Fatty Acid and Lipid Class Composition of the Microalga *Phaeodactylum tricornutum*

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Abstract: n-3 Polyunsaturated fatty acids (PUFAs) are essential for the prevention and/or risk reduction of some diseases, including cardiovascular diseases and cancer. Therefore, PUFAs-rich marine microalgae have received considerable research attention. The diatom *Phaeodactylum tricornutum*, a rich source of lipids and fucoxanthin, has commercial applications in the food and pharmaceutical industries. In this study, the lipid class composition and fatty acid distribution in *P. tricornutum* under high-density and scale-up cultivation were investigated by thin-layer chromatography and gas chromatography. The fucoxanthin content was quantified by reverse-phase high-performance liquid chromatography. Both total lipid and fucoxanthin contents were high, accounting for 321.89 and 4.47 mg/g dry weight, respectively. Neutral lipids were the major lipids, and triacylglycerol was the predominant neutral lipids (33.63% of total lipids). The glycolipids (GLs) and phospholipids (PLs) represented 20.95% and 31.39% of total lipids, respectively. Sulfoquinovosyl diacylglycerol (SQDG) was the largest GLs fraction (9.81% of total lipids). Phosphatidylglycerol (PG) was the major PLs and accounted for 13.53% of total lipids. The main fatty acids were hexadecanoic acid (C16:0), palmitoleic acid (C16:1), and eicosapentaenoic acid. In addition, docosahexaenoic acid only accumulated in the PLs fraction. These findings provide new information on the lipid types, fatty acid composition of each lipid class, and the fucoxanthin content in *P. tricornutum*.

Key words: lipid classes, fatty acids, fucoxanthin, eicosapentaenoic acid, *Phaeodactylum tricornutum*

1 Introduction

Marine microalgae are prokaryotic or eukaryotic photosynthetic microorganisms that can grow rapidly owing to their unicellular or simple multicellular structure¹. They are important primary producers in marine environments and are used extensively as aquaculture bait², potential feedstock for biodiesel³, and promising ingredients in functional foods⁴ owing to their beneficial components. They can produce high concentrations of lipids, pigments, proteins, vitamins, and other biomolecules exploited for commercial use⁵.

In recent years, microalgae have gained increasing interest as a source of n-3 polyunsaturated fatty acids (PUFAs). It is well known that n-3 PUFAs, particularly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are essential for the prevention and/or risk reduction of some diseases, such as arrhythmia⁶, atherosclerosis⁷, cardiovascular disease⁸, cancer⁹, inflammation¹⁰, and mental illness¹¹. Although fish are currently the major source of

PUFAs in the human diet, marine fish do not synthesize PUFAs by themselves, but instead obtain it from microalgae in their diets¹². Therefore, PUFAs-rich marine microalgae are a focus of research in the aquaculture, food, and pharmaceutical industries.

In addition to n-3 PUFAs, there is a growing global interest in pigments derived from microalgae, especially fucoxanthin, a major marine carotenoid. It occurs abundantly in some macro- and microalgae, such as brown seaweeds¹³.

Abbreviations: DGDG, digalactosyldiacylglycerol; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; GC, gas chromatography; GLs, glycolipids; MGDG, monogalactosyldiacylglycerol; MUFAs, monounsaturated fatty acids; NLs, neutral lipids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PLs, phospholipids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; SQDG, sulfoquinovosyl diacylglycerol; TLC, thin-layer chromatography.

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and the diatoms *Chaetoceros* sp., *Cylindrotheca closterium*, *Odontella aurita*, and *Phaeodactylum tricornutum*¹⁴⁾. Numerous studies have shown that fucoxanthin has beneficial nutritional and medicinal properties, including antioxidant, antitumor, anti-obesity, and anti-inflammatory activity^{14, 15)}.

