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EFFECT OF PHOTOPERIOD ON THE CELLULAR FATTY ACID COMPOSITION OF THREE TROPICAL MARINE MICROALGAE

LIM, K.C.* and ZALEHA, K.

Institute of Tropical Aquaculture, Universiti Malaysia Terengganu
21300, Kuala Terengganu, Terengganu
**E-mail: zizou_chin6666@yahoo.com*

ABSTRACT

The culture of microalgae in hatcheries is promoted for their high essential fatty acid (EFA) content which favors the successful rearing of aquatic animals. In this study, batch cultures of *Chaetoceros calcitrans*, *Chlorella* sp. and *Nannochloropsis* sp. were acclimated under photoperiods of 24:0h, 12:12h and 8:16h L/D regimes at 20ºC to analyze for cellular fatty acid in the logarithmic growth phase. The various photoperiods resulted in differences of cellular fatty acid content of cultivated microalgae revealing influences and strong interactions between the photoperiods studied. The photoperiod of 12:12h L/D regime is recommended for fast and economic batch culture production of *Chaetoceros calcitrans*, *Chlorella* sp. and *Nannochloropsis* sp. cells rich in EFA.

ABSTRAK

Pengkulturan mikroalga di hatceri digalakkan kerana ketinggian kandungan asid lemak perlu yang mendorong kepada kejayaan penternakan haiwan akuatik. Dalam kajian ini, pengkulturan *Chaetoceros calcitrans*, *Chlorella* sp. dan *Nannochloropsis* sp. diaklimasikan dibawah fotokala 24:0, 12:12 dan 8:16 jam rejim cahaya/gelap pada suhu 20ºC untuk dianalisis kandungan asid lemak sel dalam fasa pertumbuhan logaritma. Pelbagai fotokala telah menyebabkan perbezaan di dalam kandungan asid lemak sel mikroalga yang dikultur menunjukkan pengaruh dan interaksi kuat di antara tiga fotokala yang dikaji. Fotokala 12:12 jam rejim cahaya/gelap telah disyorkan untuk pengkulturan sel *Chaetoceros calcitrans*, *Chlorella* sp. dan *Nannochloropsis* sp. yang cepat dan berekonomi serta kaya dengan asid lemak perlu.

Key words: Cellular fatty acid content, *Chaetoceros calcitrans*, *Chlorella* sp., microalgae, *Nannochloropsis* sp., photoperiod

INTRODUCTION

Lipid nutrition is one of the keys for success in the commercial larviculture of marine fish, mollusks and crustaceans. The nutritional value of microalgae is related solely on its proximate biochemical composition, especially the lipid class and fatty acid compositions (Muller-Feuga *et al*., 2003). Fatty acids are regarded as the building blocks and important component of lipid (Matias-Peralta, 2005). These essential fatty acids (EFAs) are considered as key nutrients and important biomolecules which enhance the growth and reproduction of many marine animals (Meireles *et al*., 2003; El Abed *et al*., 2008; Marshall *et al*., 2010). Marine fish, mollusks and crustaceans produced via aquaculture generally exhibit poor ability to synthesize certain essential long chain PUFAs in quantities high enough for growth and survival especially EPA (20:5n-3) as well as DHA (22:6n-3) through chain elongation and desaturation (Becker, 2003; Martin-Creuzburg and Elert, 2004; Graham *et al*., 2009). As a consequence, it is very important that food sources provided to these animals supply all the essential PUFAs which are present or exist abundantly in microalgae in order to satisfy their dietary requirements.

Lipid content of microalgae has been studied by researchers worldwide. Evidently microalgal lipid patterns are associated with their taxonomic position and are usually species or strain specific (Becker, 2003; Muller-Feuga *et al*., 2003). Furthermore, microalgal lipids are greatly influenced by culturing conditions which include nutrient deprivation, light

* To whom correspondence should be addressed.

quality, photon flux density (PFD), photoperiod (L/D cycle) as well as temperature both qualitatively and quantitatively (Converti *et al*., 2009; Mata *et al*., 2010). Therefore, for daily nursery or hatchery practices, and for nutrition experiments, an optimization of the lipid content of microalgae is indeed required thus improving the nutritional quality of the microalgae. This study is concerned with the effect of photoperiod as part of the broader optimization study of the total fatty acid and polyunsaturated fatty acid contents of microalgae since the fact that it is scarcely reported.

MATERIALS AND METHODS

Batch cultivation

Tropical *Chlorella* sp. were isolated from the water in the vicinity of Bidong Island, Terengganu, Malaysia through successive plating on agar plates. Meanwhile, starter cultures of Malaysian tropical *Chaetoceros calcitrans* and *Nannochloropsis* sp. strains were obtained from National Prawn Fry Production and Research Centre (NAPFRE), Kedah, Malaysia. All microalgae species were stocked as monospecific cultures under optimum conditions in the laboratory of Universiti Malaysia Terengganu (UMT), Terengganu, Malaysia.

