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# Fatty acid composition of the marine micro alga *Tetraselmis chuii* Butcher in response to culture conditions

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**Abstract**

Micro alga *Tetraselmis chuii* Butcher is one of the species that are considered to be important sources of polyunsaturated fatty acids (PUFA). The aim of our experiment was to determine the effect of the light intensity and the water salinity on the growth and fatty acid composition of *Tetraselmis chuii*. There were significant correlation ($P \leq 0.05$) between the cell density and light intensity and cell grow rate and light intensity, the correlation were $r^2$=0.71; $r^2$=0.74 correspondingly. The lowest concentration of the cell density was observed at the lowest light intensity and the highest salinity. Palmitic acid among the saturated fatty acid, oleic acid, among mono unsaturated fatty acid, alpha-linoleic acid among the PUFA, and eicosapentaenoic acid (EPA) among high unsaturated fatty acid (HUFA), had the highest percentage. Variance analyses showed that the light intensity exhibited a significant correlation ($P \leq 0.05$) with PUFA, HUFA. However there was not significant correlation among light intensity and SFA, MUFA. This result showed that relationship between environment conditions and fatty acid composition in *T. chuii* was significant thus it can be used to produce specific profile of fatty acids in this strain.

Keywords : *Tetraselmis chuii,* cell density, light intensity, salinity and fatty acid.

## Introduction

Microalgae are the major food source for many aquatic organisms and the main live feed component in marine hatchery operations because they serve as a natural resource for polyunsaturated fatty acids (Makridis *et al*.,2006). *Tetraselmis chuii* **Butcher** is a green four-flagellated prasinophyte characterized by an ovoid body shape and a distinct curved body when viewed side ways. The micro alga measures 12-14µm in length,9-10µm in width and belongs to the family Chlamydomonadaceae. *Tetraselmis* is a sizeable genus (more than 50 species) of green flagellates. Most species are known from inshore marine environments, tide pools in particular, but a few freshwater species are also known (Bold and Wynne,1985). The pyrenoid is embedded in the single chloroplast, which occupies most of the volume of the cell, especially near its proximal end (Smith,1955). *Tetraselmis sp* are ideal for culture because they are euryhaline and eurythermal (Fabregas *et al*.,1984).Environmental factors such as temperature, salinity, pH, and light have been reported to affect microalgae growth (Creswell,2010). Light conditions are the main factors affecting microalgae physiology and the most important factor affecting microalgae photosynthesis kinetics. Such changes in the salinity of water often affect the growth, metabolism and photosynthesis of phytoplanktons (Moisander *et al*.,2002;Lartigue *et al*.,2003). Salt might have a direct effect upon processes involved in electron transport and / or photophosphorylation and result in a decreased in the quantum efficiency of photosynthesis (Seeman and Critchley,1985).Whereas high light intensity decreases total polar lipid content with a concomitant increase in the amount of neutral storage lipids, mainly TAG,s (Brown *et al*.,1996). Neutral lipids, such as TG, are storage substance and energy sources, While polar lipid, glycolipids and phospholipids are the structural components of cellural membranes as well as modulators of photosystem efficiency and regulators of energy flow(Thompson,1996).*Tetraselmis chuii* is one of the species of microalgae that is most extensively used in aquaculture and is considered to be an optimal source of long-chain PUFAs, and especially of eicosapentaenoic acid (EPA) (Meseck *et al*.,2005;Zaki and Saad,2010).The present study focuses on the adaptation of *Tetraselmis chuii* to varied range of salinity and different light intensity conditions and their effect on the growth and fatty acid composition.

## Materials and Methods

### *Growth conditions*

This study provides for to determine effect different light intensities, 2500,4500,6500 lux and the degree of the water salinity 20,25,35,40 ppt on the growth and fatty acid composition of *Tetraselmis chuii* in Conway medium. All the samples were incubated at constant temperature of 25-27˚C and pH of 7.5 to 8.2, creating a growth atmosphere that encouraged the growth of the sample daily. Light intensity was measured using a photometer (Model Lutran lx-107).Cell counts of *T.chuii* were measured using a hemocytometer. The specific growth rates (μ) of samples were determined with the following exponential growth equation: (Guillard, 1973).