P. tricornutum, a photosynthetic microalga, is a unicellular marine diatom that shows a high growth rate under optimal conditions¹⁶⁾. The species is considered an important potential source of EPA and therefore has important commercial applications¹⁷⁾. Additionally, *P. tricornutum* is a rich source of fucoxanthin, with at least ten times the fucoxanthin content as that in other microalgae¹⁸⁾. However, owing to the strong influence of environmental or culture conditions, there is variation in the lipid class composition and fatty acid distribution among *P. tricornutum*¹⁹⁾. Moreover, because it has been proved that PUFAs esterified into polar lipids are more effective than those esterified into nonpolar lipids as growth promoters in aquaculture¹²⁾, it is very important to determine the distribution of PUFAs within the lipid pools of target microalgae species. A full understanding of the lipid class and fatty acid composition in *P. tricornutum* may provide a basis for regulating the production yield by optimizing culture conditions. Therefore, the main objective of the present study was to investigate the lipid and fucoxanthin contents in *P. tricornutum* under high-density and scale-up cultivation. The lipid class composition and fatty acid distribution were also characterized.

2 Materials and Methods

2.1 Microalgae cultivation

The diatom *P. tricornutum* was cultured in f/2 medium. The medium (100 mL) was added to an Erlenmeyer flask (200 mL). After autoclaving the medium, the alga was inoculated into the medium and cultured for 10 days at 30°C in a 1% CO₂ atmosphere. The algal culture solution was used for high-density and scale-up cultivation. The algal culture solution (80 L) was treated by continuous centrifugation (4000 × g) to obtain a concentrated 12 L solution. The solution was further concentrated by high-speed centrifugation (13000 × g) and freeze-dried to obtain 33 g of dried sample.

2.2 Extraction of total lipids

Total lipids of *P. tricornutum* were extracted according to the modified Bligh and Dyer procedure²⁰⁾. Briefly, microalgae (ca. 2 g) were soaked in water at a ratio of 1:9 w/v for 1 hr in a dark room and lipids were then extracted with chloroform/methanol (ca. 200 mL, v/v 1:2 C/M) 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 C/M/W (v/v/v) for overnight phase separation. The lower layer containing the lipid extract was obtained in a rotary evaporator. It was weighed, resuspended in chloroform, and finally stored at –30°C under nitrogen gas to prevent lipid oxidation.

2.3 Fucoxanthin content in extracted lipids

The fucoxanthin content in lipid extracts was analyzed by high-performance liquid chromatography (HPLC). The HPLC analysis was carried out using the 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%). Purified fucoxanthin was isolated from the brown seaweed *Undaria pinnatifida* as described previously²¹⁾.

2.4 Lipid class separation

Total lipids from *P. tricornutum* were separated into neutral lipids (NLs), glycolipids (GLs), and phospholipids (PLs) by silica gel column chromatography. In brief, the silica gel column was equilibrated with 100 mL of chloroform and then 50 mg of lipids dissolved in chloroform was applied. The NLs were eluted with 500 mL of chloroform and GLs were eluted with 1000 mL of acetone. Finally, elution with 500 mL of methanol yielded the PLs. Each fraction was concentrated in a rotary evaporator, dried under nitrogen, and resuspended in chloroform at –40°C for subsequent analyses.

The aforementioned lipid fractions were subjected to one-dimensional thin-layer chromatography (TLC) using plates coated with silica gel 60 F254 (Merck, Darmstadt, Germany) for lipid class separation and identification. The solvents were hexane/diethyl ether/acetic acid (70:30:1, v/v/v) for NLs and chloroform/methanol/water (65:25:4, v/v/v) for both GLs and PLs. The lipid classes were identified by co-chromatography with pure standards (Sigma-Aldrich, St. Louis, MO, USA). NLs were stained with 50% H₂SO₄, GLs 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 PLs with Dittmer reagent (molybdenum oxide/molybdenum in H₂SO₄). The stained band intensity of individual lipid class was quantified using Image J software.

