# Biochemical characterization of eight marine microalgae grown in batch cultures

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## ABSTRACT

Among the eight marine microalgae used in this study, *Nannochloropsis* sp. exhibited the fastest growth rate of $1 \times 10^7$ cells $ml^{-1}$ and a highest protein content of 220 mg $L^{-1}$. The carbohydrate content was maximum on the 15th day of growth in *Dunaliella* sp. (120 mg $L^{-1}$). *Tetraselmis* sp. showed the maximum amount of total chlorophyll (6mg $L^{-1}$) and chlorophyll 'b' (0.9 mg $L^{-1}$) on the 25th day, whereas chlorophyll 'a' (5mg $L^{-1}$) and carotenoids (2.5mg $L^{-1}$) were highest on the 20th day. The maximum lipid content was observed in *Tetraselmis* sp. (~800μg $L^{-1}$). Highest amount of polyunsaturated fatty acid (PUFA) content was observed maximum in *Nannochloropsis* sp., eicosapentaenoic acid (EPA) in *Tetraselmis* sp. and docosahexaenoic acid (DHA) in *Isochrysis* sp. The results of this study reveal that *Nannochloropsis* sp., *Tetraselmis* sp. and *Isochrysis* sp. have good nutritional value.

## Introduction

Algae which are very simple chlorophyll-containing (Bold and Wynne, 1985) photosynthetic organisms play a key role in the productivity of ocean and constitute the basic of the marine food chain (Hillison, 1977**).** Microalgae are well known for their high nutritional values including protein, carbohydrate, lipid, essential amino acids, polyunsaturated fatty acids (PUFAs), vitamins, minerals and non-caloric dietary fibres. The beneficial value of microalgae could be transferred to animals through the food chain (Kumar and Singh, 1976). They are utilized in aquaculture as live feed for bivalve larvae and spat (Knauer and Southgate, 1999) and have wide applications in biodiesel (Chisti, 2007; Hu et al. 2008; Schenk et al. 2008), health food (Natrah et al. 2007; Plaza et al. 2008), animal feed and fertilizers (Spolaore et al. 2006), nutraceuticals and pharmaceuticals (Shahidi and Wanasundara, 1998; Horrocks and Yeo, 1999) and bioplastics (Murphy, 2006). Productivity and lipid composition of microalgae depend on their growth phase (Xu et al. 2008) and composition (Valenzuela-Espinoza et al. 2002), irradiance (Thompson et al. 1993), salinity (Renaud and Parry, 1994) and temperature (Renaud et al. 2002) of the medium. Microalgae produce a variety of lipids of nutritional importance. Long chain ω-3 polyunsaturated fatty acids (PUFAs), in particular eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are essential for the growth, development and increase in the survival percentage of larvae of both crustaceans (Kanazawa et al. 1985) and marine finfish (Reitan et al. 1997). Microalgae are the food for rotifers (Watanabe, 1983), artemia (Lavens et al. 1995), marine fish larvae (Eamta et al. 2003), shrimp (Cavalli et al. 1999) and molluscs (Knaur and Southgate, 1999).

Algal biomass can play an important role in solving the problem between the production of food and that of biofuels in the near future. The biomass can be fed to an anaerobic digester for methane production (Golueke et al. 1957; Gunaseelan, 1997; Yen and Brune, 2007), and the residual biomass from such processes can potentially be used as a fertilizer, soil amendment, or feed for fish or livestock (Mulbry and Wilkie, 2001; Mulbry et al. 2005). Microalgae rich in carbohydrates and proteins can be used as carbon sources for fermentation as a potential substrate for bioethanol production (Subhadra and Edwards, 2010; Packer, 2009). It is also possible to produce protein-rich feed for animal

and nutritional supplement for human consumption. Poly-unsaturated fatty acids (PUFAs) are a potential co-product of biodiesel production from microalgae. PUFAs of vegetable origin are alternatives to fish oils and other oils rich in omega-3 fatty acids. The composition of the algal biomass with regards to lipids, carbohydrates and proteins will greatly determine its overall value. In this present study, biochemical composition of eight native marine microalgae was evaluated at different growth phases, characterizing their chemical profile under standardized conditions.

**Materials and Methods**

*Algal Cultures*

Eight marine algal cultures, viz., *Chaetoceros* sp*., Chlorella* sp*., Dicrateria* sp*., Dunaliella* sp*., Isochrysis* sp*., Nannochloropsis* sp*., Synechococcus* sp. and *Tetraselmis* sp. were used in the present study. Cultures of each species were grown in triplicates in 5L conical flasks containing 3L of sterile f/2 culture medium (Guillard and Ryther, 1962). Algal cultures were established by inoculating 100 ml of a mother culture ($2 \times 10^6$ cells ml$^{-1}$) growing in exponential phase. These were maintained in unialgal condition in the laboratory and were kept under sufficient light (2000 lux) and temperature (26-28°C) conditions with a pH of 8.2±1 (Guillard, 1975).

*Growth Measurements*

The growth of the microalgae was measured by direct counting of the cells using a haemocytometer (Neubauer). The mean value of four counts was taken for each sample. Chlorophylls and carotenoids were quantified using the equations by Jeffrey and Humphrey (1975).

*Biochemical  analysis*

Algal cultures (50 ml for each analysis) were taken on the 5$^{th}$, 10$^{th}$, 15$^{th}$, 20$^{th}$, 25$^{th}$ and 30$^{th}$ days of growth and centrifuged at 10,000 rpm for 10 minutes. The pellets were washed thoroughly with distilled water and immediately stored at − 70°C. The biochemical composition of the microalgae was estimated by following the standard methods for total protein (Dorsey et al. 1978) and total carbohydrate (Pons et al. 1981). All the studies were performed in triplicate.

*Extraction of lipid and PUFA analysis*

Cells were harvested by centrifugation (10,000 rpm for 10 min.) and lyophilized. The dried cells were then extracted with chloroform: methanol by the method of Bligh and Dyer (1959) and the lipid extracts thus obtained were transmethylated with sodium methoxide (1% w/v) using the method described by Carreau and Dubacg (1978). The methyl esters of fatty acids were analyzed by GC (CHEMITO GC 8610) equipped with a Flame Ionization Detector and a Column BPX-70 (50% cyanopropyl, 50% methylsiloxane), injection port 250⁰, detector port 260⁰ and oven starting temperature 160⁰ and increase by 7.5⁰ per minute, the final oven temperature being 240⁰. Injections were made in the split mode (split ratio, 1:30; sample size, 0.2 µl). N$_2$ was used as the carrier gas. The fatty acids were identified by comparing with the retention times of known internal standard (17:0 fatty acid). The data were collected using Winchrom Software.

*Statistical analysis*

Comparison between species, between days and between replications was done using 3 way ANOVA based on the growth measured by number of cells, concentration of total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, carbohydrates and proteins. Students t test was applied to determine which two species/days differed significantly using trellis diagram.  The parameters, total chlorophyll, carotenoids, carbohydrates and proteins were used in discriminating between the 8 species in multivariate discriminant analysis.

**Results and discussion**
*Growth*
Eight marine microalgae such as *Chaetoceros* sp., *Chlorella* sp., *Dicrateria* sp., *Dunaliella* sp., *Isochrysis* sp., *Nannochloropsis* sp., *Synechococcus* sp. and *Tetraselmis* sp. were grown in f/2 medium. Among the eight different cultures tested, *Nannochloropsis* sp. exhibited the fastest growth rate with 1073 x 10$^4$ cells ml$^{-1}$ followed by

