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Variation of lipid class composition in *Nitzschia laevis* as a response to growth temperature change

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Abstract

The lipid composition and the distribution of fatty acids in the lipid pool were determined in eicosapentaenoic acid (EPA)-producing microalga (*Nitzschia laevis)* grown under different temperatures. Both the relative amounts of lipid classes and the degree of fatty acid unsaturation in various lipid species were not greatly changed under tested growth conditions. Higher temperature up to 23 °C benefited the growth of *N. laevis* but only had a slight influence on EPA and lipid contents. Further increasing the culture temperature caused a serious inhibition of both the cell growth and fatty acid biosynthesis. Under all temperatures tested, triacylglycerol (TAG) was the predominant lipid constituent (64.5–69.1% of total lipid) and was highly saturated. Lower temperature favored the formation of polar lipids. The highest content of phosphatidylcholine (PC), the major phospholipids component, was reached at 15 °C (10.9% of total lipid). In sharp contrast to TAG, PC was highly unsaturated and contained a higher amount of EPA under lower temperature. The highest EPA content in polar lipid was achieved at 19 °C. The results from this investigation suggested that the low temperature could improve the distribution of polyunsaturated fatty acids in phospholipids, though it could not significantly influence their amount, especially in PC.

Keywords: *Nitzschia laevis*; Temperature; Lipid; Eicosapentaenoic acid

1. Introduction

The production of algal lipids has been increasing over the past decade. The polar lipids, especially phosphatidylcholine (PC), are important lipid components in the use of cultured algae for the production of functional food and aquaculture feed (Fontagne, Geurden, Escaffre, & Bergot, 1998; Schneider, 2001). It has been reported that the different levels of polyunsaturated fatty acids (PUFAs) in algae could affect the growth rate of the bivalves significantly (Enright, Newkirk, Craigie, & Castell, 1986), and PUFAs esterified in polar lipids were even more effective than those esterified in nonpolar fractions in terms of the effect on the growth and nutritional quality of aquaculture animals (Meireles, Guedes, & Malcata, 2003). However, the content of polar lipids and the distribution of PUFAs in polar lipids in individual PUFAs producing organisms vary considerably under different environmental conditions (Harwood, 1998).

Eicosapentaenoic acid (EPA, 20:5 $n-3$), an essential fatty acid in the prevention of arrhythmia, cardiovascular disease and cancer (Pulz & Gross, 2004), has shown its ability to improve the growth of several marine fish and molluscs in aquaculture industry (Langdon & Waldock, 1981). These achievements have prompted studies concerning EPA production in microalgae. *Nitzschia laevis*, a diatom, has been determined to be the potential producer of EPA due to its higher EPA content and rapid heterotrophic growth rate based on glucose (Tan & Johns, 1996). Up to

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now, the heterotrophic growth and the cellular EPA production characteristics of this alga have been thoroughly studied (Wen & Chen, 2001, 2003), whereas the relationship between the alteration of lipid content and the distribution of EPA in the lipid pool under different culture conditions have not yet been examined.

We have recently reported the lipid class composition of *N. laevis* (Chen, Jiang, & Chen, 2007). Although the total EPA content of the investigated alga is high (11.7% of total fatty acids), the EPA content in polar lipids is relatively low (39% of total EPA). This fact will influence its application in the aquaculture industry. It is well known that the content and composition of lipid in algal cells are highly influenced by environmental conditions and can be physiologically manipulated (Harwood, 1998). It is therefore necessary to study the influence of environmental conditions on lipid syntheses in *N. laevis*. Temperature has been reported to possess a major role in the types of lipids produced by microalgae because the range of temperature at which organisms grow might be related to the fluidity and stability of their membranes. It was postulated in turn, that the physical state of the membranes, by modifying their lipid compositions, would significantly influence the temperature for growth in different organisms (Kleinschmidt & McMahon, 1970; Zhu, Lee, & Chao, 1997). Certainly, it was indicated that some plants that could grow under low temperature did regulate the unsaturation degree of their fatty acids. There was a trend that fatty acid unsaturation would increase with the decrease of the temperature (Chapman, De-Felice, & Barber, 1983; Raison & Orr, 1986). However, the modifications of lipid and fatty acid contents under different growth temperatures are species specific (Harwood & Jones, 1989). The effect of temperature on total lipid content and the distribution of fatty acids in the lipid pool of microalgae has only been reported for a few species and there is no general conclusion reported (Jiang & Chen, 2000; Roessler, 1990; Zhu et al., 1997). The aim of this study, therefore, was to investigate the variation of lipid class composition and fatty acid distribution in the lipid pool of *N. laevis* under different growth temperatures. It was expected that the contents of PC and EPA in polar lipids in *N. laevis* could rise after the cold cultivation. The result from this research would help to enhance our understanding of the physiological responses of the heterotrophic diatom to temperature change as far as lipid classes are concerned. On the other hand, the quality of *N. laevis* in terms of its polar lipids content was expected to be improved during culture process in aquaculture industry in the future.

2. Materials and methods

2.1. Heterotrophic cultivation

The diatom *N. laevis* (UTEX 2047) was used in this study. The cells were cultured in 250 ml Erlenmeyer flasks each containing 100 ml modified Lewin's marine diatom medium, based on the one optimized for *N. laevis* (Chen et al., 2007; Wen & Chen, 2001). The cultural temperatures were set as 15, 19, 23 and 27 °C, respectively.