2.5 Preparation and fatty acid analysis

After visualization and identification, lipid bands were immediately and carefully scraped out, and 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 described by Prevot and Mordret²²⁾. 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. 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 $1470 \times g$ for 5 min. The upper 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 (FID) and a capillary column (Omegawax-320; 30 m $\times$ 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). 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, Bellefonte, PA, USA).

3 Results and discussion

3.1 Total lipid and fucoxanthin contents

The total lipid content of *P. tricornutum* is presented in Table 1. High amounts of crude lipids were extracted from *P. tricornutum*, which accounted for 321.89 mg/g dry weight. The high levels of total lipids extracted from *P. tricornutum* were consistent with the results of a previous study²³⁾. In addition, HPLC results revealed that the fucoxanthin content in *P. tricornutum* was approximately 4.47 mg/g dry weight, which is higher than those of other fucoxanthin-rich sources, such as brown seaweeds¹³⁾. Accordingly, *P. tricornutum* cultured in this study is a rich source of fucoxanthin. However, Kim *et al.*¹⁸⁾ reported that the fucoxanthin yield (ethanol extract) in *P. tricornutum* is up to

Table 1 Total lipids and fucoxanthin contents in *P. tricornutum*. Values are represented as mean $\pm$ standard error (SE) of three independent measurements.

<i>P. tricornutum</i>	Content (mg/g dry weight)
Total lipids	321.89 $\pm$ 1.89
Fucoxanthin	4.47 $\pm$ 0.19

15.71 mg/g freeze-dried weight. The difference between studies may be explained by differences in culture conditions, solvent type, extraction time, temperature, and extraction method.

3.2 Lipid class composition

The observed lipid fractions are summarized in Fig. 1. The nonpolar fractions, mainly triacylglycerol (TAG), accounted for 33.63% of total lipids. The polar lipids represented 52.34% of total lipids, while GLs and PLs accounted for 20.95% and 31.39%, respectively. In addition, unidentified lipids (UN) were also detected; this fraction represented 14.03% of total lipids.

TAG was the most abundant lipid in *P. tricornutum*. TAG has already been reported as the major lipid constituent in various microalgae, such as *P. tricornutum*, *Pavlova lutheri*, and *Nitzschia laevis*^{12, 24, 25)}. However, in a previous study²⁶⁾, higher amounts of TAG (>70% of total lipids) were detected in continuous cultures of *P. tricornutum* when the microalgae were cultured in low growth rate conditions and under nitrogen limitation. In addition, culture age had no effect on the total fatty acid content, but influenced lipid class composition, i.e., the polar lipid content decreased with culture age. These previous results suggest that microalgae culture conditions can regulate the synthesis of TAG and other lipid fractions. Despite the large quantity of PUFAs esterified into TAG, PUFAs esterified into polar lipids are more effective growth promoters in aquaculture¹²⁾. In our study conditions, microalgae had a moderate growth rate and culture age, which resulted in decreased TAG and the accumulation of polar lipids. We assumed that the UN fractions in this study were NLs based on the TLC staining results; however, additional studies are required to identify these UN fractions.

The GL fractions of *P. tricornutum* in this study were composed mainly of monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG), and sulfoquinovosyl diacylglycerol (SQDG), which together accounted for 20.95% of total lipids. The total MGDG, DGDG, and SQDG repre-

Fig. 1 Individual lipid class content in *Phaeodactylum tricornutum*. Values are represented as mean $\pm$ SE of three independent measurements.

sented 3.35%, 7.79%, and 9.81% of total lipids; these estimates were lower than those of previous *P. tricornutum* studies^{19, 23)}, but higher than those of other microalga species^{12, 25)}.

The PL fractions accounted for 31.39% of total lipids. The PL fractions of *P. tricornutum* were mainly composed of phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylglycerol (PG), which accounted for 7.95%, 9.91%, and 13.53% of total lipids, respectively. PC is typically the main PL in microalgae^{19, 23, 25)}; however, PG has not previously been reported as the most abundant PL fraction. In addition, in the present study, the PE content was higher than the PC content. This observation may reflect species-specificity or culture conditions.