$\mu = In(N(t) - N_0) / (t - t_0)$

**Table 1. Chemical compounds utilized in Conway medium preparation.**

| Chemical compound | Concentration |
|---|---|
| $NaNo_3$ | 116 g |
| $Na_2EDTA$ | 45 g |
| $H_3Bo3$ | 33.6 g |
| $Na_2H_2Po_4.4H_2O$ | 20 g |
| $Fecl_3.6H_2O$ | 1.3 g |
| $Mncl_2.4H_2o$ | 0.36 g |
| $Zncl_2$ | 2.1 g |
| $Cocl_2.6H_2o$ | 2 g |
| (NH4)6MoO7.4H2O | 0.9 g |
| $Cuso_4.5H_2o$ | 2 g |
| Vitamin B1 | 200 mg |
| Vitamin B12 | 10 mg |

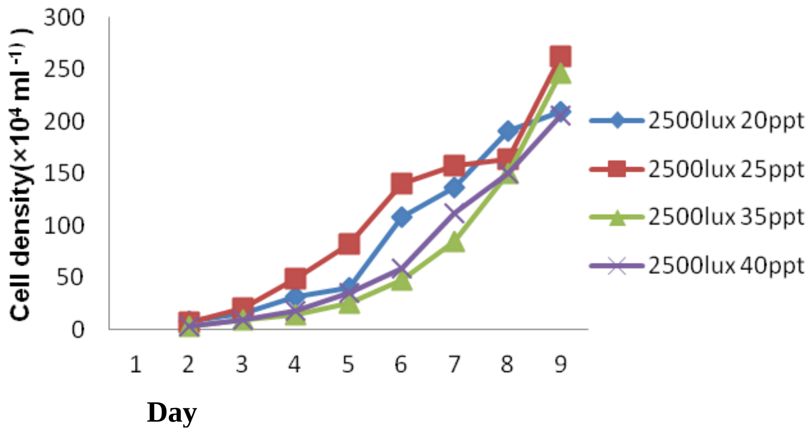
After reaching the end of an exponential phase growth, 800 ml of sample was harvested and centrifuged (model 5810R eppendrof) at 2500 rpm, for 5 min, Prior to analysis they were frozen at -70 $^{\circ}$c.

### *Lipid extraction*

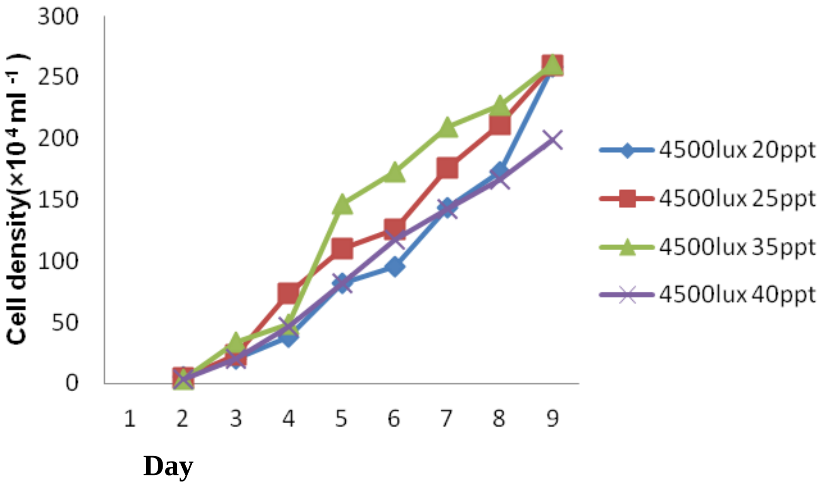
Total lipids were extracted from 1g of moist sample according to (Folch *et al*.,1957). All samples were dissolved in chloroform and methanol in ratio of 2:1. Chloroform, methanol were allowed to evaporate under nitrogen gas. The fatty acid methyl esters were prepared by direct esterification (Desvilettes *et al*.,1994) of lipid extracts. The lipid extracts were saponified in methanolic KOH (2N) for 10 min at 60°C; conversion into fatty acid methyl esters (FAME) was performed by using methanolic $H_2SO_4$. Separation and identification of the component fatty acids were done using a GC-Agilent-6890,as liquid chromatograph with a capillary column (30 m length, 0.25 mm i.d , 0.25 μm film thickness) using nitrogen as carrier gas at 1.45ml/min. The collected data were analyzed using one-way analysis of variance (ANOVA). Significant differences among the different treatments were determined using the Tukey multiple range test at 0.05 level of probability.