2.2. Determination of cell dry weight and glucose concentration

A 3 ml aliquot of the fermentation broth was sampled aseptically to determine the cell dry weight (Wen & Chen, 2001). Residual glucose concentration in the medium was determined by the 3,5-dinitrosalicylic acid method (Chen et al., 2007).

2.3. Lipid and fatty acid analyses

Lipid and fatty acid were analyzed following the process reported previously (Chen et al., 2007). Briefly, total lipids were extracted from 200 mg of lyophilized biomass according to the modified Folch procedure and then separated into neutral lipids (NLs), glycolipids (GLs) and phospholipids (PLs) using solid-phase extraction on a 500-mg Sep-Pak™ cartridge of silica gel (Waters). The lipid fractions were concentrated and subjected to one-dimensional thin-layer chromatography (TLC) for lipid class separation and identification, using TLC plates (20 × 20 cm) coated with silica gel 60 (Merck). Solvents used were hexane/diethyl ether/acetic acid (70:30:1, v/v) for NLs and chloroform/acetone/methanol/acetic acid/water (50:20:10:10:5 v/v) for both GLs and PLs (Christie, 2003).

A 0.1% (w/v) solution of 2,7-dichlorofluorescein in 95% methanol was used as general stain, which caused lipids to show up as yellow spots under UV light. Bands were identified by co-chromatography with pure standards (Sigma) and by (as specific as possible) staining when it was necessary: α-naphthol for GLs, molybdenum blue spray reagent for PLs, Dragendorff's reagent for PC, ninhydrin for phosphatidylethanolamine (PE) and phosphatidylserine (PS), cresyl violet for sulphoquinovosyldiacylglycerol (SQDG).

After visualization and identification, lipid bands were immediately and carefully scraped out, and fatty acids were direct transmethylated with sulphuric acid in methanol and then analyzed by HP-6890 gas chromatography (Hewlett–Packard) equipped with a HP-INNOWAX™ capillary column (HP 19091N-133, 30 m × 0.25 mm × 0.25 μm). The fatty acid methanol esters were identified by comparison of their retention times with those of the authentic standards (Sigma), and were quantified by comparing their peak areas with that of the internal standard (C17:0) (Chen et al., 2007).

2.4. Statistical evaluation

The data were tested for statistical significance with STATISTICA 6.0 Software (Statsoft Inc., Tusla, OK, USA), and the level of statistical significance is given as $p < 0.05$.

3. Results and discussion

3.1. Effect of temperature on cell growth of N. laevis

The kinetic growth parameters of *N. laevis* are shown in Table 1. From these data, it could be seen that the highest biomass concentration was achieved at 23 °C and the maximum specific growth rate doubled (from 0.28 d^{-1} to 0.58 d^{-1}) when temperature increased from 15 to 23 °C. Further increase of culture temperature to 27 °C caused a serious inhibition of cell growth which could be observed by the very low maximum biomass concentration (2.78 g/l). It was observed that the lag growth phase of *N. laevis* was shortened gradually according to the increase of growth temperature (data not shown). The higher growth rate under higher temperature also occurred in other microalgae such as *Isochrysis galbana* (Zhu et al., 1997) and *Chroomonas salina* (Henderson & Mackinlay, 1989). This could be due to the reduced enzyme activity in glycolysis and Krebs cycle under lower growth temperatures (Jiang & Chen, 2000).

3.2. Lipid composition related to growth temperature

In this study, only the cells grown under 15, 19 and 23 °C were used for lipid and fatty acid analyses due to their higher biomass concentrations. As shown in Fig. 1b within the temperature range of 15–23 °C, no drastic effects of temperature change on the contents of the three major lipid components were detected. However, the changes in the relative proportions of different lipid species within neutral and polar lipids have been observed (Fig. 1b). NLs of *N. laevis* were mainly composed of triacylglycerol (TAG), diacylglycerol (DAG) as well as monoacylglycerol (MAG). TAG was found to be the principal lipid fraction under all tested temperatures, which was similar to the other heterotrophic microalgae (Henderson & Mackinlay, 1989). But the content of TAG was rather constant and slightly influenced by temperature (Fig. 1b).

The major PL components of *N. laevis* were lysophosphatidylcholine (LPC), phosphatidylinositol (PI), phatidylcholine (PC), phosphatidylglycerol (PG) and diphosphatidyl glycerol (DPG). Among these lipid species, PC was the largest fraction and accounted for more than 60% of PLs under all growth temperatures. The highest PL content (16.9% of the total lipids) was achieved at 19 °C, and the proportion of PC was 10.5% of the total lipids under the same temperature (similar to that at 15 °C) (Fig. 1a and b). PC was widely reported to be the major fraction of PLs although its content varied greatly in different algal species under different culture conditions (Alonso, Belarbi, Rodriguez-Ruiz, Segura, & Gimenez, 1998; Bigogno, Khozin-Goldberg, Boussiba, Vonshak, & Cohen, 2002). PC is the major phospholipid in extraplastidic membranes and its synthesis largely depends on the endoplasmic reticulum diacylglycerol pathway. Its amount would increase along with the decreasing of temperature, which was suggested to be one of the effective strategies to regulate the membrane fluidity and the development of freezing tolerance in plants (Murphy, 2004). The gene expression of cytidine diphosphate-choline (CDP-choline) synthase, which catalyze the synthesis of the head precursor of CDP-choline, was strongly induced by cold acclimation (Charron et al., 2002). Moreover, the increased activity of cholinephosphotransferase, the terminal enzyme for the synthesis of PC, was observed under lower growth temperature (Harwood & Jones, 1989). Our results obtained from *N. laevis* were in agreement with these previous investigations.