3.3 Fatty acid composition of lipid classes

Fatty acid profiles of total lipids and various lipid classes of *P. tricornutum* are summarized in Table 2. The main lipid components were mid-chain saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), C14:0, C16:0, C16:1, and C17:1, which together represented 62.01% of total fatty acids. EPA represented 14.83% of total lipids, and was the main PUFA. The n-3/n-6 PUFA ratio was 4.19. Mounting evidence suggests that a high ratio of dietary n-3/n-6 PUFAs (or a low ratio of n-6/n-3

PUFAs) reduces the risk of several chronic diseases, such as heart disease, cancer, and metabolic disorders²⁷⁾. Therefore, the high ratio of n-3/n-6 PUFAs suggests that *P. tricornutum* is a potentially valuable source of functional food and animal feed components.

TAG is typically composed of C14:0, C16:0, and C16:1, which accounted for 76.26% of the total fatty acids in *P. tricornutum*. EPA only represented 10.67% of the TAG content; however, owing to the large amount of TAG in the total lipid fraction, EPA in TAG was still higher than that in other lipid classes. Additionally, the n-3/n-6 PUFA ratio in TAG was higher than that in the total lipid fraction. The high mid-chain fatty acid content and low percentage of EPA in TAG were generally in agreement with previous results for this species²³⁾. The fatty acid profile of UN fractions was mainly characterized by C16:0, C16:1, C17:1, and EPA. Moreover, DHA was also found in UN fractions, and accounted for 3.29% of total fatty acids.

MGDG was characteristically composed of C16:0, C16:1, and C17:1, and accounted for 55.28% of total fatty acids. EPA residue in MGDG was 16.29%. Similar results were also reported in the same species²³⁾ and other microalgae^{12, 25)}. The fatty acid composition of DGDG was similar to that of MGDG, in which C16:0, C16:1, and C17:1 together represented 68.84% of total fatty acids. However, the EPA

Table 2 Fatty acid composition of total lipids and individual lipid classes in *P. tricornutum*. Values are represented as mean $\pm$ SE of three independent measurements.