*Synechococcus* sp. (994 x 10⁴ cells ml⁻¹), *Isochrysis* sp. (924 x 10⁴ cells ml⁻¹), *Chlorella* sp. (936 x 10⁴ cells ml⁻¹) and *Tetraselmis* sp. (864 x 10⁴ cells ml⁻¹) on the 25 th day (Fig. 1A). Similar values were observed in the cell count of *Dicrateria* sp., *Isochrysis* sp., and *Tetraselmis* sp. on the 10th day, *Dunaliella* sp. and *Nannochloropsis* sp. on the 5th day, that of *Chaetoceros* sp. and *Tetraselmis* sp. on the 5th day and that of *Chaetoceros* sp. and *Isochrysis* sp. on the 15th day (P>0.05) of growth (Table 1). According to Huerlimann et al. (2010), when grown in f/2 medium, *Nannochloropsis* sp. showed a growth of ~2.5 x 10⁷ cells ml⁻¹, *Isochrysis* sp., ~6 x 10⁶ cells ml⁻¹ and *Tetraselmis* sp., ~1.9 x 10⁶ cells ml⁻¹. Abu Rezq et al. (1999) has reported maximum cell density of *Nannochloropsis* as ~32 x 10⁶ cells ml⁻¹, *Tetraselmis*, ~5 x 10⁶ cells ml⁻¹ and *Isochrysis*, ~4.5 x 10⁶ cells ml⁻¹. *T. gracilis* in Conway medium showed highest cellular density values (1.71x10⁹ cells L⁻¹) as reported by Lourenço et al. (1997), probably because Conway medium was more nutritive than f/2- Guillard. *Chaetoceros gracilis* using FeNS as the growth medium showed 95-6,415x10⁶ cells L⁻¹ as recorded by Yamashita and Magalhães (1984). According to Becker (1995) one of the best parameters to monitor microalgae production is the estimation of growth over a certain period of time. In lab grown cultures, microalgae biomass reaches a peak level after 25th day and then starts decreasing (Dayananda et al. 2007). As it happens in any organism, microalgae biomass and chemical composition can vary according to environment conditions and the age of the culture (Lourenço et al. 1997; Renaud et al. 1999; Araújo., et al. 2005).
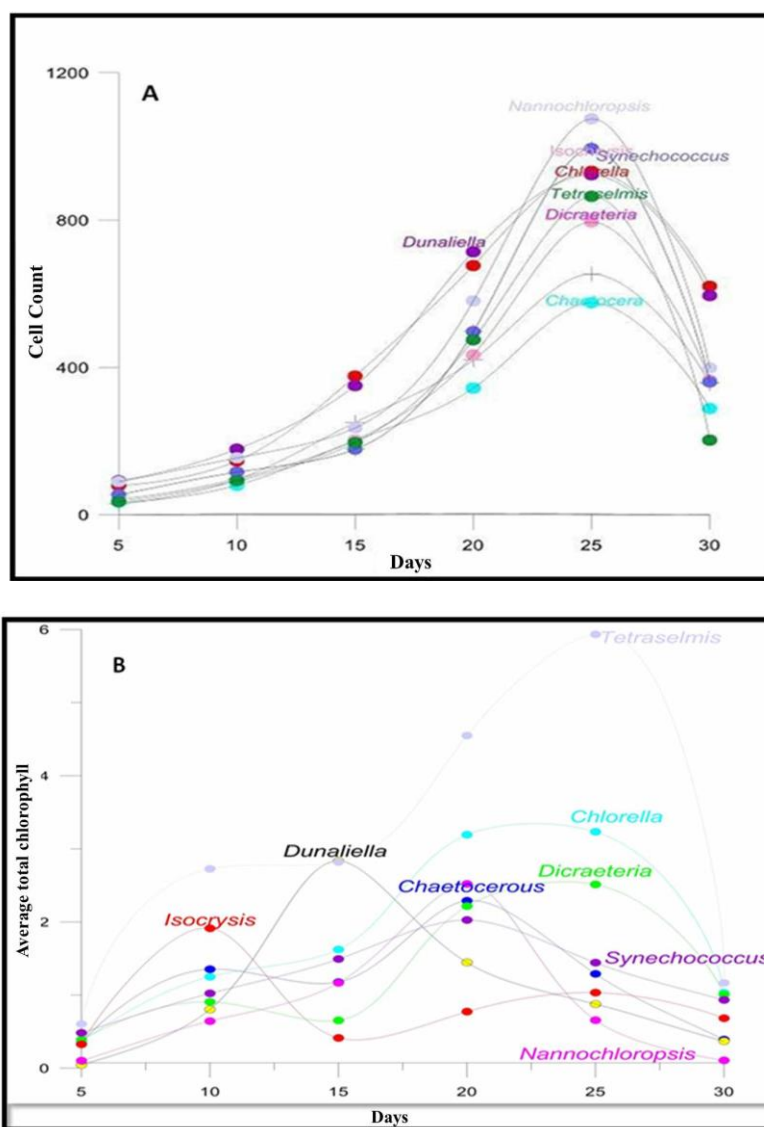


**Figure 1. A and B. Biochemical composition of eight marine microalgae grown in batch culture using f/2 medium (A) Cell Count (B) Total chlorophyll (C) Chlorophyll a (D) Chlorophyll b (E) Carotenoids (F) Protein (G) Carbohydrate**

**Figure 1. C and D. Biochemical composition of eight marine microalgae grown in batch culture using f/2 medium  (A) Cell Count (B) Total chlorophyll (C) Chlorophyll a (D) Chlorophyll b (E) Carotenoids (F) Protein (G) Carbohydrate**
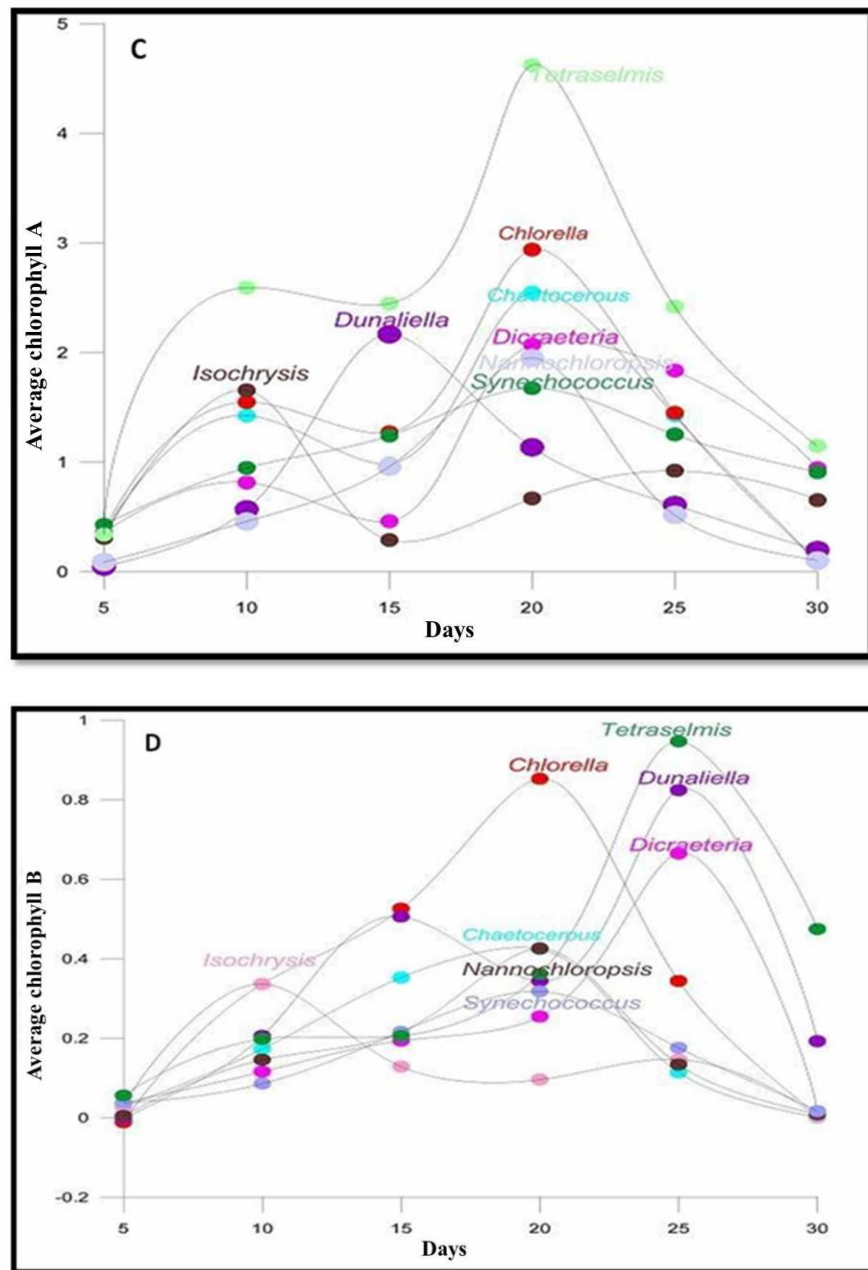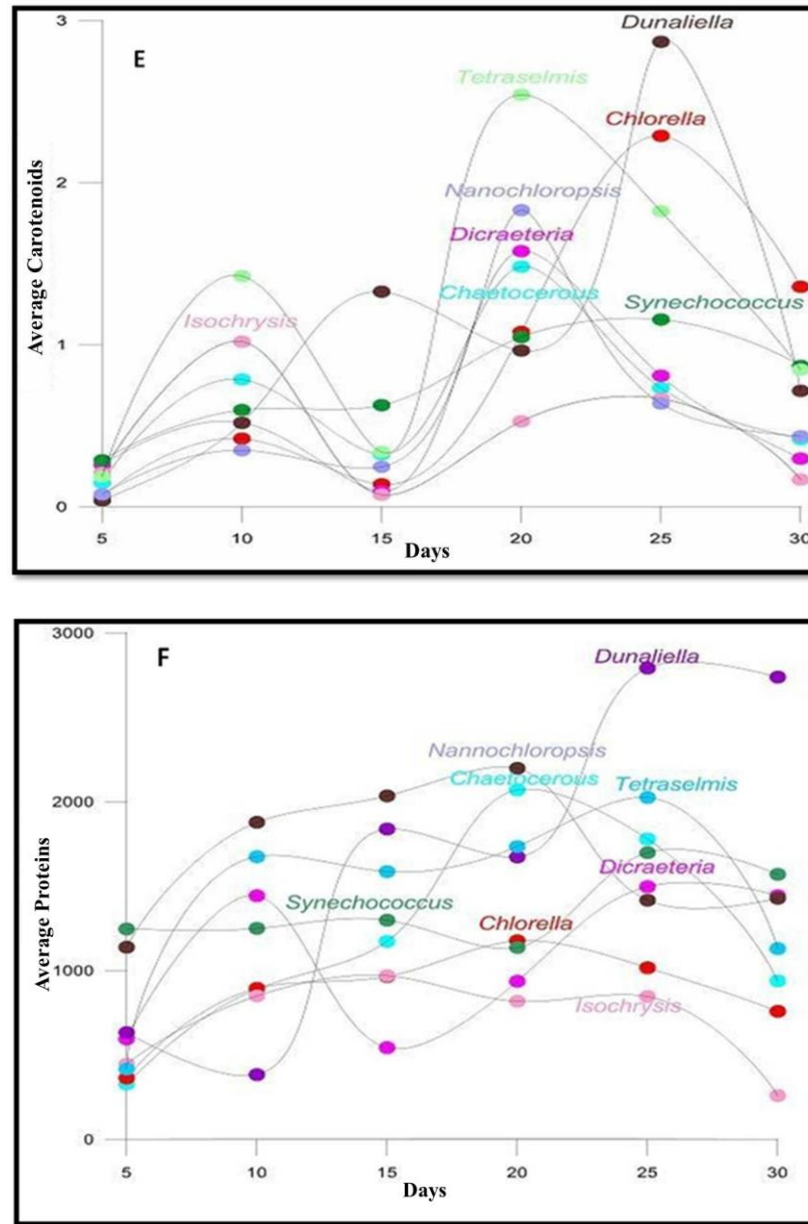
**Figure 1. E and F. Biochemical composition of eight marine microalgae grown in batch culture using f/2 medium (A) Cell Count (B) Total chlorophyll (C) Chlorophyll a (D) Chlorophyll b (E) Carotenoids (F) Protein (G) Carbohydrate**
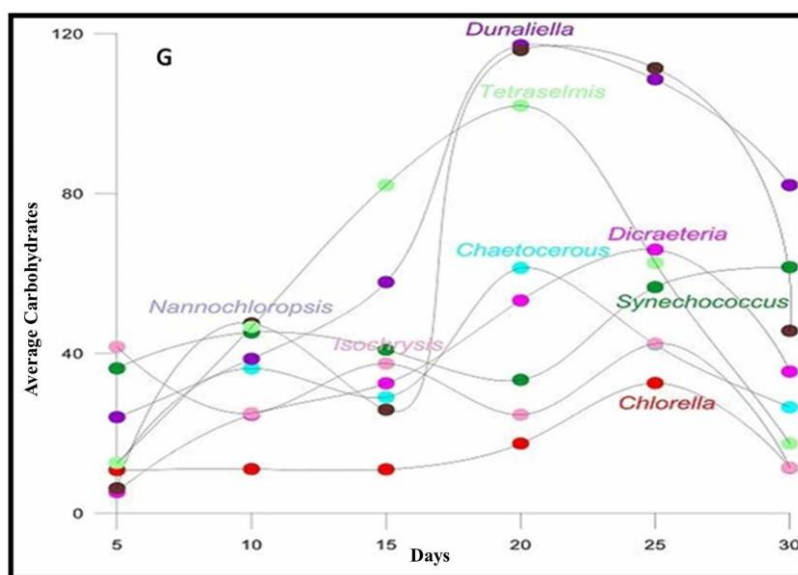
**Figure 1.G. Biochemical composition of eight marine microalgae grown in batch culture using f/2 medium  (A) Cell Count (B) Total chlorophyll (C) Chlorophyll a (D) Chlorophyll b (E) Carotenoids (F) Protein (G) Carbohydrate**