In polar lipids, the proportions of GLs in *N. laevis* were much lower than PLs, which was very different from other photoautotrophic microalgae (Lynch & Thompson, 1982). In other photosynthetic organisms, high temperature could result in increased contents of GLs and monogalactosyldiacylglycerol (MGDG) but an decreased content of digalactosyldiacylglycerol (DGDG) (Liao, Li, & He, 2004; Lynch & Thompson, 1982). Our results on GLs differed from the above observations. In this study, the contents of GLs and the major fraction DGDG increased from 1.8% to 8.1% of total lipids and 17-fold (from 0.2% to 3.5% of total lipid), respectively when the growth temperature increased from 15 °C to 23 °C (Fig. 1b). This might be due to the structure and activity changes of chloroplast in heterotrophically grown cells since *N. laevis* is a phototrophic alga in nature (Gurr, Harwood, & Frayn, 2002).

3.3. Fatty acid distribution in individual lipid classes under different growth temperatures

Fatty acid profiles of *N. laevis* under different temperatures were listed in Table 2. It could be seen that the contents of major saturated fatty acids C14:0 and C16:0

Table 1
Kinetic growth parameters of *Nitzschia laevis* at different temperatures[A]

Parameters[B]	Temperature (°C)			
	15	19	23	27
μ (d^{-1})	0.28 ± 0.05^{c}	0.39 ± 0.04^{b}	0.58 ± 0.02^{a}	0.28 ± 0.05^{c}
X_{max} (g/l)	9.07 ± 0.09^{b}	9.23 ± 0.15^{b}	10.10 ± 0.02^{a}	2.78 ± 0.12^{c}
$Y_{x/glu}$ (g/g)	0.453 ± 0.005^{b}	0.444 ± 0.011^{b}	0.479 ± 0.002^{a}	0.375 ± 0.006^{c}

[A] Data are expressed as mean ± SD of three replicates. The data marked with the same lower case letters in the same row are not significantly different ($p < 0.05$).
[B] μ, specific growth rate, d^{-1}; X_{max}, maximum biomass concentration, g/l; $Y_{x/glu}$, growth yield coefficient based on glucose, g/g.

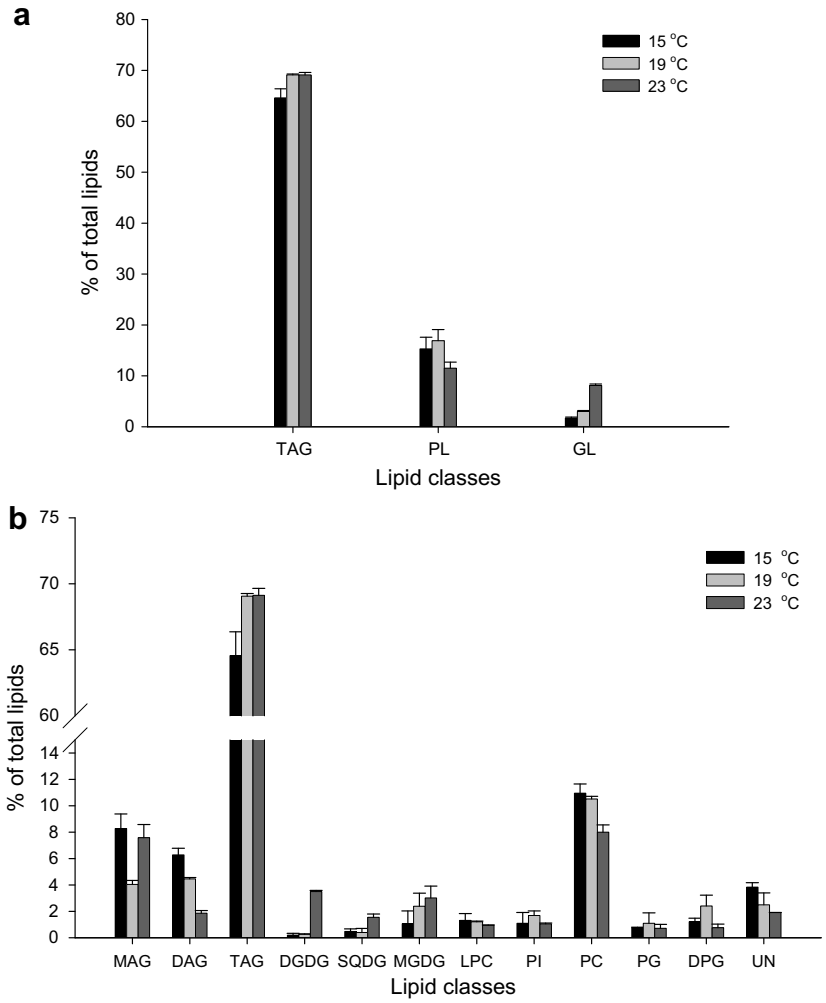

Fig. 1. The contents of different lipid classes (% of total lipids) of *Nitzschia laevis* at different temperatures. (a) Distribution of triacylglycerol (TAG), phospholipid (PL) and glycolipid (GL) in total lipids; (b) Distribution of individual lipid classes in total lipids. Values are represented as mean ± standard deviation of triplicates. MAG, monoacylglycerol; DAG, diacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulphoquinovosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; LPC, lysophosphatidylcholine; PI, phosphatidylinositol; PC, phosphatidylcholine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; UN, unidentified lipid fractions.