## Results

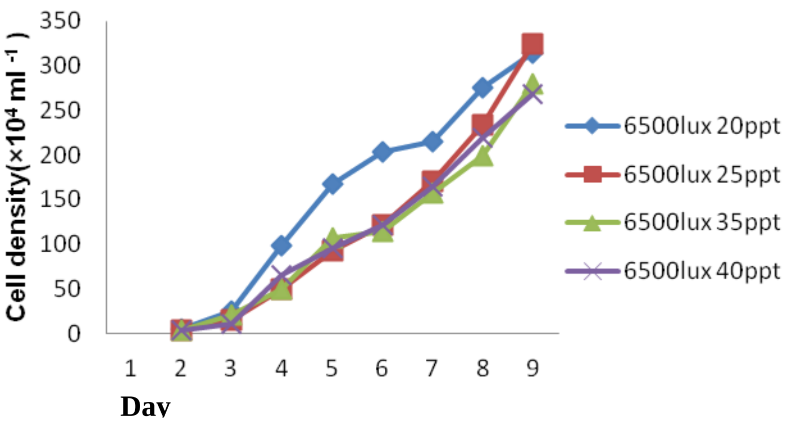
Data in table 2 shows the effect salinity and light intensity different on growth of *T.chuii*. The tests were carried out in eighth day when the alga reached maximum cell density (Figure 1). Changes in light quantity and salinity bring about the differences in fatty acids composition (Table 4). The most of SFA were C16:0, which accounted for the highest rate (28.65%) in the sample 25 ppt, 6500 lux, and the lowest rate (19.17%) in the sample 20ppt, 2500 lux determined. Ratio of PUFA to SFA was more in the sample 4500 lux, 25 ppt-6500 lux, 40ppt. The high amount of C20:4n-6 was obtained in 4500 lux,25 ppt. Among mono unsaturated fatty acids, C18:1n-9 was the most dominating. Highest percentages of n-3 showed in 6500 lux, 40 ppt (range 24.81% ) and highest percentage of n-6 in 4500 lux,20ppt (range 22.50%).




*a*




*b*




c

**Figure 1: Changes in cell density of *T.chuii* grown at three different light intensity and four different salinity : 2500lux,20,25,35,40ppt (a), 4500lux,20,25,35,40ppt (b), 6500lux,20,25,35,40ppt (c)**

 J. Algal Biomass Utln. 2015, 6 (2): 49- 55
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**Table 2: Cell density ($\times 10^4$cell ml $^{-1}$)and growth rate (division (div) day $^{-1}$) of *Tetraselmis chuii* exposed to different salinity and light in the Exponential phase.**

| Light intensity | Salinity | Cell density | Growth rate |
|---|---|---|---|
| 2500 | 20 | 210 ± 23.3 | 0.40±0.08 |
| 2500 | 25 | 262 ± 13 | 0.44±0.06 |
| 2500 | 35 | 247 ± 16.8 | 0.52±0.01 |
| 2500 | 40 | 206 ± 9.3 | 0.49±0.03 |
| 4500 | 20 | 258 ± 27.2 | 0.48±0.04 |
| 4500 | 25 | 260 ± 29.4 | 0.49±0.04 |
| 4500 | 35 | 261 ± 28.4 | 0.55±0.04 |
| 4500 | 40 | 199 ± 25.1 | 0.51±0.04 |
| 6500 | 20 | 314 ± 20.3 | 0.55±0.07 |
| 6500 | 25 | 324 ± 35.3 | 0.55±0.04 |
| 6500 | 35 | 280 ± 29.8 | 0.55±0.02 |
| 6500 | 40 | 268 ± 5.1 | 0.54±0.02 |

mean ±Standard deviations(n=3).

**Table3: Result of one way analysis of variance ANOVA.**

| Sample | Light 4 y | Salinity |
|---|---|---|
| Cell density | | 0.05* |
| Total fatty acid | 0.07 | 0.7 |
| ∑ SFA | 0.8 | 0.8 |
| ∑ MUFA | 0.01* | 0.9 |
| ∑ PUFA | 0.01* | 0.7 |
| ∑ HUFA | 0.04* | 0.7 |
| ∑ (n-3) | 0.04* | 0.9 |
| ∑ (n-6) | 0.04* | 0.8 |

*Significantly different at $P \leq 0.05$ indicate.