Three way analysis of variance applied to determine the significance of the difference between days, species and between replications observed that high significant difference exists between days of experiment viz. 5, 10, 15, 20, 25 and 30, between species and between replications for total chlorophyll, cell count, chlorophyll a, chlorophyll b, carotenoids, proteins and carbohydrates ($P<0.01$) except for replications based on chlorophyll b, carotenoids and proteins ($P>0.05$) (Table 1).

Chlorophyll is one of the cellular compounds that is used for estimating biomass of microalgae in culture and can be used to measure growth. The highest total chlorophyll content was observed in *Tetraselmis* sp. (6mg L$^{-1}$) followed by *Chlorella* sp. (3.2 mg L$^{-1}$), *Dunaliella* sp. (2.8 mg L$^{-1}$) and *Nannochloropsis* sp. (2.5 mg L$^{-1}$) (Fig. 1B). Total chlorophyll concentration in *Chlorella* sp., *Chaetoceros* sp. and *Dicrateria* sp.  did not differ much on the 5$^{th}$ day. Similarly *Dunaliella* sp. and *Tetraselmis* sp.  on the 15$^{th}$ day and *Chaetocerous* sp. and *Synechococcus* sp. on the 25$^{th}$ day had almost same concentration of total chlorophyll ($P>0.05$) (Table 1).

Chlorophyll 'a' content differed significantly between all species on all the days ($P<0.05$). *Tetraselmis* sp. (5 mg L$^{-1}$) reported the maximum amount of chlorophyll 'a' followed by *Chlorella* sp. (3 mg L$^{-1}$) (Fig. 1C). The highest chlorophyll 'b' content was observed in *Tetraselmis* sp. (0.9 mg L$^{-1}$) followed by *Chlorella* sp. (0.85 mg L$^{-1}$) and *Dunaliella* sp. (0.82 mg L$^{-1}$) (Fig. 1D). Chlorophyll 'b' concentration of *Chlorella* sp.*, Dicrateria* sp.*, Dunaliella* sp.*, Isochrysis* sp.*, Nannochloropsis* sp.  and *Synechococcus* sp.  did not differ significantly on the 5$^{th}$ day. There was also no significant difference in chlorophyll 'b' values in *Synechococcus* sp. and *Nannochloropsis* sp. on the 15$^{th}$ day, *Chaetoceros* sp. and *Nannochloropsis* sp.  on the 20$^{th}$ day and between *Chaetoceros* sp. and *Isochrysis* sp., and *Synechococcus* sp. and *Dicrateria* sp. on the 30$^{th}$ days of growth ($P>0.05$) (Table 1). Carotenoid content differed significantly between all species on all the days ($P<0.05$). *Dunaliella* sp. (2.9mg L$^{-1}$) showed high amount of carotenoids followed by *Tetraselmis* sp. (2.6mg L$^{-1}$) and *Chlorella* sp. (2.4mg L$^{-1}$) (Fig. 1E). These values were higher than those found by Lourenço et al.  (1997) who cultivated *T. gracilis* in Conway medium (1.51-3.57 mg L$^{-1}$). High chlorophyll 'a' values may occur due to high cell density, which diminishes irradiation and lead to increased production of chlorophyll 'a' (López- Muñoz et al. 1992; Sauodi-Helis et al. 1999; Valenzuela-Espinoza et al. 2002). The results of this study indicate that algal biomass increased proportionally with growth rate. This confirms that there is a good relationship between growth rate and efficient photosynthesis during the growth of the culture. On the other hand, chlorophyll content does not depend only on the cellular density, but also on the irradiance. The high cellular density of the culture produces shading which reduces the irradiance into the culture, thereby increasing the chlorophyll content per cell (Lopez-Munoz et al. 1992).

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*Biochemical composition*

In the present study the highest protein content was observed in *Dunaliella* sp. (280 mg L$^{-1}$) followed by *Tetraselmis* sp. (200 mg L$^{-1}$) on the 25$^{th}$ day (Fig. 1F). *Nannochloropsis* sp. was observed to have 220 mg L$^{-1}$ of protein content on 20$^{th}$ day. Protein content differed significantly between all species on all the days (P<0.05). Also the protein content in all the cultures showed a decline on the 30$^{th}$ day indicating the end of their growth period. The values were lower than those obtained by Fabregas et al. (1985), where the mass culture of *T. suecica* was found to have a maximum protein value of 306 mg L$^{-1}$ in logarithmic culture phase. Koening et al. (1990) reported that the soluble protein was 310 mg L$^{-1}$ in *T. tetrathele* when grown in organic fertilizer medium. The protein content of *Chlorella vulgaris* was reported to be 50% (Becker, 2007). The high protein content of various microalgal species is one of the main reasons to consider them as an unconventional source of protein (Soletto et al. 2005), which constitutes an important quality for using the marine microalgae as a single cell protein (SCP) source.

Carbohydrates have been found as intermediary reserves in some algae, due to the fact that they are required when nitrogen becomes limited in the lipid synthesis. Carbohydrate tends to accumulate in the stationary phase (Brown et al. 1993; Zhu et al. 1997) of algal growth. The highest carbohydrate content was observed in *Dunaliella* sp. (120 mg L$^{-1}$), *Tetraselmis* sp. (115 mg L$^{-1}$) and *Nannochloropsis* sp. (100 mg L$^{-1}$) on 15$^{th}$ day (Fig. 1G). Carbohydrate concentration of *Tetraselmis* sp*., Nannochloropsis* sp. and *Synechococcus* sp. on the 5$^{th}$ day, that of *Chaetoceros* sp. and *Chlorella* sp. on the 10$^{th}$ day, of *Nannochloropsis* sp. and *Dunaliella* sp. on the 20$^{th}$ day, of *Isochrysis* sp. and *Chaetoceros* sp. on the 25$^{th}$ day and *Isochrysis* sp. and *Chlorella* sp. on the 30$^{th}$ day did not show significant difference (P>0.05) (Table 1).