decreased according to the decrease of temperature in the whole cell, whereas the major unsaturated fatty acids including C16:1, C18:1 and EPA increased. As a result, the degree of unsaturation (DUS, ∇/mol) of the total fatty acids in the cell increased with the decrease of growth temperature. This phenomenon was widely observed in many other organisms because the stimulated large synthesis of unsaturated fatty acids under lower temperature could help the cell to maintain the proper membrane fluidity and functions in cold environment (Jiang & Chen, 2000; Murphy, 2004; Patterson, 1970).

As for individual lipid classes, the largest lipid fraction – TAG was mainly composed of shorter chain length fatty acids, such as C14–18 saturated and monounsaturated fatty acids (Table 2). Although nearly 40% of total EPA existed in TAG, the proportion of EPA of total fatty acids in TAG was low and only increased from 6.3% to 8.2% as a response to the decrease of temperature. This might be due to that most of the TAG accumulated in lipid bodies were used for energy storage rather than structure construction (Harwood & Jones, 1989).

In PLs, there was a quite different fatty acid profile to that of TAG. The lipid species in PLs were characterized by higher contents of PUFAs such as arachidonic acid (AA, C20:4 $n-6$) and EPA. The influences of temperature on the contents of PUFAs in different PLs fractions were obvious in contrast to those in NLs. As shown in Table 2, the highest percentage of EPA of total fatty acids was detected in PC when the alga was cultured under 15 °C (31.5% of total fatty acids in PC), though the total EPA content of PLs was achieved the highest at 19 °C (Fig. 2). In PLs, PC had high DUS values at all temperatures, which decreased from 2.6 at 15 °C to 2.3 at 23 °C. The increase of PUFA proportion along with the decrease of temperature was also observed in other microalgae such as *I. galbana* (Zhu et al., 1997). It was reported that the activity of the

Table 2
Fatty acid composition of individual lipid classes of *N. laevis* cultured at different temperatures[A]