J. Algal Biomass Utln. 2015, 6 (2): 49- 55
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**Table4: Fatty acids composition (percent of total fatty acids of fresh weight) of microalgae *T.chuii* at the exponential phase**

| Sample | 2500lux 20ppt | 2500lux 25ppt | 2500lux 35ppt | 2500lux 40ppt | 4500lux 20ppt | 4500lux 25ppt | 4500lux 35ppt | 4500lux 40ppt | 6500lux 20ppt | 6500lux 25ppt | 6500lux 35ppt | 6500lux 40ppt |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Saturated | | | | | | | | | | | | |
| C14:0 | 0.72 | 0.69 | 1.03 | 0.75 | 1.23 | 0.44 | 0.44 | 1.65 | 0.91 | 0.89 | 0.95 | 0.56 |
| C16:0 | 19.17 | 26.84 | 28.81 | 25.95 | 24.36 | 23.51 | 22.59 | 27.75 | 27.48 | 28.65 | 27.06 | 23.41 |
| C18:0 | 0.72 | 0.71 | 0.51 | 0.98 | 1.28 | 0.5 | 0.82 | 3.68 | 0.75 | 0.79 | 0.29 | 0.51 |
| C20:0 | 3.83 | 2.68 | 2.68 | 3.41 | 2.55 | 2.61 | 2.67 | 1.51 | 3.57 | 4.82 | 4.21 | 0.26 |
| C22:0 | 0.07 | 0.13 | 0.09 | 0.07 | 0.22 | 0.11 | 2.06 | 1.06 | 0.08 | 0.34 | 0.11 | 0.03 |
| Monosaturated | | | | | | | | | | | | |
| C14:1n5 | 0.15 | 0.09 | 0.15 | 0.08 | 0.04 | 0.01 | 0.03 | 0.25 | 0.15 | 0.16 | 0.15 | 0.11 |
| C16:1n7 | 3.72 | 3.79 | 4.12 | 2.63 | 1.4 | 1.33 | 2.86 | 4.1 | 1.23 | 1.73 | 1.73 | 0.89 |
| C18:1n9 | 31.37 | 25.52 | 21.32 | 21.61 | 11.41 | 12.13 | 24.33 | 14.98 | 13.61 | 15.38 | 13.31 | 14.63 |
| Polyunsaturated | | | | | | | | | | | | |
| C18:2n6 | 7.37 | 6.54 | 9.11 | 8.68 | 18.52 | 16.79 | 8.61 | 13.48 | 10.45 | 8.79 | 10.24 | 10.33 |
| C18:3n3 | 12.86 | 10.36 | 7.43 | 10.71 | 11.41 | 10.92 | 13.61 | 6.43 | 12.68 | 11.39 | 14.26 | 12.43 |
| C18:3n6 | 0.1 | 0.24 | 0.16 | 0.06 | 0.22 | 0.18 | 0.05 | 0.27 | 0.21 | 0.26 | 0.26 | 0.04 |
| C18:4n3 | 0.08 | 0.3 | 0.22 | 0.14 | 1.2 | 1.55 | 0.04 | 1.08 | 0.09 | 1.86 | 0.05 | 0.04 |
| C20: 3n6 | 1.1 | 1.93 | 1.34 | 1.77 | 0.36 | 0.14 | 0.07 | 0.22 | 1.62 | 1.77 | 1.51 | 2.7 |
| C20:3n3 | 0.96 | 1.01 | 1.48 | 0.91 | 0.06 | 0.05 | 0.01 | 0.06 | 0.05 | 0.24 | 0.08 | 0.21 |
| C20:4n6 | 0.36 | 0.23 | 0.2 | 0.12 | 3.34 | 4.74 | 1.69 | 1.18 | 1.95 | 0.2 | 1.49 | 2.83 |
| Highunsaturated | | | | | | | | | | | | |
| C20:5n3 | 3.66 | 4.37 | 4.52 | 3.72 | 4.52 | 6.56 | 5.34 | 3.05 | 5.99 | 6.55 | 6.83 | 12.22 |
| C22:5n6 | 0.13 | 0.23 | 0.01 | 0.12 | 0.04 | 0.06 | 0.04 | 0.26 | 0.07 | 0.04 | 0.04 | 0.06 |
| C22:5n3 | 0.26 | 0.03 | 0.02 | 0.08 | 0.03 | 0.04 | 0.03 | 0.05 | 0.05 | 0.09 | 0.02 | 0.02 |
| C22:6n3 | 0.18 | 0.14 | 0.11 | 0.08 | 0.12 | 0.19 | 0.12 | 0.3 | 0.08 | 0.2 | 0.06 | 0.13 |
| Sum | 86.88 | 85.92 | 83.4 | 81.95 | 82.4 | 81.95 | 85.52 | 81.54 | 81.1 | 84.22 | 82.74 | 81.48 |
| ∑SFA | 24.52 | 31.07 | 33.05 | 31.17 | 29.66 | 27.69 | 28.60 | 35.67 | 32.79 | 35.51 | 32.64 | 24.79 |
| ∑MUFA | 35.25 | 29.41 | 25.59 | 24.34 | 12.86 | 13.48 | 27.24 | 19.43 | 15 | 17.28 | 15.21 | 15.63 |
| ∑PUFA | 22.86 | 20.64 | 19.97 | 22.42 | 35.15 | 34.39 | 24.12 | 22.75 | 27.07 | 24.54 | 27.91 | 28.6 |
| ∑HUFA | 4.24 | 4.79 | 4.68 | 4.01 | 4.72 | 6.87 | 5.54 | 3.67 | 6.21 | 6.89 | 6.97 | 12.44 |
| ∑(n-3) | 16.97 | 14.92 | 12.1 | 14.6 | 16.09 | 17.74 | 19.12 | 9.83 | 18.82 | 18.24 | 21.19 | 24.81 |
| ∑(n-6) | 9.08 | 9.18 | 10.85 | 10.77 | 22.50 | 21.92 | 10.49 | 10.49 | 14.31 | 11.07 | 13.56 | 15.97 |
| (n-3/n-6) | 1.86 | 1.62 | 1.11 | 1.35 | 0.71 | 0.8 | 1.82 | 0.93 | 1.31 | 1.64 | 1.56 | 1.55 |
| DHA/EPA | 0.04 | 0.03 | 0.02 | 0.02 | 0.02 | 0.3 | 0.02 | 0.1 | 0.01 | 0.03 | 6.83 | 0.01 |
| Total lipid | 8.41% | 10.57% | 8.24% | 9.67% | 4.91% | 8.47% | 5.79% | 7.28% | 7.23% | 9.45% | 9.41% | 8.80% |

