Total Lipids Content, Lipid Class and Fatty Acid Composition of Ten Species of Microalgae

Yuhong Yang¹, Lei Du²*, Masashi Hosokawa³, and Kazuo Miyashita³,⁴

¹ School of Food Science & Engineering, Qilu University of Technology (Shandong Academy of Sciences), No.3501, Daxue Road, Jinan, 250353, CHINA
² Department of Nutrition and Food Hygiene, School of Public Health, Cheeloo College of Medicine, Shandong University, No.44 Wenhuxi Road, Jinan, Shandong, 250012, CHINA
³ Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate, Hokkaido, 041-8611, JAPAN
⁴ Center for Regional Collaboration in Research and Education, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido, 080-8555, JAPAN

Abstract: Microalgae is a potential producer of functional lipids such as n-3 polyunsaturated fatty acids (PUFA) and fucoxanthin. In the present study, lipids from ten microalgal species were analyzed especially focusing on the fucoxanthin, lipid and fatty acid compositions. The study revealed a remarkable variation in total lipids content, fucoxanthin content, lipid class composition and n-3 PUFA content in individual species, although they belong to the same genus. Among microalgae examined, Pavlova lutheri contained the highest total lipids content (313.59 mg g⁻¹ dry weight) and considerable amount of fucoxanthin (3.13 mg g⁻¹ dry weight). It also had the highest level (28.01%) of total n-3 PUFA with high level of eicosapentaenoic acid (EPA) (17.76%) and docosahexaenoic acid (DHA) (7.61%). The highest fucoxanthin content (5.19 mg g⁻¹ dry weight) was observed in Chaetoceros gracilis. C. gracilis also contained relatively high level of total lipids (228.87 mg g⁻¹ dry weight) and 10.67% EPA. The results also demonstrated that Nannochloropsis oculata contained the greatest amount of EPA (26.21%), while Isochrysis galbana had the highest level of DHA (8.76%). And both microalgae contained 1.71 and 4.44 mg g⁻¹ dry weight fucoxanthin, respectively. Microalgal lipids containing abundant fucoxanthin and n-3 PUFA such as EPA and DHA in the present study will be used as nutraceutical lipids with great commercial potential.

Key words: microalgae, total lipids, fucoxanthin, lipid class, fatty acid

1 Introduction

Microalgae is an evolutionary diverse group of unicellular and predominantly aquatic photosynthetic organisms with over 3000 different strains³. Compared to other photosynthetic organisms such as terrestrial plant, microalgae have unique advantages². Microalgae can achieve rapid proliferation within several hours¹ and show efficient ability to convert CO₂ and light into biomass and high-energy lipids, precursors of biofuels³. The phototrophic efficiency of microalgae has been reported to be 10-50 times higher than that of terrestrial plants⁴. In addition, microalgae do not compete with food crop production or available farmland. They can be cultivated on non-arable land with wastewater or reject water, saline/brackish water and even sewage as a medium⁵,⁶.

Microalgae have a great potential as a reliable and sustainable feedstock for production of biofuel and diverse valuable bioresources⁶,⁷. Microalgae produce a wide range of valuable compounds such as polysaccharides, lipids, proteins, enzymes, vitamins and pigments⁸,⁹. During the process of photosynthesis, microalgae can store nonpolar lipids like triacylglycerol (TAG) in the cells⁹. Especially, oleaginous microalgae sometimes produce intracellular oils above 20% of their dry weight that can be converted into biodiesel⁹. Some of them have the ability to accumulate

Abbreviations: AA, arachidonic acid; DGDG, digalactosyl diacylglycerol; DW, dry weight; FAME, fatty acid methyl esters; FFA, free fatty acids; GC, gas chromatography; GL, glycolipids; GLA, gamma-linolenic acid; HPLC, high performance liquid chromatography; LA, linoleic acid; MGDG, monogalactosyl diacylglycerol; NL, neutral lipids; PUFA, polyunsaturated fatty acids; PL, phospholipids; SQDG, sulfoquinovosyl diacylglycerol; TAG, triacylglycerol; TLC, thin-layer chromatography; UN, unknown lipids.