Batch cultivations of 2 L were scaled-up from 500 mL monospecific cultures of each microalgal species in 2 L conical flasks. Experiment was conducted in three replications under continuous and discontinuous light of 24:0h, 12:12h and 8:16 h L/D regimes at a constant temperature of 20°C enriched with Conway media. Cool-white fluorescent lamps (40 watts or 11900 lux) were employed as illumination source. All cultures were started with an equal inoculum (2.5 x 10^4 cells mL^{-1}) of respective species. Cultures were aerated continuously using humidified filtered air and evaporation was kept to minimum by covering the top of the culturing flasks with parafilm. The cultures were left to acclimate under these conditions until the logarithmic growth phase and harvested prior to fatty acid analysis.

Lipid extraction

Total lipids of microalgae species were extracted using the method described by Zhu *et al*. (2002) as a modification to the wet extraction procedure by Bligh and Dyer (1959). Wet cell mass was frozen overnight at -70°C and freeze-dried at -70°C under a vacuum freeze-dryer. After drying the samples, 1 g of samples were immersed into clean vials containing 9 mL mixtures of chloroform: methanol (1:2 v/v) and vortexed well using a vortex mixer. The samples were rinsed with 12 mL of chloroform and vortexed well again. A total of 24 mL distilled water was added before centrifuging at 4000 rpm for 5 minutes to give a two-phase system. The top aqueous phase was discarded and the lower organic phase was then dried in the oven at 60°C. The solvent was then evaporated and the lipid was transferred to a clean vial. Five µg of phenanthrene was used as internal standard to identify a complete lipid extraction from the microalgae species. Dilution was done with 0.5 mL of diethyl ether in 10 mL of methanol and sodium hydroxide solution (0.5 M). Samples were then idled at room temperature for 30 minutes with mild agitation. This was followed by the addition of 10 mL sodium carbonate solution (50g L^{-1}) before vortexing again. The fatty acid methyl ester (FAME) derivatives were then extracted with 10 mL of hexane. Samples were centrifuged at 4000 rpm for 5 minutes to provide complete separation giving a two-phase system. The upper phase was retained for lipid analysis via gas chromatography.

Fatty acid analysis

FAME analyses were carried out by gas chromatography equipped with flame ionization detector using DB-5HT (5% phenyl)-methypolysiloxane non-polar column (15 m x 0.32 mm ID; Agilent Tech., Palo Alto, California). Injection and detector temperature both were 370°C. Initial column temperature was 240°C, and the temperature was increased to 300°C at a temperature gradient of 15.8°C/min (Carrasco-Pancobo *et al*., 2009; Widjaja *et al*., 2009). The component FAMEs were identified by comparing their retention times and fragmentation patterns with those of the authentic standards of Supelco 37 Component FAME Mix containing 37 fatty acids well identified.

Analytical procedure

Fatty acid concentrations (CFA, mg/g of dry sample) were calculated by comparing the peak area of fatty acid in the sample with the peak area of internal standard according to the following expression (Abdulkadir and Tsuchiya, 2008):

$$C_{FA} = A_S/A_{IS} \times C_{IS}/W_S$$

where A_S = peak area of fatty acid in the sample in chromatogram, A_{IS} = peak area of internal standard in chromatogram, C_{IS} = concentration of internal standard (mg), W_S = weight of sample (g).

Statistical analysis

Statistical analysis of fatty acid composition was carried out using two-way analysis of variance (ANOVA) test to investigate for significant differences ($P<0.05$) among total fatty acid content

and variations in the weight of major fatty acid classes as proportion and per g of microalgae dry weight cultivated under different photoperiods among species. Additionally, post-hoc Tukey's multiple comparison test will only be performed if the two-way analysis of variance (ANOVA) test indicates the presence of significant differences to determine the most optimum photoperiod (Zar, 2009). Data are presented as mean ± standard error. Tests were done utilizing SPSS (Statistical Package for the Social Sciences) V11.5 statistical software package.

RESULTS

The cellular fatty acid compositions of *Chaetoceros calcitrans*, *Chlorella* sp. and *Nannochloropsis* sp. (expressed as mg g^{-1} of dry weight) cultivated under 24:0h, 12:12h and 8:16h L/D regimes were presented in Table 1, 2 and 3 respectively. On the other hand, the comparisons of the weight of major fatty acid classes under different photoperiods were depicted in Figure 1, 2 and 3 respectively. Based on the results, a total of 29 fatty acids were identified from the three species of marine microalgae with significant differences detected in total fatty acids (mg g^{-1} of algae dry weight) between species under different photoperiods. Cultivation of *Chaetoceros calcitrans*, *Chlorella* sp. and *Nannochloropsis* sp. under 12:12h L/D regime displayed noticeably higher total fatty acid contents (0.589±0.004 mg g^{-1}, 4.494±0.030 mg g^{-1} and 5.396±0.073 mg g^{-1} respectively) comparably to those cultured using 24:0h (0.443±0.016 mg g^{-1}, 3.652±0.055 mg g^{-1} and 2.473±0.004 mg g^{-1} respectively) and 6:18h (0.273±0.023 mg g^{-1}, 0.732±0.026 mg g^{-1} and 0.830±0.014 mg g^{-1} respectively) L/D regimes (Figure 1, 2 and 3 respectively). Two-way analysis of variance (ANOVA) test had demonstrated the presence of significant differences ($P<0.05$) and considerable variability of total fatty acid content between treatments. Additionally, post-hoc Tukey's multiple comparison tests revealed that total fatty acid contents were most significant ($P<0.05$) for all treatments when cultivated under 12:12h L/D regime.