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**Discussion**

Variance analyses showed that cell density in light intensity 2500 and 6500 lux was significantly different ($P<0.05$). Increasing light intensity increased cell density of *T.chuii*. With increasing light intensity, the photosynthetic rate also increased (Mendoza *et al*.,1999), In fact, researchers believe that the light intensity until the chlorophyll II molecule is damaged can cause increased cell division (Thompson *et al*.,1990;Bolch,2004). This result agrees with those of Carvalho *et al*.,2003. This micro alga in 6500 lux light intensity and 25 ppt salinity, most cell density induceded, similar to result by Garcia *et al*.,2007. That its optimum growth always occurs at salinity(25ppt),on this regard,reduced growth rates at 40ppt ,because faster nutrient depletion in the culture medium(Johnson *et al*.,1968). Nacl the essential element necessary for growth marine microalgae, thus over increase cause damage to cell microalgae.Rodolfi *et al*.,2009 and Muller- Feuga *et al*., 2003 also described in low salinity,increased total lipid content. Fatty acid composition depend to their cell growth ( De la Pena *et al*.,2005) .Therefore, increase polyunsaturated fatty acid in high light intensity by reason of increase cell density. Our results on fatty acid showed that an increase in light intensity was associated with increased PUFA,HUFA,n-3,n-6 and decreased MUFA.On the other hand,Molina- Grima *et al*.,1994 also described Decreases in the content of EPA with increasing light,in Batch cultures of *Isochrysis galbana*.It is widely accepted that low levels of light intensity bring about increases in the amount of thylakoid membranes, thus promoting systhesis of its.Lipid constituents-galactolipids(which contain a high percentage of EPA). Therefore, any comparison of the intensity of light, they respond differently. Oleic acid, linoleic acid, alpha linolenic acid, an important fatty acids are unsaturated fatty acid (Pratoomyot *et al*.,2005;Patil *et al*.,2007).The significant presence of polyunsaturated fatty acids in these species, the nutritional value of food fish has doubled. Arachidonic acid and EPA are precursors of eicosanoid compounds. However, the eicosanoids from these two fatty acids are different both structurally and functionally, and are sometimes even antagonistic in their effects (Gill and Valivety, 1997) .High ratios of n-3 to n-6 polyunsaturated fatty acids have been used as an index of high nutritional value to aquaculture animals (Watanabe *et al.,* 1983). The percentage differences in fatty acid composition in our study are similar to those reported by Pratoomyot *et al*.,2005 for the *Tetraselmis sp* grown in laboratory culture with irradiances of 4719lux. One difference was that their did not find DHA.

According to the result, a salinity of 25 ppt, light intensity 6500 lux seems more adequate for enhanced growth and fatty acid composition of *T. chuii*.

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7


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