*Correspondence to: Lei Du, Department of Nutrition and Food Hygiene, School of Public Health, Cheeloo College of Medicine, Shandong University, No.44 Wenhuxi Road, Jinan, Shandong, 250012, CHINA
E-mail: dulei@sdu.edu.cn
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considerable amounts (20-50% per dry cell weight) of TAG under adverse environmental conditions. The biomass and lipid productivity even vary for the same species depending on the growing conditions such as light, temperature, nitrogen level, salt stress and the growth stages at which they are harvested. When microalgae are subjected to these stress conditions, they can produce large amounts of lipids up to 85% \(^{12}\). However, wild-type microalgae under environmental conditions are usually not capable of producing high amount of TAG and biodiesel obtained from microalgae is still not economically sustainable since its production price is still not competitive compared with fossil fuel price. \(^{9}\)

Lipids can be categorized as neutral lipids (NL) and polar lipids. The NL in microalgae, which are primarily referred as TAG and free fatty acids (FFA), are great potential as a source of biofuel substitute. Microalgae also comprise high level of polar lipids, such as glycolipids (GL) and phospholipids (PL), which are the major components of chloroplast lipids in microalgae. They function as membrane structural lipids and signaling molecules in the cells. The algal GL, including monogalactosyl diacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyl diacylglycerol (SQDG), are known to have various biological activities such as antiviral, antitumor and anti-inflammatory effects. \(^{14}\) The PL are also known to show various beneficial health effects such as improvement of cognition, \(^{15}\) metabolic disorders, \(^{16}\) and prevention against oxidative stress. \(^{17}\) Certain PL are applied in the pharmaceutical and nutritional fields to improve human health. \(^{18, 19}\) Moreover, being amphiphilic molecules and natural surfactants, PL are also used as food additives to improve baking, enhance wetting, reduce viscosity, stabilize margarine and prevent crystallization in chocolate. \(^{20, 21}\)

Among lipid components produced by microalgae, much attention has been paid to n-3 polyunsaturated fatty acids (PUFA) and carotenoids. \(^{12, 22, 23}\) A mixture of C16 and C18 saturated and unsaturated fatty acids are known as dominant fatty acids of microalgae lipids, whereas longer carbon-chain PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are also found as major fatty acids. \(^{12, 24, 25}\) Based on epidemiological studies, randomized controlled trials, and laboratory studies, EPA and DHA have been shown to exhibit several kinds of important biological roles, such as promotion the development of the neural system and prevention of cardiovascular disease. \(^{27}\) Hence, a large number of global and national health agencies and associations, and government bodies recommended the intake of EPA and DHA for a healthy human diet. \(^{25}\)

Although fish oil is known as the most common source of EPA and DHA, the interest has been also focused on microalgae because of the reduction of marine animal supplies and the increased demand for EPA and DHA. \(^{25}\) Fatty acid composition, including EPA and DHA content, of microalgae varies with the species. Thus, more data is required on the EPA and DHA content of various kinds of microalgae.

Carotenoids are a group of fat-soluble pigments responsible for the red and yellow colors of natural products. They can show many kinds of benefits for human health, which are usually related to their antioxidant activities. \(^{28, 29}\) Microalgae have also the capacity to produce carotenoids. They include the most well-known \(\beta\)-carotene and lutein found in terrestrial plants together with a variety of specific carotenoids to only found in algae, cyanobacteria, and several species of yeast. \(^{30}\) Among these carotenoids, fucoxanthin is known to reduce the risk of a surprisingly wide variety of dysfunctions and diseases, including metabolic syndrome, obesity, heart disease, diabetes, cancer, hypertension, as well as reactive oxygen species- and inflammation-associated disorders. \(^{31}\) Brown seaweeds are possible source of fucoxanthin, but the content is not high ranging up-to 0.7 mg g\(^{-1}\) dry weight. \(^{32}\) On the other hand, fucoxanthin was also found in several kinds of microalgae such as Phaeodactylum tricornutum, Isochrysis galbana, Cylindrotheca closterium, Tisochrysis lutea, and Odontella aurita, \(^{28, 33, 34}\) showing the possibility of the use of microalgae as fucoxanthin source.

The objective of this study was to evaluate the lipid content and lipid composition of ten species microalgae, with special attention to the level of n-3 PUFA and fucoxanthin. The results obtained will be useful in the application of functional lipids from microalgae.

### 2 Experimental Procedures

#### 2.1 Microalgae sample

*Chaetoceros ceratoporum*(C. ceratoporum, CC), *Chaetoceros gracilis*(C. gracilis, CG), *Chaetoceros species-1*(C. species-1, CS1), *Chaetoceros species-2*(C. species-2, CS2), *Chaetoceros species-3*(C. species-3, CS3), *Isochrysis galbana*(I. galbana, 1G), *Nannochloropsis oculata*(N. oculata, NO), *Pavlova lutheri*(P. lutheri, PL), and *Spirulina*(Arthrospira platensis) were provided by DIC Corporation (Tokyo, Japan). Phaeodactylum tricornutum No. 1055/1 (P. tricornutum, PT) was obtained from The Culture Collection of Algae and Protozoa (Scotland, UK). *P. tricornutum* was cultivated at large scale (finally 70 L scale of cultivation), whereas other microalgae were cultivated at a small scale (finally 0.7 L scale of cultivation).