The major fatty acid classes which consisted of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) also displayed significant differences among fatty acid concentrations (mg g^{-1} of algae dry weight) when acclimatized under different photoperiods. All the cultivated microalgae species exhibited similar patterns of variation in the concentration of fatty acid classes according to photoperiod acclimation. In fact, the cultivated *Chaetoceros calcitrans*, *Chlorella* sp. and *Nannochloropsis* sp. under 12:12h L/D regime had all denoted remarkably higher values of SFA, MUFA and PUFA than under 24:0h and 6:18h L/D regimes. Those variables reached the lowest values when acclimatized under the 6:18h L/D regime. Significant differences ($P<0.05$) were detected by two-way analysis of variance (ANOVA) test with considerable variability in the weights of major fatty acid classes between treatments. Furthermore, post-hoc Tukey's multiple comparison tests had also shown the most significant difference ($P<0.05$) and best optimization of cellular fatty acid content for all treatments under 12:12h L/D regime.

DISCUSSION

Quite a number of factors influencing the cellular fatty acid content of microalgae have been proposed which include temperature (James *et al*., 1989; Renaud *et al*., 2002; Chen *et al*., 2008), growth rate (Saoudi-Helis *et al*., 1994; Muller-Feuga *et al*., 2003), nutrient deprivation (Harrison *et al*., 1990; Mata *et al*., 2010), pH (Cohen *et al*., 1988; Borges *et al*., 2011), light quality (Sanchez Saavedra and Voltolina, 1994; Richmond, 2004; Mata *et al*., 2010), photon flux density (Renaud *et al*., 1991; Sukenik *et al*., 1993; Chen *et al*., 2011) and photoperiod (Sicko-Goad and Andresen, 1991; Tzovenis *et al*., 1997; Hu, 2004).

One of the most interesting aspects that have emerged from this study was the obvious effect of photoperiod or day length on the cellular fatty acid composition of three tropical microalgae species cultivated. At 20°C, *Chaetoceros calcitrans*, *Chlorella* sp. and *Nannochloropsis* sp. had demonstrated a preference of 12:12h L/D regime for optimal production or yield of total cellular fatty acid content, SFA, MUFA and PUFA. Numerous studies with microalgae of various groups had proposed that the cellular lipid constituent and total SFA, MUFA and PUFA including the essential eicosapentaenoic acid (C20:5n-3) (EPA) are inversely related to growth light intensity (Cohen, 1999; Hu, 2004). This is in turn related to day-length exposure where a longer time resulted in greater light intensity which might be received for photosynthesis. It has been previously documented by Sukenik *et al*. (1989), Tzovenis *et al*. (1997) and Hu (2004) that microalgae cells contain high lipid and polyunsaturated fatty acid (PUFA) when cultivated under light-limiting conditions sufficient for photosynthesis. Low light enhanced the productions of essential fatty acids (EFAs) including PUFAs which are usually coupled with a

Table 1. Cellular fatty acid composition (value expressed as mg g^{-1} of dry weight) of *Chaetoceros calcitrans* cultivated under 24:0h, 12:12h and 8:16h L/D regimes. Data are mean value and standard deviation of three repetitions

Fatty acids	Designation	Concentration (mg g^{-1} of dry weight)		
		24:0h L/D	12:12h L/D	8:16h L/D
Saturated fatty acids (SFA)				
Capric acid	C10:0	0.022±0.004	0.018±0.003	0.018±0.007
Undecanoid acid	C11:0	0.012±0.003	0.008±0.001	0.010±0.001
Lauric acid	C12:0	0.007±0.004	0.008±0.005	0.020±0.005
Tridecanoic acid	C13:0	0.023±0.005	0.014±0.002	0.030±0.007
Myristic acid	C14:0	0.104±0.016	0.086±0.006	0.042±0.003
Pentadecanoic	C15:0	0.003±0.002	0.002±0.001	0.002±0.001
Palmitic acid	C16:0	0.072±0.006	0.035±0.012	0.024±0.005
Stearic acid	C18:0	0.010±0.003	0.005±0.001	0.002±0.001
Behenic acid	C22:0	–	0.018±0.003	–
Lignoceric acid	C24:0	–	0.105±0.005	–
Subtotal		***0.253±0.019***	***0.299±0.006***	***0.148±0.018***
Monounsaturated fatty acids (MUFA)				
Myristoleic acid	C14:1(n-5)	0.002±0.001	0.005±0.002	0.006±0.001
Cis-10-Pentadecenoic acid	C15:1	0.016±0.003	0.015±0.001	0.017±0.003
Palmitoleic acid	C16:1(n-7)	0.064±0.007	0.063±0.006	0.015±0.003
Cis-10-Heptadecenoic acid	C17:1(n-7)	0.004±0.001	0.006±0.002	–
Subtotal		***0.086±0.011***	***0.089±0.008***	***0.038±0.004***
Polyunsaturated fatty acids (PUFA)				
Linoleic acid *(Omega-6)*	C18:2(n-6)	0.003±0.001	0.002±0.001	0.007±0.003
Linolenic acid (*Omega-3*)	C18:3(n-3)	0.056±0.007	0.102±0.006	0.031±0.005
Eicosapentaenoic acid (***EPA***) *(Omega-3)*	C20:5(n-3)	0.045±0.001	0.104±0.004	0.049±0.003
Subtotal		***0.104±0.007***	***0.208±0.005***	***0.087±0.004***
Total		**0.443±0.016**	**0.596±0.004**	**0.273±0.023**