A large part of the data available on chemical composition of marine microalgae has been obtained using f/2 culture medium (Guillard, 1975; Enright et al. 1986; Brown et al. 1998; Mc Causland et al. 1999; Renaud et al. ,1999; Knuckey et al. 2002; Lafarga-De la Cruz et al. 2006). Hence in the present study the marine algae were grown in batch culture in f/2 medium to analyze the biochemical composition with respect to different stages of growth. It has been observed that in *Nannochloropsis* sp. the total chlorophyll, chlorophyll a and carotenoids increased in the same rate as that of growth, but the peak values were obtained on the 20$^{th}$ day unlike the cell count which was on the 25$^{th}$ day. Protein showed a stationary phase between 10$^{th}$ and 20$^{th}$ day whereas for carbohydrates the stationary period was observed between 20$^{th}$ and 25$^{th}$ day (Fig. 2). In *Synechococcus* sp. the pigment concentrations were in the same pattern as observed for *Nannochloropsis* sp. whereas protein and carbohydrates showed a stationary phase beyond the 25$^{th}$ day after a decline at the end of the exponential phase, on 20$^{th}$ day. Protein concentration remained stationary at 1250 mg/l in the initial phase up to the 15$^{th}$ day (Fig. 2). In *Tetraselmis* sp. total chlorophyll followed the same pattern as indicated by cell count whereas chlorophyll 'a' and carotenoids exhibited a pattern similar to that of *Nannochloropsis* sp. with a sharp decline on the 15$^{th}$ day. Protein concentration remained almost stationary during the exponential phase of growth with peak value coinciding with the growth peak value, on the 25$^{th}$ day, whereas carbohydrates showed a steady increase with the growth reaching the peak value on the 20$^{th}$ day (Fig. 2). In *Chaetoceros* sp., pigments as well as proteins and carbohydrates followed the same growth pattern with a sharp decline on the 15$^{th}$ day of growth and with a lag period of 5 days to peak growth as observed in the case of *Tetraselmis* sp. (Fig. 2). In *Dunaliella* sp., the decline phase of growth started from the 25$^{th}$ day whereas pigments showed reduction from the 15$^{th}$ day onwards except carotenoids which showed the same trend as that of growth. Protein showed a stationary phase after a peak value on the 25$^{th}$ day while carbohydrate was maximum on the 20$^{th}$ day with a steep gradient of increase before 20$^{th}$ day and a low gradient of decrease after 20$^{th}$ day towards the death phase (Fig. 2). In *Chlorella* sp., the three pigments followed the same trend as that of growth with a stationary phase after 10$^{th}$ day, up to the 20$^{th}$ day followed by a decline. Carbohydrates showed a steep gradient from the initial phase to a peak value on the 25$^{th}$ day followed by sharp decrease on the 30$^{th}$ day (Fig. 2). In *Isochrysis* sp., the three pigments were maximum on the 10$^{th}$ day followed by a sharp decrease to the value of 0.5 mg/l on the 15$^{th}$ day afterwards reaching the death phase. Protein was nearly doubled after 5$^{th}$ day which continued at the same level till the maximum growth is attained where as carbohydrates reached the maximum on the day of maximum growth maintaining the level between 30 and 40 during the initial and exponential phase of growth (Fig. 2). In *Dicrateria* sp., the pigments were initially found to be moderately increasing, with a minimum value on the 15$^{th}$ day followed by a steady increase to the maximum value on the 20$^{th}$ day and a stationary phase up to the 25$^{th}$ day where the maximum growth is attained. Protein concentration of this species exhibited a pattern which is different from all other species with a bimodal peak on the 10$^{th}$ and 25$^{th}$ day with a sharp decline to the minimum value on the 15$^{th}$ day. Carbohydrates maintained a perfect positive correlation with growth pattern. Total chlorophyll, protein and

carbohydrates can be delineated as the optimal discriminative parameters effective enough to discriminate the 8 species during their growth phases by multivariate discriminant analysis. In this analysis the algae such as *Dunaliella* sp*., Nannochloropsis* sp*., Synechococcus* sp. and *Chlorella* sp. were discriminated at different stages of their growth (Fig. 3). In batch culture system with sufficient amount of nutrients the synthesis of both protein and chlorophyll will increase, whereas, during nutrient limitation the concentration of carbohydrate and lipid will increase. This is in accordance with the report of earlier researchers that there is a coupling between protein and carbohydrates in algal cells, which also reflects the budget of carbon and nitrogen available to the cells (Geider et al. 1993; Turpin, 1991). According to Enright et al.  (1986), when the rate of cell division in a phytoplankton culture is limited by nutrients, cells alter their metabolism and convert energy to produce reserve substances.
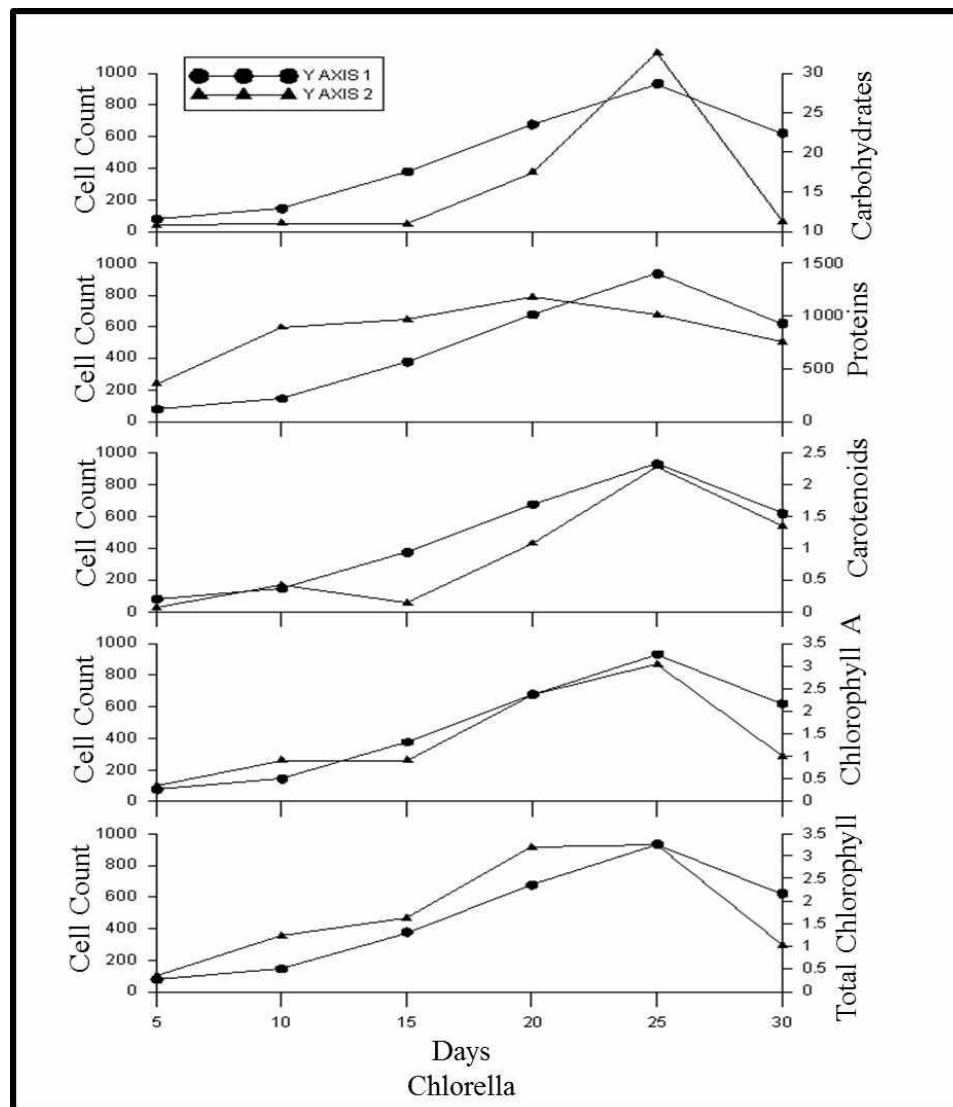


