2.52), Ca (0.97), and Co (< 0.10), while boron was undetectable. Importantly, the contents of heavy metals, such as Cd, Pb, As, and Hg were lower than required levels of Vietnamese standards for aquaculture feed.

Thalassiosira pseudonana has high nutritional values and can be used this biomass as quality feed for aquaculture.

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