Lipid class[B]	Temperature (°C)	Fatty acid composition											Unsatd[C]	DUS (∇/mol)[D]
		C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3 $n-6$	C18:3 $n-3$	C20:4 $n-6$	C20:5 $n-3$		
Biomass	15	12.8 ± 0.1^{b}	1.5 ± 0.1^{a}	25.8 ± 0.1^{b}	26.6 ± 0.3^{a}	0.4 ± 0.1^{b}	6.8 ± 0.1^{a}	4.4 ± 0.0^{ab}	1.8 ± 0.0^{b}	2.0 ± 0.0^{a}	5.3 ± 0.0^{a}	12.6 ± 0.1^{a}	60.9 ± 0.1^{a}	1.4 ± 0.0^{a}
	19	13.5 ± 0.2^{b}	1.6 ± 0.0^{a}	29.0 ± 0.3^{a}	23.2 ± 0.3^{b}	0.4 ± 0.0^{b}	5.9 ± 0.0^{a}	5.6 ± 0.2^{a}	1.9 ± 0.0^{ab}	2.3 ± 0.1^{a}	4.7 ± 0.2^{a}	11.9 ± 0.3^{b}	57.0 ± 0.5^{b}	1.3 ± 0.0^{b}
	23	16.9 ± 1.2^{a}	0.9 ± 0.4^{a}	28.5 ± 0.5^{a}	23.9 ± 0.0^{b}	0.7 ± 0.1^{a}	5.2 ± 1.0^{a}	3.4 ± 0.7^{b}	2.1 ± 0.1^{a}	2.0 ± 0.8^{a}	5.0 ± 1.2^{a}	11.7 ± 0.4^{b}	54.0 ± 0.8^{c}	1.3 ± 0.0^{b}
TAG	15	15.1 ± 0.7^{b}	1.6 ± 0.0^{a}	28.5 ± 1.9^{b}	28.1 ± 0.3^{a}	0.1 ± 0.0^{a}	8.5 ± 0.7^{a}	4.5 ± 0.0^{b}	1.3 ± 0.0^{b}	0.8 ± 0.1^{b}	3.3 ± 0.3^{a}	8.2 ± 0.1^{a}	56.3 ± 1.2^{a}	1.1 ± 0.0^{a}
	19	15.1 ± 0.1^{b}	1.7 ± 0.0^{a}	34.6 ± 0.1^{a}	23.2 ± 0.5^{c}	0.2 ± 0.0^{a}	7.0 ± 0.0^{a}	5.5 ± 0.2^{a}	1.5 ± 0.1^{ab}	1.3 ± 0.2^{a}	2.8 ± 0.3^{ab}	7.2 ± 0.3^{b}	50.1 ± 0.3^{b}	1.0 ± 0.0^{b}
	23	20.0 ± 0.0^{a}	0.9 ± 0.0^{b}	34.1 ± 0.1^{a}	25.4 ± 0.1^{b}	0.1 ± 0.0^{a}	5.0 ± 0.0^{b}	3.1 ± 0.0^{c}	1.7 ± 0.0^{a}	1.2 ± 0.0^{ab}	2.4 ± 0.0^{b}	6.3 ± 0.2^{c}	45.9 ± 0.4^{c}	0.9 ± 0.0^{c}
MAG	15	6.7 ± 0.0^{a}	1.4 ± 0.4^{a}	21.3 ± 3.3^{a}	27.3 ± 2.4^{a}	0.9 ± 0.3^{a}	3.8 ± 1.6^{a}	2.7 ± 0.4^{a}	3.6 ± 0.8^{a}	8.0 ± 0.8^{a}	3.6 ± 0.4^{b}	20.8 ± 3.5^{a}	71.1 ± 4.5^{a}	1.9 ± 0.3^{a}
	19	6.6 ± 0.7^{a}	1.1 ± 0.2^{a}	14.6 ± 4.3^{a}	27.3 ± 1.2^{a}	1.6 ± 0.9^{a}	3.3 ± 0.1^{a}	3.2 ± 0.3^{a}	3.5 ± 0.3^{a}	9.6 ± 0.5^{a}	5.0 ± 1.0^{ab}	24.2 ± 3.6^{a}	77.2 ± 4.4^{a}	2.2 ± 0.3^{a}
	23	7.2 ± 2.5^{a}	0.5 ± 0.0^{a}	8.8 ± 0.8^{a}	27.2 ± 3.1^{a}	0.2 ± 0.6^{a}	1.8 ± 0.8^{a}	1.7 ± 0.2^{a}	3.4 ± 2.3^{a}	6.9 ± 0.8^{a}	7.5 ± 0.1^{a}	34.8 ± 1.3^{a}	83.8 ± 2.0^{a}	2.7 ± 0.1^{a}
DAG	15	7.6 ± 0.6^{b}	1.0 ± 0.2^{a}	30.4 ± 2.4^{a}	31.7 ± 5.0^{a}	2.3 ± 0.1^{a}	5.4 ± 0.5^{a}	4.1 ± 0.7^{b}	1.5 ± 0.7^{a}	1.4 ± 0.5^{a}	7.2 ± 0.5^{a}	7.4 ± 1.7^{a}	59.8 ± 4.4^{a}	1.2 ± 0.2^{a}
	19	9.7 ± 1.3^{b}	2.3 ± 1.1^{a}	22.8 ± 1.8^{b}	34.7 ± 0.2^{a}	2.5 ± 0.4^{a}	5.8 ± 0.6^{a}	5.5 ± 0.2^{b}	1.9 ± 0.1^{a}	2.0 ± 0.1^{a}	5.7 ± 1.7^{ab}	7.2 ± 0.5^{a}	65.0 ± 2.8^{a}	1.2 ± 0.1^{a}
	23	14.5 ± 0.3^{a}	1.1 ± 0.2^{a}	21.1 ± 1.2^{b}	29.4 ± 2.0^{a}	1.8 ± 0.4^{a}	7.8 ± 1.0^{a}	12.9 ± 0.2^{a}	1.7 ± 0.5^{a}	1.3 ± 0.0^{a}	2.1 ± 0.2^{b}	6.2 ± 0.4^{a}	62.6 ± 0.9^{a}	1.1 ± 0.0^{a}