Figure 2 : Comparison between species, between days and between replications was done using 3 way ANOVA based on the growth measured by number of cells, concentration of total chlorophyll, chlorophyll a, chlorophyll b, carotenoids, carbohydrates and proteins
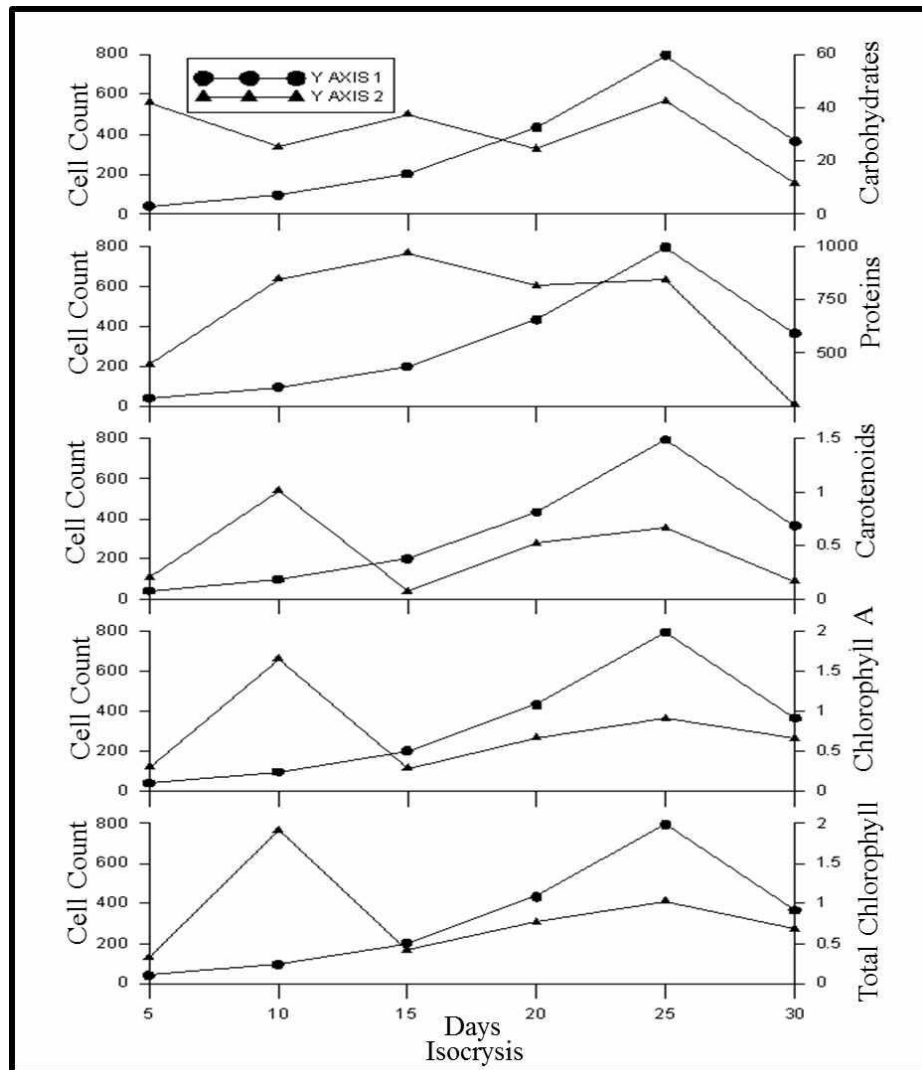
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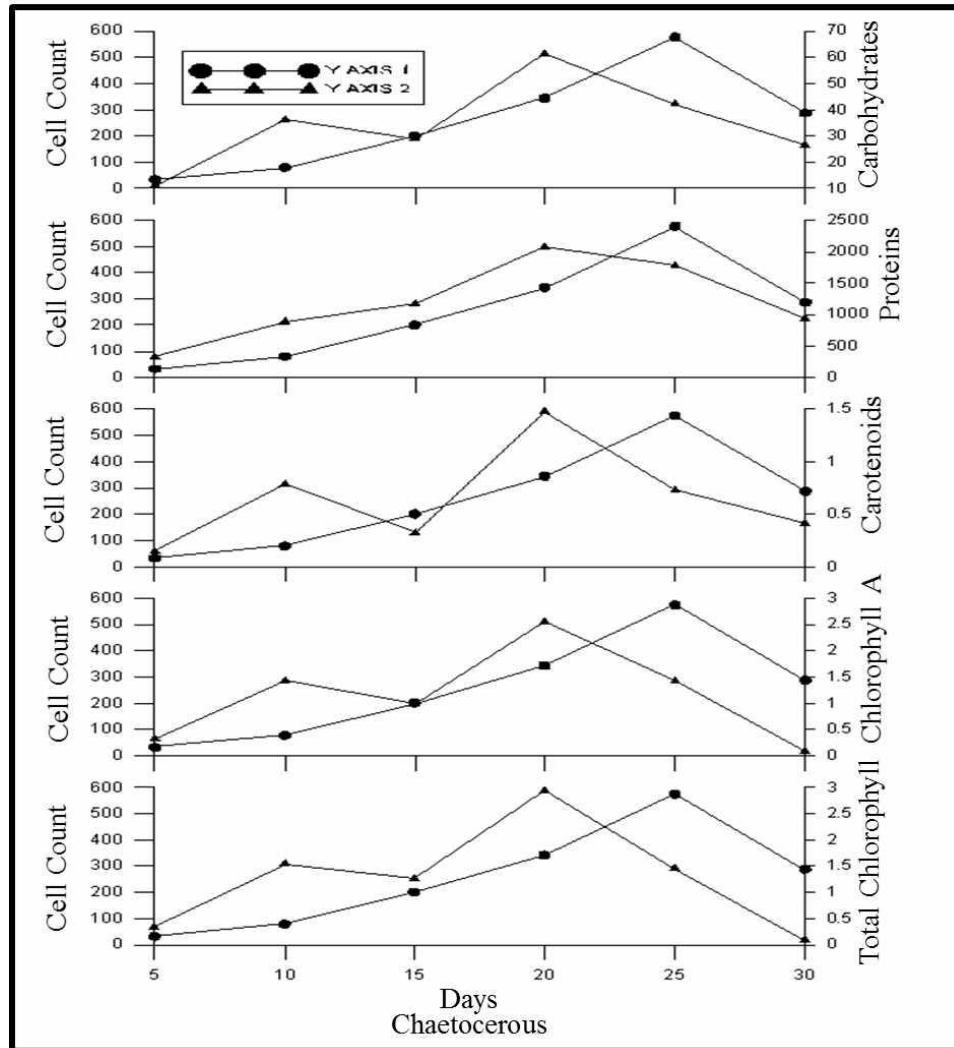
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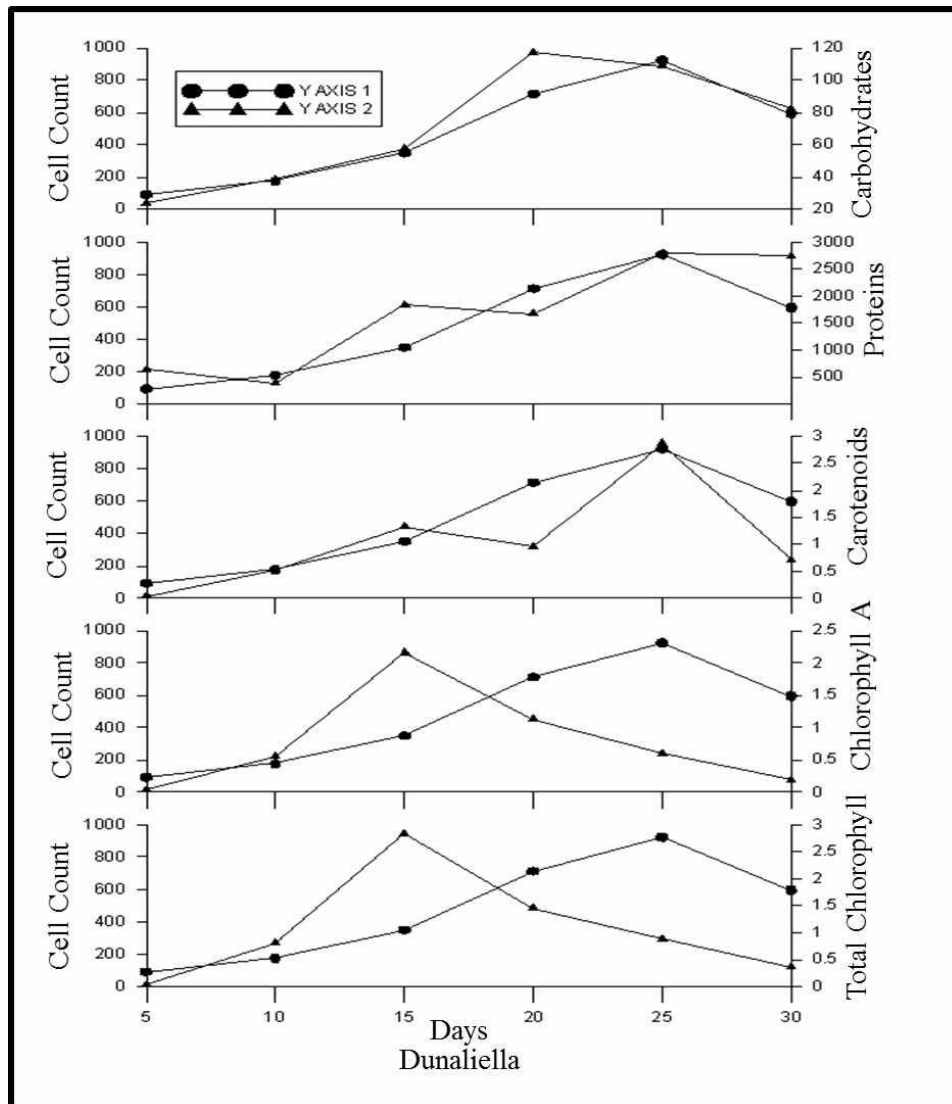
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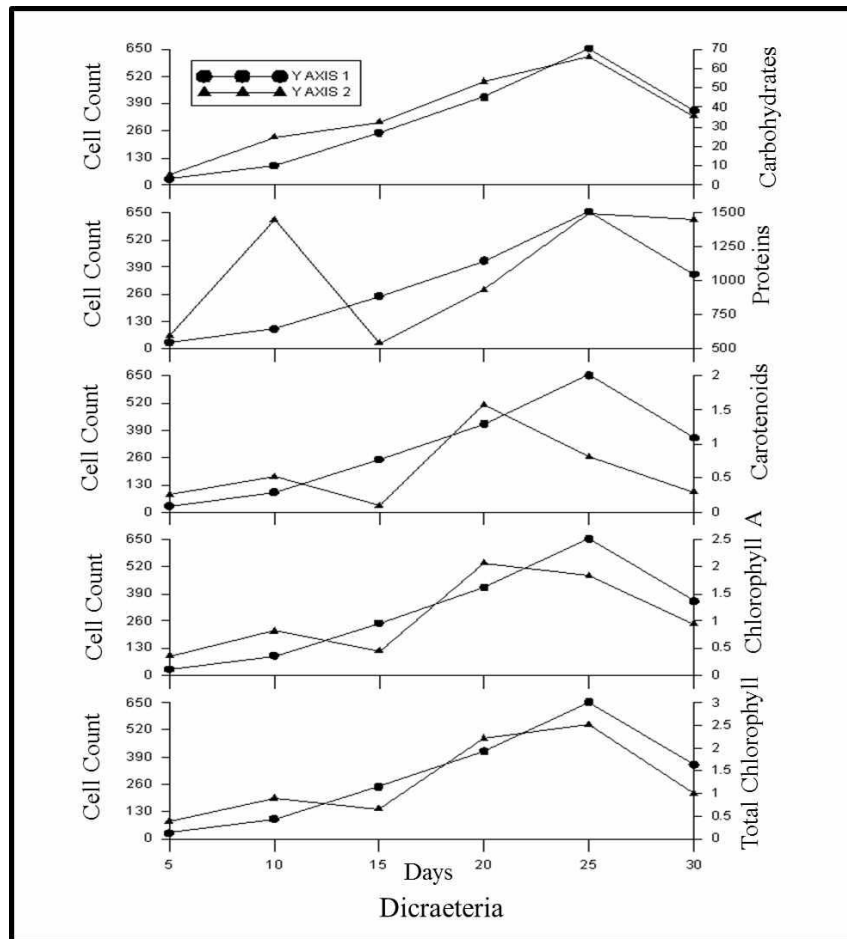