Fatty acids ^a	Total lipids	TAG	UN	MGDG	DGDG	SQDG	PC	PE	PG
C14:0	3.64 $\pm$ 0.05	5.25 $\pm$ 0.10	3.35 $\pm$ 0.03	2.27 $\pm$ 0.20	0.63 $\pm$ 0.11	5.15 $\pm$ 0.46	2.37 $\pm$ 0.06	2.37 $\pm$ 0.06	2.69 $\pm$ 0.07
C16:0	20.45 $\pm$ 0.09	25.46 $\pm$ 0.02	18.43 $\pm$ 0.33	9.68 $\pm$ 0.27	14.08 $\pm$ 0.02	34.70 $\pm$ 1.09	25.35 $\pm$ 0.19	10.22 $\pm$ 0.09	19.84 $\pm$ 0.33
C16:1	33.42 $\pm$ 0.26	45.55 $\pm$ 0.03	31.02 $\pm$ 0.10	19.79 $\pm$ 0.16	16.72 $\pm$ 0.32	10.76 $\pm$ 0.28	27.39 $\pm$ 0.32	18.19 $\pm$ 0.08	16.21 $\pm$ 0.73
C16:2n-4	1.45 $\pm$ 0.04	1.13 $\pm$ 0.01	1.80 $\pm$ 0.10	5.48 $\pm$ 0.07	7.90 $\pm$ 0.57	0.52 $\pm$ 0.01	1.56 $\pm$ 0.02	1.59 $\pm$ 0.01	1.31 $\pm$ 0.05
C17:1	4.50 $\pm$ 0.06	1.21 $\pm$ 0.01	6.01 $\pm$ 0.52	25.81 $\pm$ 0.99	38.04 $\pm$ 1.58	0.86 $\pm$ 0.01	7.28 $\pm$ 0.11	6.74 $\pm$ 0.08	6.16 $\pm$ 0.43
C18:0	0.56 $\pm$ 0.00	0.65 $\pm$ 0.01	1.08 $\pm$ 0.35	0.32 $\pm$ 0.10	0.18 $\pm$ 0.03	0.67 $\pm$ 0.02	0.55 $\pm$ 0.01	0.45 $\pm$ 0.03	0.81 $\pm$ 0.06
C18:1n-9	2.01 $\pm$ 0.02	1.87 $\pm$ 0.02	2.45 $\pm$ 0.24	0.67 $\pm$ 0.01	0.52 $\pm$ 0.05	0.31 $\pm$ 0.01	2.63 $\pm$ 0.06	3.10 $\pm$ 0.05	2.62 $\pm$ 0.05
C18:1n-7	0.72 $\pm$ 0.01	0.69 $\pm$ 0.01	0.68 $\pm$ 0.02	0.34 $\pm$ 0.01	0.38 $\pm$ 0.05	0.33 $\pm$ 0.01	1.21 $\pm$ 0.03	2.04 $\pm$ 0.07	2.04 $\pm$ 0.12
C18:2n-6	2.53 $\pm$ 0.02	1.14 $\pm$ 0.01	3.46 $\pm$ 0.18	1.25 $\pm$ 0.11	0.71 $\pm$ 0.07	0.36 $\pm$ 0.01	5.61 $\pm$ 0.10	6.80 $\pm$ 0.03	5.27 $\pm$ 0.35
C18:3n-6	0.65 $\pm$ 0.01	0.37 $\pm$ 0.00	0.98 $\pm$ 0.08	0.93 $\pm$ 0.10	0.89 $\pm$ 0.08	0.11 $\pm$ 0.00	0.44 $\pm$ 0.04	1.59 $\pm$ 0.03	0.71 $\pm$ 0.01
C18:3n-3	0.58 $\pm$ 0.03	0.37 $\pm$ 0.00	0.68 $\pm$ 0.06	0.33 $\pm$ 0.16	0.27 $\pm$ 0.04	0.13 $\pm$ 0.01	1.31 $\pm$ 0.03	1.31 $\pm$ 0.02	0.88 $\pm$ 0.01
C20:4n-6	1.12 $\pm$ 0.02	0.59 $\pm$ 0.01	1.54 $\pm$ 0.06	0.68 $\pm$ 0.08	0.28 $\pm$ 0.05	0.76 $\pm$ 0.02	1.42 $\pm$ 0.05	3.21 $\pm$ 0.03	1.58 $\pm$ 0.04
C20:5n-3	14.83 $\pm$ 0.15	10.67 $\pm$ 0.13	14.40 $\pm$ 0.19	16.29 $\pm$ 0.70	5.90 $\pm$ 0.11	32.80 $\pm$ 1.06	5.51 $\pm$ 0.20	20.99 $\pm$ 0.05	17.41 $\pm$ 0.37
C22:6n-3	2.60 $\pm$ 0.09	0.79 $\pm$ 0.00	3.29 $\pm$ 0.04	1.56 $\pm$ 0.05	1.35 $\pm$ 0.01	0.36 $\pm$ 0.03	4.68 $\pm$ 0.23	10.98 $\pm$ 0.05	3.89 $\pm$ 0.25
C24:0	0.67 $\pm$ 0.15	—	—	0.04 $\pm$ 0.04	0.09 $\pm$ 0.09	7.57 $\pm$ 0.12	1.74 $\pm$ 0.09	0.28 $\pm$ 0.02	7.83 $\pm$ 1.70
SFAs	25.32 $\pm$ 0.11	31.36 $\pm$ 0.12	22.86 $\pm$ 0.71	12.30 $\pm$ 1.24	14.95 $\pm$ 0.14	48.09 $\pm$ 1.41	30.00 $\pm$ 0.15	13.31 $\pm$ 0.10	31.17 $\pm$ 1.37
MUFAs	40.65 $\pm$ 0.28	49.32 $\pm$ 0.05	40.15 $\pm$ 0.37	46.61 $\pm$ 1.12	55.66 $\pm$ 1.80	12.25 $\pm$ 0.27	38.51 $\pm$ 0.34	30.07 $\pm$ 1.02	27.02 $\pm$ 1.86
PUFAs	23.76 $\pm$ 0.39	15.05 $\pm$ 0.14	26.12 $\pm$ 0.51	26.51 $\pm$ 0.87	14.79 $\pm$ 1.13	35.02 $\pm$ 0.73	20.52 $\pm$ 1.03	46.46 $\pm$ 0.11	31.04 $\pm$ 0.98
n-3/n-6	4.19	5.66	3.08	6.48	3.72	27.28	1.54	2.87	2.94