F/2 medium containing NaNO\(_3\), Na\(_2\)SiO\(_3\)·9H\(_2\)O and NaH\(_2\)PO\(_4\) as macronutrient was used for the cultivation. The medium (0.1 L) was put in an erlenmeyer flask (0.2 L). After autoclaving the medium, the alga was inoculated to medium, and then cultured for 10 days at 30°C under 1% \(\text{CO}_2\) atmosphere. The cultivation was done by shaking the flask at 120 rpm with a 12 h light/12 h dark cycle. After the
small-scale cultivation, 5 mL cultures were added to 0.7 L F/2 medium and grown in a 1 L flask by aeration of 1% CO₂. After 11 days’ incubation, the final solution was further concentrated with high-speed centrifuge and the residue was lyophilized.

In case of *P. tricornutum*, after the 11 days’ incubation, all the cultures were mixed with 10 L F/2 medium and grown at 20°C by aeration of 1% CO₂ and agitation at 300 rpm under constant fluorescent light for 9 days. Finally, all the cultures containing 25 mg alga was mixed with 70 L F/2 medium in acryl tank and grown at 20°C by aeration of 1% CO₂ under constant fluorescent light for 8 days. The photon flux density changed from 70 mol m⁻² s⁻¹ (day 0 to day 2), 140 mol m⁻² s⁻¹ (day 3 to day 4) and finally to 210 mol m⁻² s⁻¹ (day 5 to day 8). To monitor the growth rate of alga in the final cultivation, a small amount of culture medium was taken to measure the optical density at 750 nm every two days. The time-course measurement showed that the alga growth reached to stationary phase after 8 days’ cultivation. At day 8, alga weight was 685 mg L⁻¹. The final solution was concentrated and lyophilized to obtain 33 g dried sample.

2.2 Lipid extraction

Total lipids of microalgae were extracted according to the modified method of Folch et al.⁵⁰. Briefly, microalgae (ca. 2 g) was extracted with chloroform/methanol (ca. 0.2 L, 2:1, v/v) and allowed to stand overnight at room temperature in the dark. The extract was filtered and mixed with chloroform and distilled water to obtain a final concentration of 1:1:0.9 of chloroform/methanol/water (v/v/v). The solution was placed into a separatory funnel and allowed the funnel to stand overnight. The lower layer containing the lipid extract was evaporated under reduced pressure in a rotary evaporator. The last traces of organic solvent and water were removed in a desiccator under high vacuum to obtain total lipids. After weighing the total lipids, it was re-suspended in chloroform/methanol (1:1, v/v), and finally stored at −30°C under nitrogen gas to prevent lipid oxidation.

2.3 Analysis of fucoxanthin content

The fucoxanthin content in total lipids was analyzed by high-performance liquid chromatography (HPLC). The HPLC analysis was carried out using a Hitachi L-7000 HPLC System (Tokyo, Japan) equipped with a pump (L-7100), an autosampler (L-7200), a photodiode array detector (L-7455) and online analysis software (Model D-7000). The analysis was carried out at 25°C using a reversed-phase column (Develosil-ODS UG-5, 250×4.6 mm i.d., 5.0 μm particle size; Nomura Chem. Co., Seto, Aichi, Japan) protected with a guard column with the same stationary phase. The mobile phase was methanol/acetonitrile (70:30, v/v) and the flow rate was 1.0 mL/min. Fucoxanthin was detected at 450 nm and its content was estimated using a standard calibration curve for purified fucoxanthin (purity >98%). The purified fucoxanthin was isolated from the brown seaweed *Undaria pinnatifida* as described previously²⁴.