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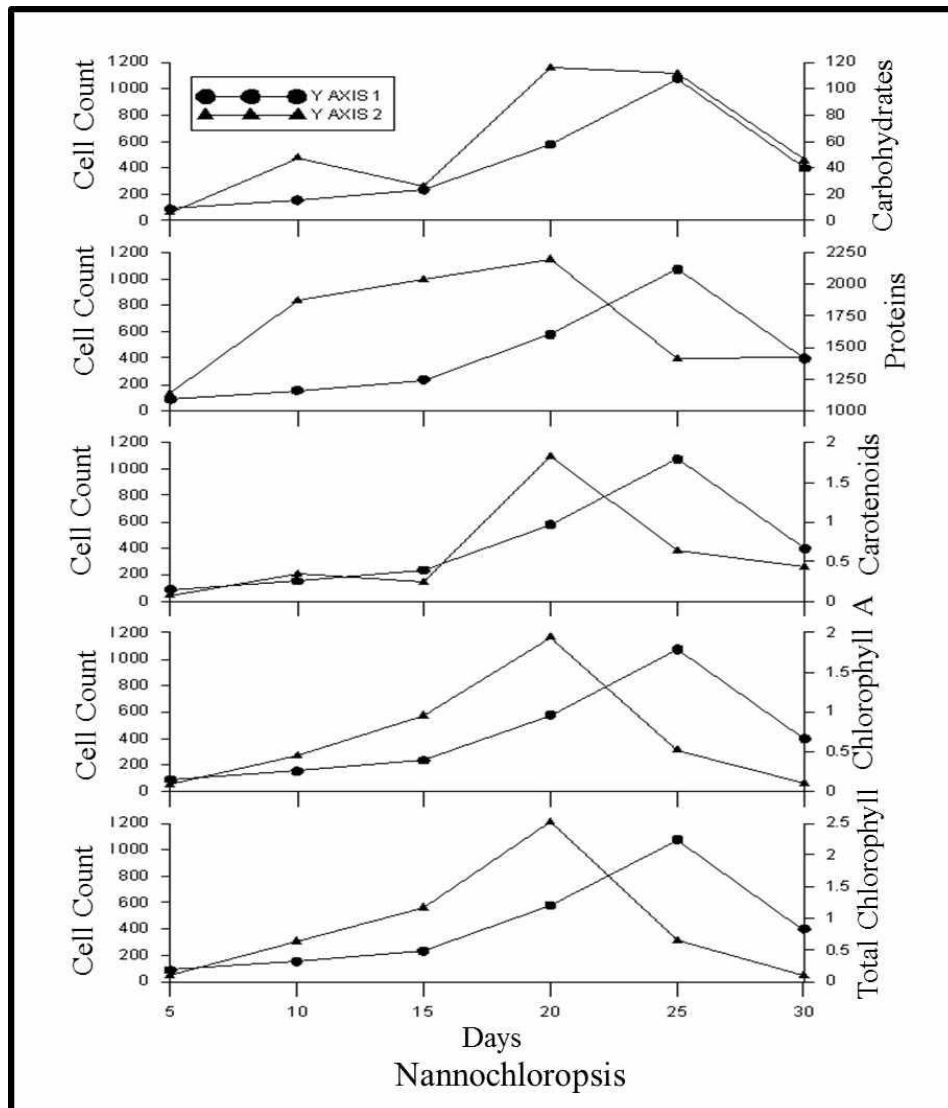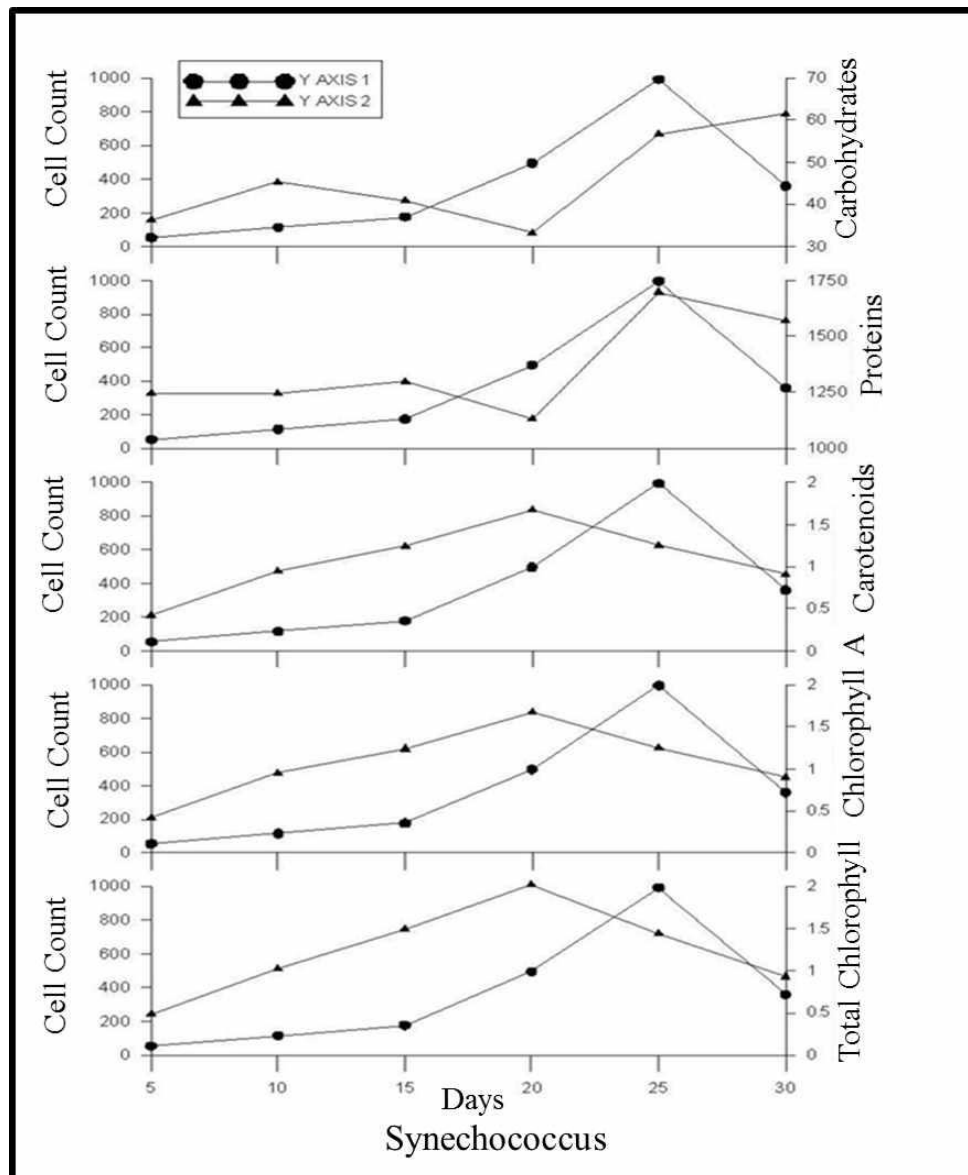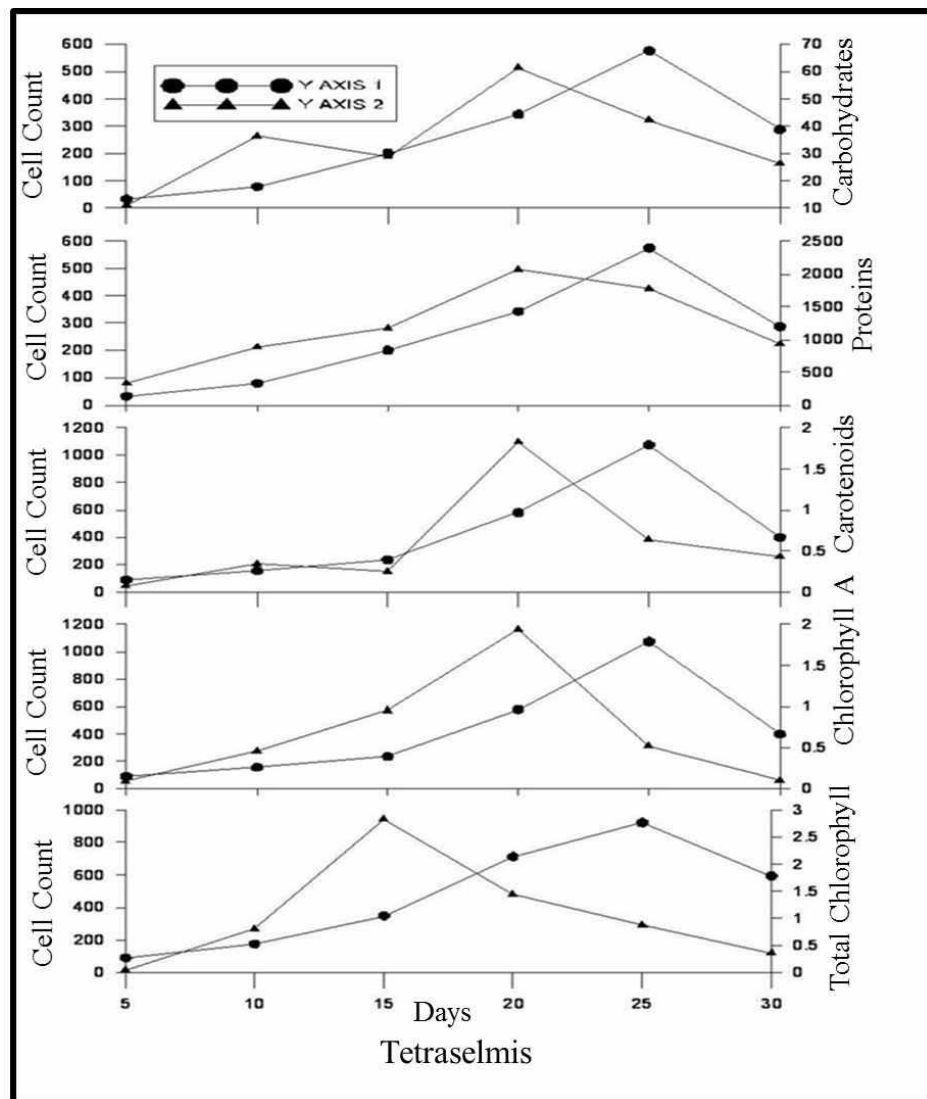
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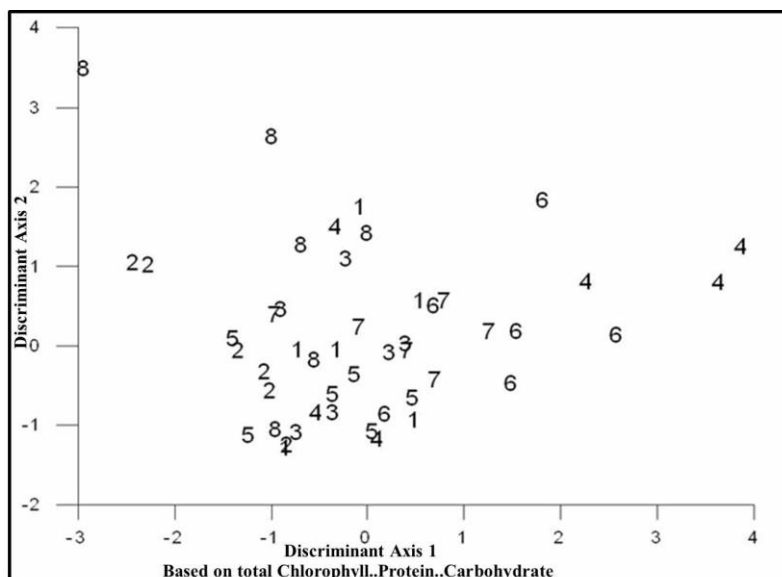
**Figure 3: Multivariate discriminant analysis between the 8 species [(1) Chaetoceros sp., (2) Chlorella sp., (3) Dicrateria sp., (4) Dunaliella sp., (5) Isochrysis sp., (6) Nannochloropsis sp., (7) Synechococcus sp. (8) Tetraselmis sp. ] were used in discriminating  for Total chlorophyll, Carotenoids, Carbohydrates and Proteins**