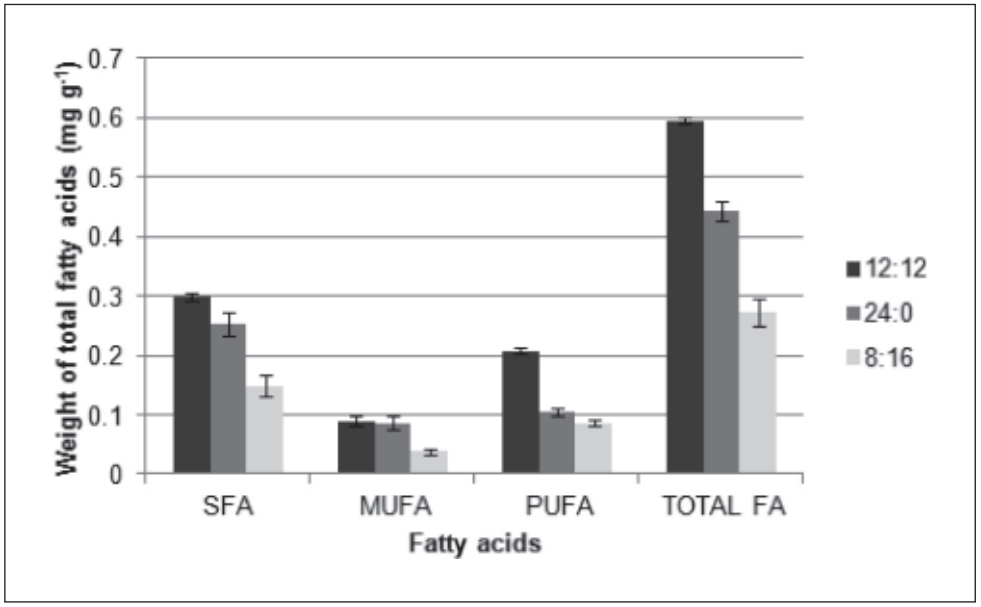

Fig. 1. Weight of total fatty acids (expressed as mg g^{-1} of dry weight) of *Chaetoceros calcitrans* cultivated under different photoperiods (L/D hours). SAFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids. Data are mean value and standard deviation of three repetitions.

Table 2: Cellular fatty acid composition (value expressed as mg g^{-1} of dry weight) of *Chlorella* sp. cultivated under 24:0h, 12:12h and 8:16h L/D regimes. Data are mean value and standard deviation of three repetitions

Fatty acids	Designation	Concentration (mg g^{-1} of dry weight)		
		24:0h L/D	12:12h L/D	8:16h L/D
Saturated fatty acids (SFA)				
Caprylic acid	C8:0	0.003±0.001	–	–
Capric acid	C10:0	0.009±0.002	0.014±0.002	0.007±0.002
Undecanoid acid	C11:0	0.010±0.001	0.013±0.002	0.006±0.003
Lauric acid	C12:0	0.027±0.003	0.013±0.002	0.018±0.005
Tridecanoic acid	C13:0	0.004±0.002	0.016±0.002	0.007±0.002
Myristic acid	C14:0	0.315±0.008	0.238±0.017	0.049±0.002
Pentadecanoic acid	C15:0	0.012±0.003	0.016±0.004	0.004±0.002
Palmitic acid	C16:0	1.578±0.044	2.335±0.024	0.267±0.006
Heptadecanoic acid	C17:0	0.009±0.002	0.007±0.002	0.002±0.001
Stearic acid	C18:0	0.018±0.001	0.022±0.003	0.006±0.002
Elaidic acid	C18:1(n-9)	0.274±0.008	0.318±0.003	0.010±0.002
Arachidic acid	C20:0	–	0.002±0.001	–
Heneicosanoic acid	C21:0	0.014±0.002	–	–
Behenic acid	C22:0	–	0.042±0.003	–
Lignoceric acid	C24:0	–	–	–
Subtotal		***2.000±0.041***	***2.718±0.028***	***0.366±0.014***
Monounsaturated fatty acids (MUFA)				
Myristoleic acid	C14:1(n-5)	0.004±0.002	0.011±0.001	0.009±0.002
Cis-10-Pentadecenoic acid	C15:1	0.005±0.002	0.012±0.003	0.001±0.000
Palmitoleic acid	C16:1(n-7)	1.077±0.008	1.071±0.033	0.182±0.012
Cis-10-Heptadecenoic acid	C17:1(n-7)	–	0.005±0.002	0.004±0.002
Oleic acid	C18:1(n-7)	0.079±0.007	0.114±0.004	–
Cis-11-Eicosenoic acid	C20:1(n-9)	0.002±0.001	0.001±0.001	–
Subtotal		***1.441±0.007***	***1.532±0.039***	***0.206±0.012***
Polyunsaturated fatty acids (PUFA)				
Linoleic acid *(Omega-6)*	C18:2(n-6)	0.038±0.003	0.030±0.003	0.008±0.002
Linolenic acid (*Omega-3*)	C18:3(n-3)	0.078±0.009	0.095±0.002	0.107±0.002
ã-linolenic acid *(Omega-6)*	C18:3(n-6)	0.004±0.002	0.002±0.001	0.006±0.002
Eicosadienoic acid (*Omega-6*)	C20:2(n-6)	–	0.001±0.001	–
Eicosatrienoic acid (*Omega-3*)	C20:3(n-3)	–	0.002±0.001	0.003±0.001
Arachidonic acid (***AA***) (*Omega-6*)	C20:4(n-6)	0.031±0.007	0.018±0.004	0.006±0.003
Eicosapentaenoic acid (***EPA***) *(Omega-3)*	C20:5(n-3)	0.060±0.003	0.095±0.005	0.030±0.003
Subtotal		***0.211±0.009***	***0.244±0.004***	***0.160±0.005***
Total		**3.652±0.055**	**4.494±0.030**	**0.732±0.026**