2.4 Lipid class analysis

Total lipids obtained from each microalga were subjected to one-dimensional thin-layer chromatography (TLC) using plates coated with silica gel 60 F254 (Merck, Darmstadt, Germany) for identification of carotenoids and lipid class. The solvents were *n*-hexane/acetonitrile (70:30, v/v) for carotenoids, *n*-hexane/diethyl ether/acetic acid (70:30:1, v/v/v) for NL and chloroform/methanol/water (65:25:4, v/v/v) for both GL and PL. The lipid class was identified by co-chromatography with pure standards (Sigma-Aldrich, St. Louis, MO, USA). NL were stained with 50% H₂SO₄. GL with Bial’s reagent (consists of 0.4 g orcinol, 200 mL of concentrated hydrochloric acid and 0.5 mL of 10% solution of ferric chloride) and PL with Dittmer reagent (molybdenum oxide/molybdenum in H₂SO₄). The stained band intensity of individual lipid class was quantified using Image J software (National Institutes of Health, Bethesda, MA, USA).

2.5 Analysis of fatty acid composition

After visualization and identification on TLC, each lipid band corresponding to different kind of lipid class was immediately and carefully scraped out, and the fatty acids were analyzed by gas chromatography (GC) after the conversion of fatty acyl groups in the lipid to fatty acid methyl esters (FAME), according to the methods of our previous study.³⁷ Briefly, 1 mL of *n*-hexane and 0.2 mL of 2 N NaOH in methanol were added to lipids (ca. 10 mg), vortexed and incubated at 50°C for 20 to 30 second. After incubation, 0.2 mL of 2 N HCl in methanol solution was added to the solution and vortexed. The mixture was separated by centrifugation at 3000 rpm for 5 min. The upper *n*-hexane layer containing FAME was recovered and subjected to GC analysis. The GC analysis was performed using a Shimadzu GC-14B (Shimadzu Seisakusho, Kyoto, Japan) equipped with a flame-ionization detector and a capillary column (Omegawax-320; 30 m × 0.32 mm i.d.; Supelco Inc., Bellefonte, PA, USA). The detector, injector and column temperatures were 260°C, 250°C and 200°C, respectively. The carrier gas was helium at a flow rate of 50 KPa. The peaks were identified by comparisons with the retention of FAME for different fatty acid standards (Nu-Check-Prep, Inc., Elysian, MN, USA) and were quantified using an online integrator (Shimadzu Chromatopack C-R8A, Kyoto, Japan). The fatty acid contents are expressed as weight percentages of the total fatty acids by comparing the retention times with a standard fatty acid mix. Heptadecanoic acid (C17:0) was used as an internal standard (Supelco Inc., Bellefonte, PA, USA).
2.6 Statistical analyses

Values in the present study represent the mean ± standard error of three independent measurements.

3 Results and Discussion

3.1 Total lipids and fucoxanthin contents

Total lipids content of individual microalgae is presented in Table 1. Higher amounts of total lipids could be extracted from P. lutheri, I. galbana, N. oculata and C. gracilis, which accounted for 313.59 mg g⁻¹ DW, 286.83 mg g⁻¹ DW, 266.07 mg g⁻¹ DW and 228.87 mg g⁻¹ DW, respectively. On the other hand, the levels of total lipids in C. species-2, P. tricornutum and C. species-3 were less than 100 mg g⁻¹ DW. The low level of total lipids extracted from P. tricornutum was inconsistent with the result of a previous study⁴⁰, and the total lipids content of I. galbana found in this study was higher than that described by Brown et al.⁴⁰. Furthermore, the lipid content of P. tricornutum and Chaetoceros species (C. ceratosporum, C. gracilis, C. species-1, C. species-2 and C. species-3) showed high variability in individual species, although they belong to the same genus, suggesting that lipid content greatly influenced by species and growth conditions such as nutrients, temperature, salinity, and light intensity, as well as interactions among these factors⁴⁰,⁴¹.

Fucoxanthin is a photosynthetic pigment mainly found in brown seaweed and some kinds of microalgae. It has a distinctive structure with an allenic bond, a 5, 6-monoepoxide, and nine conjugated double bonds. Fucoxanthin is known to show a unique and wide variety of biological activities, therefore, good sources of this marine carotenoid have been explored⁴¹. Although fucoxanthin can be synthesized chemically, extraction from brown seaweeds and microalgae is a more accessible, safe, and economic method⁴². The results determined by HPLC revealed that the fucoxanthin content greatly varied from microalgae species (Table 1). Among the ten species analyzed, C. gracilis contained the highest amount of fucoxanthin (5.19 mg g⁻¹ DW). Kim et al.⁴³ reported that the fucoxanthin yield obtained by ethanol extraction from P. tricornutum was up to 15.71 mg g⁻¹ freeze-dried weight, which was much higher than that (1.23 mg g⁻¹ DW) obtained from the same species in the present study. The discrepancy may be explained by differences in culture conditions and extraction procedure.