MGDG	15	8.7 ± 0.5^{a}	2.7 ± 1.6^{a}	14.5 ± 0.4^{a}	27.7 ± 2.6^{a}	1.6 ± 0.2^{b}	6.8 ± 4.8^{a}	3.1 ± 0.9^{a}	2.8 ± 0.3^{a}	6.3 ± 1.7^{a}	5.7 ± 3.5^{a}	20.3 ± 2.6^{b}	75.3 ± 0.7^{a}	2.0 ± 0.0^{b}
	19	9.0 ± 0.7^{a}	1.5 ± 0.4^{a}	10.4 ± 0.6^{b}	27.0 ± 0.2^{a}	1.2 ± 0.2^{b}	2.6 ± 0.2^{b}	3.5 ± 0.2^{a}	3.4 ± 0.1^{a}	10.1 ± 1.0^{a}	4.2 ± 0.1^{a}	27.0 ± 1.5^{ab}	79.4 ± 1.4^{a}	2.3 ± 0.1^{ab}
	23	5.7 ± 0.1^{b}	0.5 ± 0.0^{a}	11.9 ± 1.7^{ab}	23.6 ± 0.3^{a}	4.5 ± 0.9^{a}	3.4 ± 0.3^{b}	1.9 ± 0.0^{a}	3.8 ± 0.7^{a}	9.1 ± 0.9^{a}	4.5 ± 1.8^{a}	31.2 ± 2.0^{a}	78.0 ± 1.9^{a}	2.4 ± 0.1^{a}
DGDG	15	7.0 ± 1.9^{ab}	6.2 ± 0.1^{a}	13.7 ± 3.9^{a}	26.0 ± 5.0^{b}	9.8 ± 0.3^{a}	2.0 ± 0.4^{a}	2.9 ± 0.2^{b}	2.6 ± 3.6^{a}	3.3 ± 4.6^{a}	10.6 ± 4.3^{a}	16.0 ± 4.1^{a}	69.5 ± 4.3^{a}	1.8 ± 0.2^{a}
	19	5.5 ± 0.1^{b}	4.8 ± 0.4^{b}	11.9 ± 2.3^{a}	30.6 ± 2.5^{ab}	6.3 ± 2.0^{ab}	5.6 ± 3.5^{a}	7.9 ± 0.1^{a}	3.4 ± 4.9^{a}	1.3 ± 1.8^{a}	6.0 ± 1.7^{a}	16.6 ± 4.8^{a}	76.3 ± 4.4^{a}	1.8 ± 0.1^{a}
	23	11.1 ± 0.6^{a}	0.7 ± 0.0^{c}	20.5 ± 2.8^{a}	30.9 ± 3.7^{a}	1.9 ± 1.3^{b}	1.3 ± 2.3^{a}	2.4 ± 0.0^{b}	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	10.2 ± 3.7^{a}	19.7 ± 2.2^{a}	66.5 ± 2.6^{a}	1.8 ± 0.1^{a}
SQDG	15	1.6 ± 1.4^{b}	1.6 ± 1.4^{a}	6.4 ± 0.6^{b}	28.3 ± 2.3^{a}	1.6 ± 0.6^{a}	2.7 ± 0.7^{b}	2.2 ± 0.1^{a}	11.5 ± 1.2^{a}	34.3 ± 3.6^{a}	4.3 ± 1.8^{a}	5.5 ± 0.1^{a}	90.4 ± 2.6^{a}	2.2 ± 0.2^{a}
	19	2.7 ± 0.3^{b}	4.1 ± 3.0^{a}	9.3 ± 2.0^{b}	25.3 ± 3.5^{b}	3.5 ± 2.7^{a}	1.6 ± 0.6^{b}	3.0 ± 0.8^{a}	9.0 ± 1.5^{a}	25.9 ± 0.9^{b}	6.0 ± 1.9^{a}	9.5 ± 4.4^{a}	84.5 ± 5.0^{a}	2.1 ± 0.2^{ab}
	23	6.9 ± 0.4^{a}	2.8 ± 0.0^{a}	30.5 ± 3.6^{a}	7.4 ± 2.5^{c}	9.1 ± 1.7^{a}	19.7 ± 2.4^{a}	3.6 ± 1.4^{a}	4.5 ± 1.9^{a}	1.8 ± 0.3^{c}	5.8 ± 0.4^{a}	7.9 ± 2.5^{a}	53.5 ± 3.8^{b}	1.2 ± 0.2^{b}
PC	15	10.9 ± 0.7^{b}	0.9 ± 0.2^{a}	8.8 ± 1.3^{b}	21.7 ± 1.0^{b}	0.1 ± 0.1^{a}	3.6 ± 0.2^{b}	6.2 ± 0.1^{b}	1.9 ± 0.2^{b}	1.4 ± 0.1^{b}	12.9 ± 1.1^{ab}	31.5 ± 0.6^{a}	80.2 ± 0.6^{a}	2.6 ± 0.0^{a}
	19	10.9 ± 0.9^{b}	1.1 ± 0.0^{a}	8.6 ± 0.5^{b}	24.2 ± 4.2^{a}	0.2 ± 0.1^{a}	3.3 ± 0.0^{b}	8.5 ± 0.3^{a}	2.4 ± 0.1^{b}	3.4 ± 1.2^{a}	9.6 ± 2.3^{b}	27.8 ± 4.5^{b}	80.3 ± 1.5^{a}	2.4 ± 0.2^{b}
	23	14.1 ± 0.2^{a}	0.7 ± 0.0^{a}	13.5 ± 0.5^{a}	15.7 ± 0.3^{c}	0.3 ± 0.0^{a}	4.2 ± 0.0^{a}	5.6 ± 0.1^{c}	3.1 ± 0.1^{a}	1.5 ± 0.1^{b}	18.1 ± 0.3^{a}	23.3 ± 0.5^{c}	72.1 ± 0.6^{b}	2.3 ± 0.0^{b}
LPC	15	4.8 ± 0.4^{b}	1.0 ± 0.1^{a}	13.5 ± 2.0^{a}	16.4 ± 2.6^{a}	0.4 ± 0.3^{c}	6.0 ± 0.3^{a}	14.7 ± 1.9^{a}	5.1 ± 1.5^{a}	2.6 ± 0.6^{a}	11.8 ± 2.4^{a}	23.7 ± 2.0^{a}	81.3 ± 3.7^{a}	2.4 ± 0.3^{a}
	19	3.4 ± 1.6^{b}	1.3 ± 0.2^{a}	17.2 ± 5.0^{a}	14.0 ± 2.2^{a}	1.4 ± 1.1^{b}	8.6 ± 4.2^{a}	16.7 ± 4.2^{a}	6.1 ± 0.5^{a}	3.4 ± 0.6^{a}	8.3 ± 2.7^{a}	19.6 ± 3.3^{ab}	78.0 ± 4.7^{a}	2.2 ± 0.5^{a}