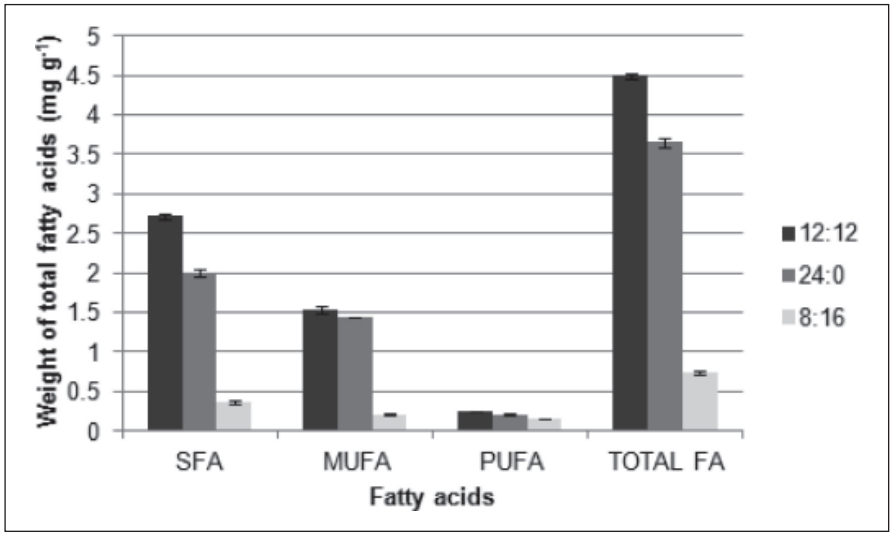

Fig. 2. Weight of total fatty acids (expressed as mg g^{-1} of dry weight) of *Chlorella* sp. cultivated under different photoperiods (L/D hours). SAFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids. Data are mean value and standard deviation of three repetitions.

Table 3: Cellular fatty acid composition (value expressed as mg g^{-1} of dry weight) of *Nannochloropsis* sp. cultivated under 24:0h, 12:12h and 8:16h L/D regimes. Data are mean value and standard deviation of three repetitions

Fatty acids	Designation	Concentration (mg g^{-1} of dry weight)		
		24:0h L/D	12:12h L/D	8:16h L/D
Saturated fatty acids (SFA)				
Capric acid	C10:0	0.015±0.003	0.019±0.002	0.020±0.002
Undecanoid acid	C11:0	0.012±0.002	0.010±0.003	0.010±0.002
Lauric acid	C12:0	0.015±0.002	0.014±0.001	0.015±0.002
Tridecanoic acid	C13:0	0.026±0.003	0.007±0.002	0.021±0.002
Myristic acid	C14:0	0.138±0.003	0.229±0.004	0.049±0.001
Pentadecanoic acid	C15:0	0.005±0.001	0.021±0.003	0.005±0.001
Palmitic acid	C16:0	1.259±0.006	2.906±0.074	0.322±0.005
Heptadecanoic acid	C17:0	0.005±0.001	0.013±0.002	0.001±0.000
Stearic acid	C18:0	0.017±0.003	0.041±0.002	0.013±0.002
Behenic acid	C22:0	–	–	0.040±0.003
Subtotal		***1.492±0.006***	***3.260±0.076***	***0.496±0.011***
Monounsaturated fatty acids (MUFA)				
Myristoleic acid	C14:1(n-5)	0.009±0.002	0.008±0.001	0.007±0.002
Cis-10-Pentadecenoic acid	C15:1	0.016±0.003	0.013±0.002	0.012±0.002
Palmitoleic acid	C16:1(n-7)	0.479±0.005	0.970±0.005	0.115±0.003
Oleic acid	C18:1(n-7)	0.123±0.003	0.150±0.008	0.094±0.003
Elaidic acid	C18:1(n-9)	0.233±0.005	0.291±0.008	–
Cis-11-Eicosenoic acid	C20:1(n-9)	0.001±0.000	-	–
Subtotal		***0.861±0.005***	***1.432±0.008***	***0.228±0.008***
Linoleic acid *(Omega-6)*	C18:2(n-6)	0.077±0.003	0.508±0.009	0.014±0.002
Linolenic acid (*Omega-3*)	C18:3(n-3)	0.014±0.002	0.091±0.007	0.029±0.003
ã-linolenic acid *(Omega-6)*	C18:3(n-6)	0.006±0.001	0.016±0.002	0.003±0.002
Eicosatrienoic acid (*Omega-3*)	C20:3(n-3)	–	0.002±0.001	0.002±0.001
Arachidonic acid (***AA***) (*Omega-6*)	C20:4(n-6)	0.006±0.002	0.008±0.002	0.008±0.002
Eicosapentaenoic acid (***EPA***) *(Omega-3)*	C20:5(n-3)	0.017±0.002	0.079±0.009	0.050±0.002
Subtotal		***0.120±0.005***	***0.704±0.008***	***0.106±0.008***
Total		**2.473±0.004**	**5.396±0.073**	**0.830±0.014**