Fucoxanthin has been reported from more than 20 species of brown seaweeds and the contents varied from 0.17 to 0.72 mg g⁻¹ DW⁴²,⁴³. These fucoxanthin levels are much lower than those found in microalgae analyzed in the present study except for C. species-2 (Table 1). Other studies have also reported that fucoxanthin content in microalgae is generally higher than those in brown seaweeds²⁸, ³³, ³⁴. Regardless of species and growth conditions, fucoxanthin content in total lipids is often approximately 5% by weight in brown seaweeds⁴⁰. Therefore, the content generally increases with increasing the total lipids content of brown seaweeds. On the other hand, the fucoxanthin content in total lipids greatly varied from 0.05% to 2.37% by weight in microalgae (Table 1).

3.2 Lipid class composition

The composition of lipid class is presented in Table 2. There was a considerable variation among the different species. Previous studies have also demonstrated that microalgal lipid content and the lipid composition varies considerably one species to another⁴⁵. Among different kinds of lipid class, NL and TAG estimated as 16-41% and 9-31% of total lipids in ten species of microalgae, respectively (Table 2). Microalgae have been regarded as a potential source of biofuel¹³, ⁴⁵. NL, especially TAG, are the best sub-

### Table 1 Total lipids and fucoxanthin contents in ten species of microalgae.

| Species                          | Total lipids content (mg g⁻¹ DW) | Fucoxanthin (mg g⁻¹ DW) |
|----------------------------------|----------------------------------|-------------------------|
| Chaetoceros ceratosporum (CC)    | 198.02 ± 4.20                    | 3.63 ± 0.23             |
| Chaetoceros gracilis (CG)        | 228.87 ± 1.53                    | 5.19 ± 0.38             |
| Chaetoceros species -1 (CS1)     | 178.84 ± 5.70                    | 4.24 ± 0.20             |
| Chaetoceros species -2 (CS2)     | 40.54 ± 0.88                     | 0.02 ± 0.00             |
| Chaetoceros species -3 (CS3)     | 74.06 ± 3.36                     | 1.47 ± 0.14             |
| Isochrysis galbana (IG)          | 286.83 ± 3.81                    | 4.44 ± 0.46             |
| Nannochloropsis oculata (NO)     | 266.07 ± 1.87                    | 1.71 ± 0.14             |
| Pavlova lutheri (PL)             | 313.59 ± 3.57                    | 3.13 ± 0.14             |
| Phaeodactylum tricornutum (PT)   | 52.93 ± 1.62                     | 1.23 ± 0.04             |
| Spirulina                        | 117.82 ± 4.31                    | N.D.                    |

Values are represented as mean ± SE of three independent measurements.
strate to produce biodiesel in this case. TAG serves as energy storage to microalgal cells and the contents can increase by the method based on the manipulation of the nutritional and/or cultivation conditions including exposure to different wavelengths and light intensity, carbon dioxide levels, temperature, available nutrients, stress to heavy metals and salinity. Meng et al. found that the total lipids content in *Nannochloropsis oceanica* reached 59% of its dry weight under nitrogen replete conditions during the cultivation period, while it was 31.2% under nitrogen replete conditions. They also found that the increase in the total lipids was almost derived from the increase in TAG but not in polar lipids. Therefore, the content of NL such as TAG found in Table 2 could be changed by the growth conditions.

As seen from the Table 2, several kinds of GL and PL are also found from the microalgal lipids. These polar lipids have an important role to maintain cell structure. In this study, TLC analysis confirmed the presence of MGDG, DGDG, SQDG, phosphatidylethanolamine (PE), phosphatidylglycerol (PG) in microalgae by comparison with commercial standards. GL formed the major lipid class, being the most abundant in *C. ceratoporum*, *C. gracilis*, *C. species-1*, *C. species-2*, and *Spirulina*. Microalgae except for *C. species-2* and *P. lutheri* contained 19-32% PL as major lipid class. On the other hand, PL was not found in *C. species-2*, which showed very low level of DGDG, and of total lipids and fucoxanthin (Table 1). This is very different from those of other kinds of *Chaetoceros species* (*C. ceratoporum*, *C. gracilis*, *C. species-1* and *C. species-2*). Furthermore, *C. species-2* had an unusually high content of unknown compounds estimated to be 44.25% of total lipids. Most of the unknown compounds were found in polar fractions based on the TLC staining. Further studies are required to identify these unknown compounds. Although identification of *C. species-1*, 2, and 3 could not be done in the present study, the results suggested a big variation of lipid composition of different kinds of microalgal species corresponding to the same genus.