	23	11.2 ± 1.3^{a}	1.2 ± 0.4^{a}	29.0 ± 3.6^{a}	15.7 ± 1.4^{a}	3.6 ± 1.8^{a}	13.3 ± 1.0^{a}	3.6 ± 2.7^{b}	2.0 ± 1.5^{b}	1.0 ± 0.5^{a}	8.7 ± 3.6^{a}	10.9 ± 2.3^{b}	56.3 ± 1.8^{b}	1.4 ± 0.5^{b}
PI	15	12.7 ± 0.4^{a}	0.9 ± 0.2^{a}	29.4 ± 4.0^{a}	29.2 ± 2.0^{a}	0.1 ± 0.0^{c}	2.7 ± 0.7^{b}	3.3 ± 0.8^{b}	2.1 ± 0.2^{a}	1.6 ± 0.0^{b}	7.7 ± 0.8^{a}	10.4 ± 1.0^{a}	57.8 ± 3.7^{ab}	1.3 ± 0.1^{a}
	19	14.0 ± 0.6^{a}	0.9 ± 0.1^{a}	33.5 ± 1.7^{a}	24.1 ± 1.0^{a}	0.2 ± 0.0^{b}	1.8 ± 0.2^{b}	2.7 ± 0.6^{b}	2.1 ± 0.2^{a}	1.5 ± 0.0^{b}	8.6 ± 0.7^{a}	10.5 ± 1.6^{a}	52.3 ± 2.3^{b}	1.3 ± 0.1^{a}
	23	4.8 ± 0.2^{b}	1.6 ± 0.5^{a}	31.5 ± 3.4^{a}	16.1 ± 4.6^{b}	3.4 ± 2.0^{a}	16.8 ± 0.1^{a}	5.6 ± 0.7^{a}	1.8 ± 0.0^{a}	5.6 ± 0.2^{a}	4.2 ± 2.5^{b}	8.7 ± 1.2^{b}	60.3 ± 1.4^{a}	1.3 ± 0.3^{a}
PG	15	5.7 ± 0.5^{b}	1.4 ± 0.3^{a}	11.0 ± 0.1^{b}	20.2 ± 4.4^{a}	1.1 ± 0.6^{a}	6.1 ± 0.1^{b}	5.8 ± 0.1^{a}	8.7 ± 0.8^{a}	21.8 ± 4.0^{a}	5.9 ± 0.5^{b}	12.3 ± 0.9^{b}	82.2 ± 0.0^{a}	2.2 ± 0.1^{a}
	19	12.1 ± 2.8^{a}	1.6 ± 0.6^{a}	11.2 ± 3.3^{b}	9.0 ± 1.8^{b}	2.4 ± 1.6^{a}	7.9 ± 2.3^{b}	7.1 ± 1.3^{a}	6.5 ± 0.0^{a}	5.2 ± 2.7^{b}	11.2 ± 3.7^{a}	25.8 ± 4.1^{a}	74.3 ± 5.0^{b}	2.4 ± 0.2^{a}
	23	5.5 ± 0.4^{b}	2.5 ± 0.4^{a}	34.6 ± 2.9^{a}	12.8 ± 4.0^{b}	3.7 ± 0.6^{a}	20.2 ± 4.3^{a}	2.4 ± 0.0^{b}	2.2 ± 0.6^{b}	1.4 ± 0.1^{c}	3.2 ± 1.2^{b}	11.4 ± 0.8^{b}	56.1 ± 3.7^{c}	1.2 ± 0.2^{b}
DPG	15	13.7 ± 2.2^{a}	1.6 ± 0.3^{a}	10.6 ± 2.2^{b}	7.5 ± 0.5^{ab}	0.6 ± 0.2^{b}	3.9 ± 1.5^{b}	8.9 ± 0.8^{a}	4.3 ± 0.7^{b}	3.4 ± 0.2^{b}	17.6 ± 3.9^{a}	28.1 ± 2.6^{a}	75.1 ± 4.3^{a}	2.6 ± 0.3^{a}
	19	11.2 ± 1.0^{a}	1.3 ± 0.4^{a}	9.3 ± 2.6^{b}	10.7 ± 1.5^{a}	0.4 ± 0.1^{b}	3.2 ± 0.2^{b}	7.8 ± 0.1^{a}	5.0 ± 0.7^{a}	5.1 ± 1.4^{a}	14.6 ± 3.2^{a}	31.6 ± 3.2^{a}	79.2 ± 3.5^{a}	2.8 ± 0.2^{a}
	23	14.0 ± 1.9^{a}	1.2 ± 0.1^{a}	25.8 ± 2.4^{a}	5.8 ± 0.4^{b}	4.1 ± 0.9^{a}	21.5 ± 2.7^{a}	4.1 ± 0.7^{b}	3.1 ± 0.6^{c}	2.9 ± 0.5^{b}	6.0 ± 1.5^{c}	11.5 ± 2.1^{b}	56.1 ± 3.0^{b}	1.4 ± 0.2^{b}
UN	15	7.4 ± 0.7^{a}	1.6 ± 0.1^{a}	17.9 ± 3.9^{a}	10.6 ± 0.8^{a}	1.6 ± 0.1^{a}	2.0 ± 0.4^{a}	2.6 ± 1.7^{a}	1.5 ± 0.4^{a}	0.8 ± 0.3^{b}	16.4 ± 3.5^{b}	37.6 ± 4.6^{a}	73.2 ± 3.8^{a}	2.8 ± 0.4^{a}
	19	7.7 ± 0.4^{a}	3.0 ± 1.6^{a}	17.7 ± 4.5^{a}	10.4 ± 0.5^{a}	3.3 ± 2.9^{a}	2.2 ± 1.2^{a}	4.1 ± 2.2^{a}	3.1 ± 3.3^{a}	1.5 ± 0.9^{ab}	16.2 ± 2.9^{b}	30.7 ± 4.7^{a}	71.3 ± 4.3^{a}	2.6 ± 0.2^{a}
	23	7.0 ± 1.2^{a}	0.5 ± 0.2^{a}	9.5 ± 0.2^{a}	7.1 ± 1.4^{b}	2.0 ± 0.8^{a}	3.4 ± 0.8^{a}	5.5 ± 0.4^{a}	4.3 ± 0.4^{a}	2.8 ± 0.1^{a}	27.5 ± 0.4^{a}	30.3 ± 1.1^{a}	81.4 ± 0.4^{a}	3.0 ± 0.1^{a}