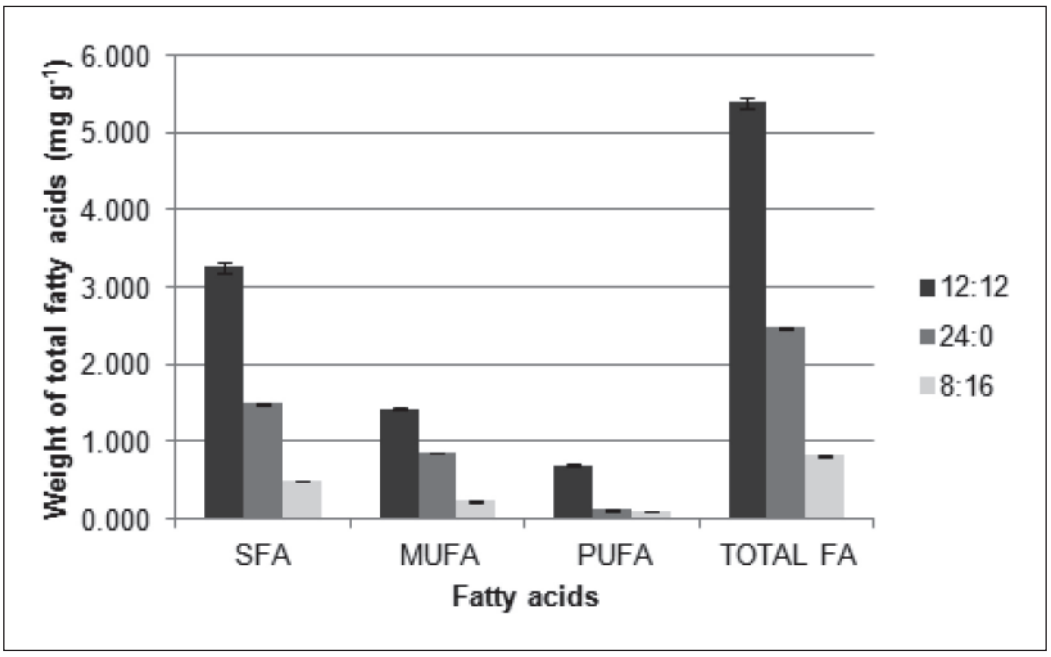

Fig. 3. Weight of total fatty acids (expressed as mg g^{-1} of dry weight) of *Nannochloropsis* sp. cultivated under different photoperiods (L/D hours). SAFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids. Data are mean value and standard deviation of three repetitions.

concomitant increase in total thylakoid membrane in the microalgae cells since the fact that all essential fatty acids (EFA) are the major constituents of the thylakoid membranes (Saoudi-Helis *et al*., 1994; Tzovenis *et al*., 1997; Muller-Feuga *et al*., 2003). The outcome of this study was also in accordance with the study conducted by Tzovenis *et al*. (1997) in which maximal docosahexanoic acid (DHA) content of *Isochrysis* aff. *galbana* was obtained when grown under 12:12h L/D regime. In addition, Brown *et al*. (1993) had also suggested that microalgae cells grown under 12:12h L/D regime may provide optimal nutritional value for aquaculture animals. On the contrary, more light energy supply will in fact decrease the number of the chloroplast thylakoids causing decrement of cellular fatty acid content (Tzovenis *et al*., 1997; Masojidek *et al*., 2004; Poerschmann *et al*., 2004; Sasaki and Nagano, 2004). Hence, notable changes in the cellular fatty acid content is expected under the conditions that affect the membrane metabolism (cellular and photosynthetic). Nevertheless, this could possibly attributed to the trend of substantially pronounced amount of total fatty acid and essential fatty acid contents detected in the microalgae species when grown under 12:12h L/D regime than 24:0h L/D regime in comparison. On the other hand, the acclimatization of microalgae cultures under 8:16h L/D regime seems to be insufficient for the optimization of cellular fatty acid content in the batch cultivation of microalgae mainly due to the significantly lower amount of cellular fatty acids recorded in this study. This could possibly be explained by the reason that lipids and fatty acids were oxidized when cells require energy in the dark during insufficiency of light for photosynthesis (Amblard and Bourdier, 1988; Sicko-Goad and Andresen, 1991; Sasaki and Nagano, 2004).