*I. galbana* and *N. oculata* had similar lipid class composition in terms of NL and polar lipids, but the minor amount of MGDG (4.17%) in *I. galbana* was observed. Owing to the exceptionally high level of unknown fractions (50.85%), the TLC analysis showed that *P. lutheri* contained the least amounts of GL (13.26%) and PL (8.41%). On the other hand, Meireles et al. reported that the polar fraction of *P. lutheri* was mainly composed of MGDG. The discrepancy with the present study may reflect species-specificity or culture conditions. *Spirulina* contained the greatest amounts of total GL (51.96%), SQDG (13.25%) and PG (10.9%) among the examined microalgae, but little PC was found from this microalgae. The same result was also obtained by Tropis et al. They reported SQDG

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**Table 2. Lipid class composition in ten species of microalgae.**

| Lipid class | CC (%) of total lipid | Cs1 | CS2 | CS3 | PT | NO | PL |
|-------------|----------------------|-----|-----|-----|----|----|----|
| TAG         | 21.68 ± 0.37         | 10.62 ± 0.15 | 5.43 ± 2.01 | 5.48 ± 4.20 | 10.25 ± 0.18 | 10.25 ± 0.18 | 10.25 ± 0.18 |
| DGDG        | 21.68 ± 0.37         | 10.62 ± 0.15 | 5.43 ± 2.01 | 5.48 ± 4.20 | 10.25 ± 0.18 | 10.25 ± 0.18 | 10.25 ± 0.18 |
| SQDG        | 21.68 ± 0.37         | 10.62 ± 0.15 | 5.43 ± 2.01 | 5.48 ± 4.20 | 10.25 ± 0.18 | 10.25 ± 0.18 | 10.25 ± 0.18 |
| PC          | 21.68 ± 0.37         | 10.62 ± 0.15 | 5.43 ± 2.01 | 5.48 ± 4.20 | 10.25 ± 0.18 | 10.25 ± 0.18 | 10.25 ± 0.18 |
| Unkonwn     | 21.68 ± 0.37         | 10.62 ± 0.15 | 5.43 ± 2.01 | 5.48 ± 4.20 | 10.25 ± 0.18 | 10.25 ± 0.18 | 10.25 ± 0.18 |

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## Table 3  Fatty acid composition of total lipids in ten species of microalgae.