[A] Data are expressed as mean ± SD of three replicates. The data marked with the same lower case letters in the same column of each lipid class are not significantly different ($p < 0.05$).

[B] MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; DGDG, digalactosyldiacylglycerol; SQDG, sulphoquinovosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; LPC, lysophosphatidylcholine; PI, phosphatidylinositol; PC, phosphatidylcholine; PG, phosphatidylglycerol; DPG, diphosphatidylglycerol; UN, unidentified lipid fractions; Biomass, fatty acid composition in whole microalgal biomass.

[C] Unsatd: percentage of unsaturated fatty acids of the total fatty acids.

[D] DUS (∇/mol): the degree of fatty acid unsaturation, DUS = (1.0 (% monoene) + 2.0 (% diene) + 3.0 (triene) + 4.0 (% tetraene) + 5.0 (% pentaene))/100.

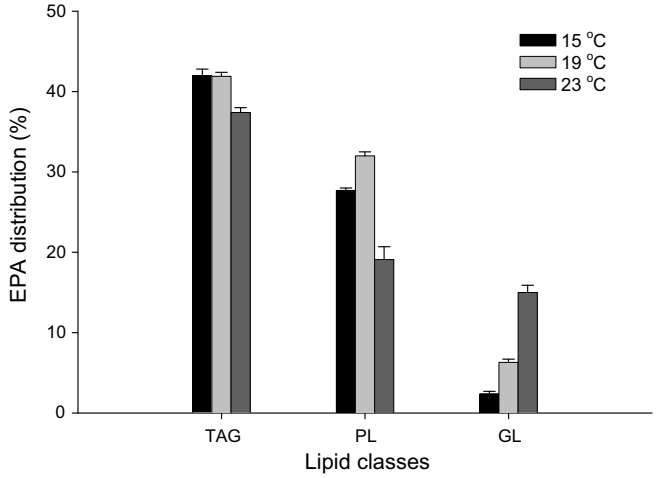

Fig. 2. Distribution of eicosapentaenoic acid (EPA) in Triacylglycerol (TAG), phospholipid (PL) and glycolipid (GL) of *Nitzschia laevis* at different temperatures. Values are represented as mean ± standard deviation of triplicates.

desaturase was sensitive to the temperature change and were greatly stimulated by the increase of dissolved oxygen concentration at low temperature (Jiang & Chen, 2000; Seto, Wang, & Hesseltine, 1984). The increase of PUFA proportion and the modification of the molecular species in PLs were suggested to be the major strategies to maintain membrane fluidity and to stabilize the fluid bilayers under low temperature. Moreover, the higher degree of membrane fatty acid saturation was suggested to be involved in maintaining the integrity of cell membranes at elevated temperatures in the alga (Kleinschmidt & McMahon, 1970; Murphy, 2004). Since PLs were the major membrane lipids in the investigated microalga (Chen et al., 2007), the relatively high growth activity of *N. laevis* under low temperature such as 15 °C, in terms of its X_{max} and $Y_{x/glu}$ (Table 1) was attributed to the increased value of DUS, which played an important role in the maintenance of the membrane stability.

In photosynthetic algae and plants, the high degree of chloroplast membrane lipid unsaturation was required for some aspect of chloroplast biogenesis. The contents of PUFAs were generally very high in DGDG and MGDG (Hugly & Somerville, 1992). In this investigation, the GLs of heterotrophic *N. laevis* also had high proportion of unsaturated fatty acids such as EPA in their molecular species (Table 2), although the EPA in GLs was only accounted for a very low part of the total cellular EPA.

4. Conclusion

The effects of temperature on the lipid class composition and the distribution of fatty acids in the lipid pool of *N. laevis* were investigated. It was shown that temperature greatly affected the cell growth of *N. laevis* but had less influence on its lipid and fatty acid contents such as PC and EPA. TAG was found to be the predominant lipid fraction and the amount decreased along with the decrease of temperature. PC was the largest polar lipid component and low temperature improved its accumulation. Moreover, polar lipids, especially PLs contained high percentage of PUFA and low temperature favored the distribution of PUFAs in PLs. The highest PL content was accumulated at 19 °C and the EPA content in PL at this temperature was higher than that at other temperatures. The culture temperature of 19 °C will be suitable for the cultivation of *N. laevis* with higher PC content, although the fact was unsatisfactory that the change of PC content under different growth temperatures was slight in this study. On the other hand, the effect of temperature shift (such as shift from high to low temperature) will be investigated in the future to get high EPA and lipid productivity with best lipid and fatty acid composition, because high temperature contributes to higher biomass concentration whereas cold environment gives better lipid composition. This strategy will be practical and economic for the aquaculture and functional food industries if the test is successful.

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