CONCLUSION

Photoperiods or light regimes pose a significant interactive effect on the cellular fatty acid composition of cultivated microalgae cells. Cultivation of microalgae under a photoperiod of 12:12h L/D regime should be a basis for a batch culture production system for maximal cellular fatty acid content of microalgae cells. Therefore, a photoperiod of 12:12h L/D regime is an attractive alternative to continuous illumination since it is more economical for hatchery application and permits outdoor use.

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REFERENCES

Abdulkadir, S. and Tsuchiya, M. 2008. One-step method for quantitative and qualitative analysis of fatty acids in marine animal samples. *J. Exp. Mar. Biol. Ecol*., **354**, pp. 1-8.

Amblard, C. and Bourdier, G. 1988. Evidence for dial changes in adenine nucleotides, fatty acid contents and primary productivity in lacustrine phytoplankton. *Arch. Hydrobiol*., **113**, pp. 1-14.

Becker, E.W. 2003. Microalgae in human and animal nutrition. In: *Live Feeds in Marine Aquaculture* (eds. Stottrup, J.G and Mcevoy, L.A.). *Blackwell Publishing Ltd.*, USA, pp. 312-350.

Bligh, E.G. and Dyer, W.J. 1959. A rapid method for total lipid extraction and purification. *Can. J. Biochem. Physiol*., **37**, pp. 911-917.

Borges, L., Moron-Villarreyes, J.A., D'Oca, G. and Abreu, P.C. 2011. Effects of flocculants on lipid extraction and fatty acid composition of the microalgae *Nannochloropsis oculata* and *Thalassiosira weissflogii*. *Biomass and Bioenergy*, **35**, pp. 4449-4454.

Brown, M.R., Dunstan, G.A., Jeffrey, S.W., Volkman, J.K., Barret, S.M. and Leroi, J.M. 1993. The influence of irradiance on the biochemical composition on the prymnesiophyte *Isochrysis* sp. (clone T-ISO). *Journal of Phycology*, **29**, pp. 601-612.

Carrasco-Pancobo, A., Navas-Iglesias, N. and Cuadros-Rodriguez, L. 2009. From lipid analysis towards lipidomics, a new challenge for the analytical chemistry of the 21st century. Part 1: Modern lipid analysis. *Trends in Analytical Chemistry*, **28**, pp. 3-9.

Chen, G.Q., Jiang, Y. and Chen, F. 2008. Variation of lipid class composition in *Nitzschia laevis* as a response to growth temperature change. *Food Chemistry*, **109**, pp. 88-94.

Chen, C.Y., Yeh, K.L., Aisyah, R., Lee, D.J. and Chang, J.S. 2011. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. *Bioresource Technology*, **102**, pp. 71-81.

Cohen, Z., Vonshak, A. and Richmond, A. 1988. Effect of environmental conditions on fatty acid composition of the red algae *Phorphyridium cruentum*: correlation to growth rate. *Journal of Phycology*, **24**, pp. 328-332.

Cohen, Z. 1999. Phorphyridium cruentum. In: *Chemicals from microalgae* (ed. Cohen, Z.). *Taylor and Francis*, London, pp. 41-56.

Converti, A., Casazza, A.A., Ortiz, E.Y., Perego, P. and Borghi, M.D. 2009. Effect of temperature and nitrogen concentration on the growth and lipid content of *Nannochloropsis oculata* and *Chlorella vulgaris* for biodiesel production. *Chemical Engineering and Processing*, **48**, pp. 1146-1151.

El Abed, M.M., Marzouk, B., Medhioub, M.N., Helal, A.N. and Medhioub, A. 2008. Micro-algae: a potential source of polyunsaturated fatty acids. *Nutritional health*, **19**, pp. 221-226.

Graham, L.E., Graham, J.M. and Wilcox, L.W. 2009. Algae, 2nd edition. *Pearson*, San Francisco, 616 pp.

Harrison, P.J., Thompson, P.A. and Calderwood, G.S. 1990. Effects of nutrient and light limitation on the biochemical composition of phytoplankton. *Journal of Applied Phycology*, **2**, pp. 45-56.

Hu, Q. 2004. Environmental effects on cell composition. In: *Handbook of Microalgal Mass Culture, Biotechnology and Applied Phycology* (ed. Richmond, A.). *Blackwell Science Ltd.*, Oxford, UK, pp. 83-93.

James, C.M., Ai-Hinty, S. and Salman, A.E. 1989. Growth and ù3 fatty acid and amino acid composition of microalgae under different temperature regimes. *Aquaculture*, **77**, pp. 337-351.

Marshall, R., McKinley, S. and Pearce, C.M. 2010. Effects of nutrition on larval growth and survival in bivalves. *Reviews in Aquaculture*, **2**, pp. 33-55.