| Fatty acids\(^a\) | CC       | CG       | CS1      | CS2      | CS3      | IG       | NO       | PL       | PT       | Spirulina |
|-------------------|----------|----------|----------|----------|----------|----------|----------|----------|----------|-----------|
| C14:0             | 17.42 ± 0.44 | 16.12 ± 0.76 | 10.77 ± 0.25 | 21.68 ± 0.11 | 12.49 ± 0.16 | 20.95 ± 0.17 | 5.42 ± 0.01 | 14.49 ± 0.08 | 2.50 ± 0.10 | N.D.      |
| C16:0             | 5.10 ± 0.03 | 5.39 ± 0.29 | 5.32 ± 0.08 | 27.33 ± 0.05 | 9.52 ± 0.15 | 6.79 ± 0.04 | 21.86 ± 0.14 | 16.95 ± 0.07 | 11.51 ± 0.14 | 46.66 ± 0.30 |
| C16:1             | 33.33 ± 0.74 | 24.46 ± 1.21 | 17.30 ± 0.13 | 38.09 ± 0.01 | 13.74 ± 0.08 | 1.96 ± 0.01 | 24.52 ± 0.13 | 15.96 ± 0.11 | 25.42 ± 0.06 | 2.31 ± 0.03 |
| C17:1             | 12.83 ± 0.14 | 17.72 ± 0.98 | 22.41 ± 0.25 | N.D.      | 4.03 ± 0.01 | 0.23 ± 0.00 | 0.66 ± 0.00 | 0.42 ± 0.00 | 13.20 ± 0.06 | N.D.      |
| C18:0             | 0.38 ± 0.01 | 0.43 ± 0.03 | 0.18 ± 0.00 | 0.86 ± 0.02 | 2.03 ± 0.08 | 0.28 ± 0.01 | 0.19 ± 0.00 | 0.18 ± 0.01 | 0.33 ± 0.00 | 0.77 ± 0.00 |
| C18:1n-9          | 0.43 ± 0.01 | 0.59 ± 0.03 | 1.11 ± 0.01 | 0.43 ± 0.01 | 1.17 ± 0.05 | 9.34 ± 0.04 | 3.10 ± 0.01 | 0.20 ± 0.00 | 0.35 ± 0.00 | 1.89 ± 0.04 |
| C18:1n-7          | 0.26 ± 0.01 | 0.26 ± 0.01 | 0.27 ± 0.00 | 0.73 ± 0.00 | 8.67 ± 0.33 | 1.67 ± 0.02 | 0.38 ± 0.01 | 3.06 ± 0.03 | 0.57 ± 0.01 | 0.24 ± 0.00 |
| C18:2n-6          | 0.83 ± 0.02 | 1.19 ± 0.06 | 2.06 ± 0.00 | 0.27 ± 0.01 | 1.27 ± 0.03 | 5.86 ± 0.01 | 4.05 ± 0.02 | 0.70 ± 0.01 | 1.67 ± 0.01 | 21.59 ± 0.22 |
| C18:3n-6(GLA)     | 0.25 ± 0.01 | 0.57 ± 0.03 | 1.36 ± 0.01 | 0.43 ± 0.00 | 0.44 ± 0.01 | 11.75 ± 0.04 | 0.25 ± 0.00 | 0.35 ± 0.01 | 0.20 ± 0.00 | 18.54 ± 0.41 |
| C18:3n-3(ALA)     | 0.10 ± 0.00 | 0.10 ± 0.01 | 0.08 ± 0.00 | N.D.      | 7.96 ± 0.39 | N.D.      | N.D.      | 2.64 ± 0.03 | 0.69 ± 0.00 | 0.04 ± 0.00 |
| C20:4n-6(AA)      | 1.58 ± 0.04 | 2.11 ± 0.08 | 5.61 ± 0.13 | 1.08 ± 0.01 | 1.10 ± 0.04 | 0.12 ± 0.04 | 3.74 ± 0.03 | 0.32 ± 0.01 | 0.46 ± 0.01 | N.D.      |
| C20:5n-3(EPA)     | 11.49 ± 0.20 | 10.67 ± 0.45 | 15.95 ± 0.35 | 1.88 ± 0.02 | 3.85 ± 0.09 | 0.12 ± 0.01 | 26.21 ± 0.19 | 17.76 ± 0.42 | 22.63 ± 0.36 | N.D.      |
| C22:6n-3(DHA)     | 1.41 ± 0.07 | 0.78 ± 0.01 | 1.23 ± 0.03 | N.D.      | N.D.      | 8.76 ± 0.07 | 0.52 ± 0.09 | 7.61 ± 0.18 | 1.94 ± 0.04 | N.D.      |
| Unknown           | 14.59 ± 0.61 | 19.61 ± 0.77 | 16.35 ± 0.16 | 7.22 ± 0.22 | 33.73 ± 1.08 | 32.17 ± 0.62 | 9.10 ± 0.20 | 19.36 ± 0.35 | 18.53 ± 0.22 | 7.98 ± 0.31 |
| SFA               | 22.90 ± 0.41 | 21.94 ± 1.08 | 16.27 ± 0.33 | 49.87 ± 0.55 | 24.04 ± 0.60 | 28.02 ± 0.22 | 27.47 ± 0.24 | 31.62 ± 0.34 | 14.34 ± 0.15 | 47.43 ± 0.30 |
| MUFA              | 46.85 ± 0.58 | 43.03 ± 2.24 | 41.09 ± 0.36 | 39.25 ± 0.62 | 27.61 ± 0.47 | 13.20 ± 0.04 | 28.66 ± 0.42 | 19.64 ± 0.12 | 39.54 ± 0.69 | 4.44 ± 0.03 |
| PUFA              | 15.66 ± 0.38 | 15.42 ± 0.62 | 27.29 ± 0.51 | 3.66 ± 0.03 | 14.62 ± 0.54 | 26.61 ± 0.80 | 34.77 ± 0.23 | 29.38 ± 0.37 | 29.53 ± 0.40 | 40.17 ± 0.42 |
| n-3 PUFA          | 13.00 ± 0.31 | 11.55 ± 0.32 | 17.26 ± 0.40 | 1.88 ± 0.02 | 11.81 ± 0.48 | 8.88 ± 0.09 | 26.73 ± 0.37 | 28.01 ± 0.39 | 25.26 ± 0.39 | 0.04 ± 0.00 |
| n-6 PUFA          | 2.66 ± 0.07 | 3.87 ± 0.07 | 9.03 ± 0.11 | 1.78 ± 0.08 | 2.81 ± 0.05 | 17.73 ± 0.28 | 8.04 ± 0.24 | 1.37 ± 0.02 | 2.33 ± 0.02 | 40.13 ± 0.61 |
| n-3/n-6 PUFA      | 4.89 ± 0.04 | 2.98 ± 0.03 | 1.91 ± 0.02 | 1.05 ± 0.06 | 4.20 ± 0.20 | 0.50 ± 0.11 | 3.32 ± 0.13 | 20.44 ± 0.41 | 10.84 ± 0.16 | 0.00 ± 0.00 |
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There have been many studies on the beneficial health effect of GL and PL from natural products including microalgae. Banskota et al. has reported that MGDG from Tetrasselmis chu ish showed strong nitric oxide inhibitory activity in RAW 264.7 macrophage cells. An experimental investigation indicates that MGDG derived from I. galbana could be an emerging therapeutic strategy for the treatment of inflammatory skin pathologies such as psoriasis. Especially, GL and PL derived from microalgae are rich in n-3 PUFA such as EPA and DHA. Judging from the fatty acid analysis in this study, several kinds of microalgae such as C. ceratosporum, C. gracilis, C. species-1, I. galbana, N. oculata, P. lutheri, and P. tricornutum would contain GL and PL having considerable amount of EPA and/or DHA. These polar lipids are known to show beneficial health effects; therefore, lipids produced by the microalgae will be used as functional food ingredients.