*Lipid and fatty acid profiles*

The highest lipid content was observed in *Tetraselmis* sp. (~800µg L$^{-1}$) followed by *Nannochloropsis* sp. (~600µg L$^{-1}$) and *Isochrysis* sp. (~500µg L$^{-1}$), while *Synechococcus* sp. had the lowest lipid content (Fig. 4). In the present study *Dunaliella* sp. showed high amount of carotenoids on the 25$^{th}$ day whereas *Tetraselmis* sp. and *Nannochloropsis* sp. had the maximum on the 20$^{th}$ day. ß-carotene serves as an essential nutrient and has high demand in the market as a natural food colouring agent, as an additive to cosmetics and also as a health food (Raja et al. 2007)  with pro-vitamin A activity (Baker and Gunther,  2004).
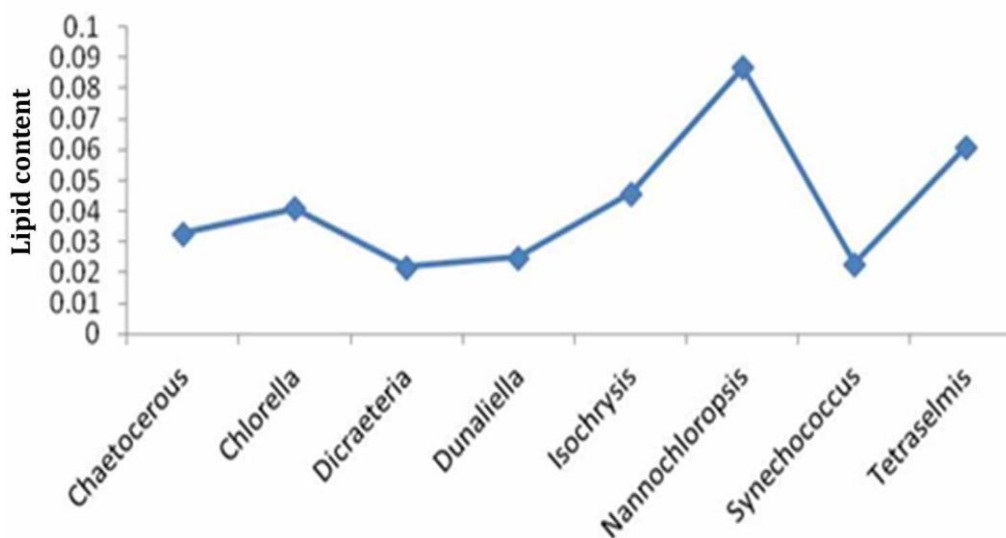


**Figure 4:  Lipid content of eight marine microalgae grown in batch culture using f/2 medium**

Interestingly, the highest biomass productivity was observed for *Tetraselmis* sp. due to its large cell size, although it showed the lowest specific growth rate. Similar result was also reported by Huerlimann et al.  (2010). There have been many studies investigating the lipid content and fatty acid profiles of various microalgae during the late logarithmic or stationary growth phase using a single medium (Zhukova and Aizdaicher, 1995; Renaud et al. 2002; Martines-Fernandez et al. 2006; Natrah et al. 2007).  Quite a number of factors influencing the lipid content of microalgae have been proposed. They include nutrient deprivation (Mortensen et al. 1988), temperature (James et al. 1989), photoperiod (Sicko-Goad and Anderson, 1991), pH (Cohen et al. 1988), growth rate (Sauodi-Helis et al. 1999), light quality (S´anchez Saavedra and Voltolina, 1994) and photon flux density (Sukenik et al. 1993).