Martin-Creuzburg, D. and Elert, E. V. 2004. Impact of 10 dietary sterols on growth and reproduction of *Daphnia galeata*. *J. Chem. Ecol*., **30**, pp. 483-500.

Masojidek, J., Koblizek, M. and Torzillo, G. 2004. Photosynthesis in microalgae. In: *Handbook of Microalgal Mass Culture, Biotechnology and Applied Phycology* (ed. Richmond, A.). *Blackwell Science Ltd.*, Oxford, UK, pp. 20-37.

Mata, T.M., Martins, A.A. and Caetano, N.S. 2010. Microalgae for biodiesel production and other applications: a review. *Renew. Sust. Energ. Rev*., **14**, pp. 217-232.

Matias-Peralta, H., Fatimah, M. Y., Mohamed, S. and Aziz, A. 2005. Effect of some environmental parameters on the reproduction and development of a tropical marine harpacticoid copepods *Nitocra affinis* f. *californica* Lang. *Marine Pollution Bulletin,* **51**, pp. 722-728.

Meireles, L.A., Guedes, A.C. and Malcata, F.X. 2003. Lipid class composition of the microalga *Pavlova lutheri*: Eicosapentaenoic and docosahexaenoic acids. *Journal of Agricultural and Food Chemistry*, **51**, pp. 2237-2241.

Muller-Feuga, A., Moal, J. and Kaas, R. 2003. The microalgae of aquaculture. In: *Live Feeds in Marine Aquaculture* (ed. Stottrup, J.G. and Mcevoy, L.A.). *Blackwell Publishing Ltd*., USA, pp. 206-252.

Poerschmann, J., Spijkerman, E. and Langer, U. 2004. Fatty acid patterns in *Chlamydomonas* sp. as a marker for nutritional regimes and temperature under extremely acidic conditions. *Microbial Ecology*, **48**, pp. 78-89.

Renaud, S.M., Parry, D.L., Thinh, L.V., Kuo, C., Padovan, A. and Sammy, N. 1991. Effect of light intensity on the proximate biochemical and fatty acid composition of *Isochrysis* sp. and *Nannochloropsis oculata* for use in tropical aquaculture. *Journal of Applied Phycology*, **3**, pp. 43-53.

Renaud, S.M., Thinh, L.V., Lambrinidis, G. and Parry, D.L. 2002. Effect of temperature on growth, chemical composition and fatty acid composition of tropical Australian microalgae grown in batch cultures. *Aquaculture*, **211**, pp. 195-214.

Richmond, A. 2004. Biological principles of mass cultivation. In: *Handbook of Microalgal Mass Culture, Biotechnology and Applied Phycology* (ed. Richmond, A.). *Blackwell Science Ltd*., Oxford, UK, pp. 125-177.

Sanchez Saavedra, M.D.P. and Voltolina, D. 1994. The chemical composition of *Chaetoceros* sp. (Bacillariophyceae) under different light conditions. *Comparative Biochemistry and Molecular Biology*, **107**, pp. 39-44.

Saoudi-Helis, L., Dubacq, J.P., Marty, Y., Samain, J.F. and Gudin, C. 1994. Influence of growth rate on pigment and lipid composition of the microalgae *Isochrysis* aff. *galbana* clone T-ISO. *Journal of Applied Phycology*, **6**, pp. 315-322.

Sasaki, Y. and Nagano, Y. 2004. Plant acetyl-CoA carboxylase: structure, biosynthesis, regulation, and gene manipulation for plant breeding. *Bioscience Biotechnology and Biochemistry*, **68**, pp. 1175-1184.

Sicko-Goad, L. and Andreson, N.A. 1991. Effect of growth and light/dark cycles on diatom lipid content and composition. *Journal of Phycology*, **27**, pp. 710-718.

Sukenik, A., Carmeli, Y. and Berner, T. 1989. Regulation of fatty acid composition by irradiance level in eustigmatophyte *Nannochloropsis* sp. *J. Phycol*., **25**, pp. 686-692.

Sukenik, A., Zmora, O. and Carmeli, Y. 1993. Biochemical quality of marine unicellular algae with special emphasis on lipid composition. II. *Nannochloropsis* sp. *Aquaculture*, **117**, pp. 313-326.

Tzovenis, I., De Pauw, N. and Sorgeloos, P. 1997. Effect of different light regimes on the docosahexanoic acid (DHA) content of *Isochrysis* aff *galbana* (clone T-ISO). *Aquaculture International*, **5**, pp. 489-507.

Widjaja, A., Chien, C.C. and Ju, Y.H. 2009. Study on increasing lipid production from fresh water microalgae *Chlorella vulgaris*. *Journal of the Taiwan Institute of Chemical Engineers*, **40**, pp. 13-20.

Zar, J.H. 2009. Biostatistical Analysis, 5th edition. *Prentice-Hall*, New Jersey. 663 pp.

Zhu, M., Zhou, P.P. and Yu, L.J. 2002. Extraction of lipids from *Mortierella alpine* and enrichment of arachidonic acid from the fungal lipids. *Bioresour. Technol*., **84**, pp. 93-98.