3.3 Fatty acid composition of ten species of microalgae

Major fatty acids in C. ceratosporum were C14:0 (17.42%), C16:1 (33.33%), C17:1 (12.83%) and EPA (11.49%), representing 75.07% of total fatty acids. C. ceratosporum contained the highest amount of monounsaturated fatty acids (MUFA) (46.85%) among the examined microalgae. The fatty acid composition of C. gracilis also showed the similar fatty acid profile. C. species-1 was also rich in C14:0 (10.77%), C16:1 (17.30%), C17:1 (22.41%) and EPA (15.95%), which accounted for 66.43% of total fatty acids. C. species-2 had a narrow distribution of fatty acids, with the highest amount of SFA (49.87%) and lowest level of PUFA (3.66%). The main fatty acids were C14:0 (21.68%), C16:0 (27.33%) and C16:1 (38.09%). C. species-3 was rich in C14:0 (12.49%) and C16:1 (13.74%), and presented the highest amount of alpha-linolenic acid (ALA, C18:3n-3).

The main fatty acids in I. galbana were C14:0 (20.95%) and GLA (11.75%), while P. lutheri was rich in C14:0 (14.49%), C16:0 (16.95%), C16:1 (15.96%) and EPA (17.76%). Although a little content of DHA was found in most of microalgae analyzed in the present study, I. galbana and P. lutheri contained a considerable amount of DHA. In addition, P. lutheri contained more than 17.76% EPA, which was the third highest after P. tricornutum (22.63%) and N. oculata (26.21%), respectively. In Spirulina, C16:0 was the major fatty acid (46.66%). It was also rich in LA (21.59%) and GLA (18.54%). Similar distributions were also reported by Otleś and Pire.

It is well-known that n-3 PUFA such as EPA and DHA have significant biochemical and physiological effects and primarily exhibit a positive influence on human nutrition and health. When focusing on total n-3 PUFA content, P. lutheri showed the highest content (28.01%), followed by N. oculata (26.73%) and P. tricornutum (25.26%), respectively. Among these three microalgae, P. lutheri showed the highest total lipids content and considerable amount of fucoxanthin (Table 1). The relatively higher levels of total lipids and fucoxanthin content were also found in C. ceratosporum, C. gracilis and C. species-1 (Table 1). These three kinds of Chaetoceros species contained more than 10% EPA (Table 3). Considering the high nutritional impact of n-3 PUFA and fucoxanthin, microalgae having these lipid components at higher level will be used as an ideal substrate for the production of functional lipids with great commercial potential.

4 Conclusion

The growing importance of functional food has driven research into the physiological effects of high biological value components of natural origin. The photosynthetic microorganisms have been regarded as promising natural sources of these bioactive compounds including lipids, proteins, and others. In the present study, the attention has been paid to microalgal functional lipids such as n-3 PUFA and fucoxanthin. The results showed that several kinds of microalgae could produce high amount of n-3 PUFA and fucoxanthin. In addition, a large part of n-3 PUFA would be present as GL and PL. These polar lipids are known to show beneficial health effects; therefore, lipids produced by the microalgae will be used as functional food ingredients.

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Conflicts of Interest

The authors declare that they have no conflicts of interest.

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