Fatty acid composition of the eight species of microalgae used in this study is shown in Table 2. Saturated fatty acid (SFA) content was higher in *Tetraselmis* sp. (59.68 mg g$^{-1}$), *Isochrysis* sp. (39.64 mg g$^{-1}$), *Dunaliella* sp. (37.57 mg g$^{-1}$) and *Nannochloropsis* sp. (34.61 mg g$^{-1}$).  Monounsaturated fatty acid (MUFA) content was higher in *Dunaliella* sp. (41.86 mg g$^{-1}$), *Nannochloropsis* sp. (31.93 mg g$^{-1}$), *Chlorella* sp. (29.96 mg g$^{-1}$), *Isochrysis* sp. (28.46 mg g$^{-1}$) and *Tetraselmis* sp. (17.78 mg g$^{-1}$). The highest amount of PUFA content was observed in *Nannochloropsis* sp. (33.39 mg g$^{-1}$) followed by *Isochrysis* sp. (31.88 mg g$^{-1}$) and *Tetraselmis* sp. (22.7 mg g$^{-1}$). Palmitic acid, stearic acid, oleic acid and linoleic acid comprised 60 to 80% of the total lipids in *Nannochloropsis* sp., *Isochrysis* sp. and *Tetraselmis* sp. The highest amount of EPA was found in *Nannochloropsis* sp. (15.8 mg g$^{-1}$) followed by *Tetraselmis* sp. (5.37 mg g$^{-1}$), while DHA was abundant only in *Isochrysis* sp. (12.26 mg g$^{-1}$). Arachidonic acid was present in *Tetraselmis* sp. (2.05 mg g$^{-1}$) and *Nannochloropsis* sp. (1.17 mg g$^{-1}$). The findings reveal that palmitic acid was the major saturated fatty acid in all the algae. This is in accordance with previous reports by Patil et al.  (2007). The fatty acid distribution was in accordance with that of previous studies for *Nannochloropsis* sp., *Isochrysis* sp. and *Tetraselmis* sp.  (Ackman et al. 1968 ; Volkman et al. 1989 ; Reitan et al. 1994).

The main fatty acids found in *Nannochloropsis* sp. were palmitic acid, oleic acid, stearic acid and EPA. This study is in concurrence with Patil et al.  (2007) who has reported that *Nannochloropsis oceanica* is rich in EPA (23.4 mg g$^{-1}$) and therefore has been proposed for its commercial production of EPA (Sukenik et al. 1993). *Nannochloropsis* sp. are widely used as food in aquaculture (Maruyama et al. 1986; Apt and Behrens, 1999). *Isochrysis* consisted of mostly palmitic, oleic, linoleic and DHA. Similar result was reported by Patil et al.  (2007) that DHA was abundant only in *Isochrysis galbana* (15.8 mg g$^{-1}$). Fatty acid profile of *Isochrysis* sp. is in accordance with the earlier studies (Volkman et al. 1989; Shamsudin, 1992; Zhu et al. 1997). Lipid content of *I. galbana* culture grown in door ranged from 10 to 28% of dry weight (Brown et al. 1993; Molina Grima et al. 1994). In the present study *Tetraselmis* sp. showed high amount of palmitic acid, stearic acid, arachidic acid, oleic acid and linoleic acid. The fatty acid profile for *Tetraselmis* sp. was in accordance with those of earlier reports (Ackman et al. 1968; Volkman et al. 1989; Reitan et al. 1994; Patil et al. 2007), the EPA content being 5.37 mg g$^{-1}$. Usually the relative EPA content of *Tetraselmis* sp. ranges between 4-8% (Dunstan et al. 1992; Lourenço et al. 1997; Renaud et al. 1999; Milke et al. 2004; Rivero-Rodríguez et al. 2007; Patil et al. 2007) or 13–14% (Tzovenis et al. 2009), but rarely exceeds 10% ( Patil et al. 2007). When considering biomass productivity and lipid content, *Tetraselmis* sp. can be considered as the best species in this study. The results of this study will aid aquaculture farmers in choosing microalgae for their nutritional value.

**Conclusion**

The study provides information on the chemical profile of eight native marine microalgae, which might be useful for the selection of suitable native species in aquaculture and bioenergy production. Since microalgae may be limited by one or more essential nutrients, a mixed algal culture supplies a better equilibrium of nutrient properties, contributing for a better success in aquaculture **(**Brown et al. 1997; Rico-Villa et al. 2006**)** probably because their combined nutrient is more likely to meet the nutritional requirements of the target species (Cerón-Ortiz et al. 2009). *Nannochloropsis* sp. and *Tetraselmis* sp. can be used as suitable feed for aquaculture from the 10-20$^{th}$ day of their growth. The size of *Nannochloropsis* sp. and its high PUFA content was found to be the best nutritional option for aquaculture. This study also identifies *Tetraselmis* sp. as the species of choice for carotenoids, biomass and lipid production.

**Acknowledgements**

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Table 1

Showing the significance of the difference between days, species and replications based on (a) Total chlorophyll (b) Cell count (c) Chlorophyll a (d) Chlorophyll b (e) Carotenoids (f) Proteins (g) Carbohydrates using 3way ANOVA

| source | Total chlorophyll F ratio | Cell count F ratio | Chlorophyll A F ratio | Chlorophyll B F ratio | Carotenoids F ratio | Proteins F ratio | Carbohydrates F ratio | d o f Of F ratio |
|---|---|---|---|---|---|---|---|---|
| Days (A) | 14674.3** | 43081.7** | 11626.1** | 3896.20** | 13365.4** | 84090.1** | 3246.41** | (5,70) |
| Species (B) | 26659.8** | 8781.228** | 18454.2** | 4111.39** | 8952.81** | 241553.0** | 7494.54** | (7,70) |
| Replications ( C) | 6.4238** | 2.4848* | 3.5714* | 1.9422 | 1.2784 | 0.8333 | 2.8116* | (2,70) |
| AB interaction | 5668.40** | 1365.36** | 4429.75** | 2092.34** | 5247.80** | 63475.1** | 1709.71** | (35,70) |
| BC interaction | 0.7895 | 1.2475 | 0.9694 | 1.2456 | 0.8401 | 1.5476 | 1.5476 | (14,70) |
| AC interaction | 1.3036 | 0.9939 | 1.3214 | 1.2314 | 0.6136 | 1.6667 | 0.8063 | (10,70) |
| | | | | | | | | |
| Error | 0.000323 | 14.0857 | 0.0002563 | 0.000046158 | 0.00013428 | 9.6000 | 0.7558 | 70 |
| Total | 493.154 | 31064900 | 312.977 | 15.6353 | 149.593 | 275117000 | 396554.0 | 143 |

**Table 2        Fatty acid profile (FA) (wt %) of Marine microalgae**

| Fatty acid | Structure | *Chaetoceros* sp | *Chlorella* sp | *Dicrateria* sp | *Dunaliella* sp | *Isochrysis* sp | *Nannochloropsis* sp | *Synechococcus* sp | *Tetraselmis* sp |
|---|---|---|---|---|---|---|---|---|---|
| **Saturated** | | | | | | | | | |
| **Lauric acid** | **C12:0** | 0.11 | 0.06 | 0.2 | 0.24 | 0.19 | 0.15 | 0.4 | 0.27 |
| **Myristic acid** | **C14:0** | 1.22 | 1.37 | 0.2 | 3.51 | 0.98 | 0.13 | 2.6 | 2.18 |
| **Palmitic acid** | **C16:0** | 17.32 | 18.17 | 3.8 | 26.46 | 28.09 | 24.89 | 3.4 | 26.86 |
| **Stearic acid** | **C18:0** | 5.1 | 5.11 | 0 | 4.44 | 6.62 | 5.91 | 0 | 10.93 |
| **Arachidic acid** | **C20:0** | 0.2 | 10.87 | 0 | 0.87 | 1.73 | 1.17 | 0 | 18.38 |
| **Behenic acid** | **C22:0** | 0.9 | 1.82 | 0 | 2.05 | 2.03 | 2.36 | 0 | 1.06 |
| | **Sum** | 24.85 | 37.4 | 4.2 | 37.57 | 39.64 | 34.61 | 6.4 | 59.68 |
| **Monounsaturated** | | | | | | | | | |
| **Palmitoleic acid** | **C16:1** | 1.8 | 1.72 | 1.5 | 7.51 | 0.25 | 1.64 | 1.02 | 1.17 |
| **Oleic acid** | **C18:1 (ω-9)** | 9.47 | 27.72 | 2.1 | 27.42 | 28.02 | 28.98 | 0.84 | 14.08 |
| **Erucic acid** | **C22:1** | 0.1 | 0.52 | 0 | 6.93 | 0.19 | 1.31 | 0 | 2.53 |
| | **Sum** | 11.37 | 29.96 | 3.6 | 41.86 | 28.46 | 31.93 | 1.86 | 17.78 |

**Polyunsaturated**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Linoleic acid** | **C18:2 (ω-6)** | 0.7 | 14.84 | 0.5 | 18.27 | 18.45 | 15.07 | 0 | 12.32 |
| **Linolenic acid** | **C18:3 (ω-3)** | 1.1 | 0 | 0.12 | 0 | 0.55 | 1.35 | 0 | 0 |
| **Arachidonic acid (AA)** | **C20:4 (ω-6)** | 0.1 | 0 | 0 | 0 | 0.2 | 1.17 | 0 | 2.05 |
| **Eicosapentanoic acid (EPA)** | **C20:5 (ω-3)** | 1.8 | 0.38 | 0 | 0.93 | 0.42 | 15.8 | 0 | 5.37 |
| **Docosahexanoic acid (DHA)** | **C22:6 (ω-3)** | 0.8 | 0.4 | 0 | 0 | 12.26 | 0 | 0 | 2.96 |
| | **Sum** | 4.5 | 15.62 | 0.62 | 19.2 | 31.88 | 33.39 | 0 | 22.7 |

0 = Not Detected