Note: ^aWeight %/total fatty acids

content in DGDG was only 5.90%, which was lower than that of MGDG and previous estimates.

In addition, the fatty acid profile of SQDG in this study was unique compared with that of other GLs, with high levels of EPA and C24:0 (which accounted for 32.80% and 7.57% of this class, respectively) and a low C17:1 content (0.86% of total fatty acids). The n-3/n-6 PUFA ratio in SQDG reached 27.28, which was the highest among the lipid classes.

With respect to PLs, the fatty acid composition of PC in this study was typical, containing C16:0, C16:1, and C17:1, which together accounted for over 60% of total fatty acids. The major PUFAs in PC were C18:2 (5.61% of total fatty acids), EPA (5.51% of total fatty acids), and DHA (4.68% of total fatty acids), and these differed from the major PUFAs in previous reports^{19, 23)}, which indicated a high EPA content in PC. Additionally, in this study, PLs exhibited a unique fatty acid profile characterized by high levels of DHA.

The fatty acid profile of PE was somewhat similar to that of PC, but had more EPA and DHA as well as less C16:0 and C16:1. High contents of EPA and DHA (which together accounted for 31.97% of total fatty acids) in PE have been observed previously in this species¹⁹⁾. The fatty acid composition of PG in the present study was essentially similar to that of PE, but with significantly more C16:0 and C24:0 as well as less DHA. A similar fatty acid profile of PG has been described for several species, including *P. tricornutum*^{12, 19, 25, 28)}.

Conclusion

The lipid classes and fatty acid composition in the lipid pool of *P. tricornutum* under high-density and scale-up cultivation were investigated in the present study. *P. tricornutum* exhibited high total lipid and fucoxanthin contents, i.e., 321.89 and 4.47 mg/g dry weight, respectively. NLs were the major lipid constituents, and TAG (33.63% of total lipids) was the predominant component. The GL and PL fractions of *P. tricornutum* represented 20.95% and 31.39% of total lipids, respectively. SQDG constituted the largest GL fraction. With respect to PLs, PG was the major component and accounted for 13.53% of total lipids. In addition, the fatty acid profiles of individual lipid classes were measured. TAG, the main class of lipids, contained high proportions of C14:0, C16:0, C16:1, and EPA. The lipid class with the highest EPA content in *P. tricornutum* was SQDG. Moreover, the n-3/n-6 PUFA ratio in SQDG was much higher than that of other lipid classes. The fatty acid profile of PLs showed an accumulation of DHA as a distinctive feature. These results clarify the lipid types, fatty acid composition of each lipid class, as well as the fucoxanthin content in *P. tricornutum*. It may provide a basis for

further studies of differences in lipid classes and fatty acid composition in microalgae under different culture processes and conditions. The present results also suggest that *P. tricornutum* has beneficial properties for human health and is a candidate ingredient for natural functional foods.

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