



# A close monitoring of Variations in Fatty acid profile of *Isochrysis galbana* under conditions of limited Nutrition

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## Abstract

The current study was aimed at investigating the possible deviations in the degree of unsaturation of fatty acid components in the marine microalga *Isochrysis galbana*, under conditions of low nitrogen as well as low phosphorus concentrations. Standard f/2 medium in filtered and sterilized sea water of salinity 33ppt was used as control/N<sub>100</sub>. The other treatments used were f/2 medium with 25% nitrogen source (N<sub>25</sub>) and f/2 medium with 25% phosphorus source (P<sub>25</sub>). For control cultures, saturated fatty acids (SFAs) accounted for over 70.66% of the total fatty acids, whereas it was only 62.96% in nitrogen limited cultures and 60.17% in phosphorus limited ones. The present study reveals that nutrient stress provided a remarkable elevation in the degree of unsaturation. The value of monounsaturated fatty acids (MUFAs) was maximum for N<sub>25</sub> cultures (22.20%) followed by P<sub>25</sub> (19.62%) whereas it was 16.81% for control. Among the three treatments, the highest value of polyunsaturated fatty acids (PUFAs) observed in the P<sub>25</sub> cultures (20.21%) followed by 14.84% (N<sub>25</sub>) while it was only 12.52% for control cultures. One way analysis of data brought to light that nutrient stress can influence fatty acid pattern of the microalga significantly ( $p < 0.05$ ). The present observations suggest that low availability of macronutrients such as nitrogen or phosphorus can shift the fatty acid profile from saturated to unsaturated.

**Keywords:** *Isochrysis galbana*, nutrient limitation, saturated fatty acids, monounsaturated fatty acids, polyunsaturated fatty acids

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## Introduction

The marine microalga, *Isochrysis galbana* is a commonly used planktonic feed in mariculture industry (Sukenik et al., 1991). Assimilation of microalgal cells by shell fish larvae is very easy mainly due to their tininess and lack of rigid cell wall. Because of their high proliferation rate, easiness in large scale cultivation, wide temperature and salinity endurance and lack of toxins (Jeffrey et al., 1994) *Isochrysis* spp. have been used as a possible source of very long chain polyunsaturated fatty acids (VLC PUFAs) such as arachidonic acid, AA (C20:4n-6), eicosapentaenoic acid, EPA (C20:5n-3), docosahexaenoic acid, DHA (C22:6n-3) etc. for nurturing many aquatic species such as fishes, crustaceans etc. as well as their larvae (Guedes et al., 2012).

The dominance of microalgae over conventional crops credits to their potentiality to change cellular compositions according to changing culture conditions by different aspects such as age of the culture (Collins & Kalnins, 1969; Pugh, 1971; Conover, 1975; Bajpai, 1993), temperature (Renaud et al., 2002; Raghavan et al., 2008; Converti et al., 2009; Taoka et al., 2009), salinity (Lee et al., 1988; Cowan, 1991; Kirrolia et al., 2011), light intensity (Klok et al., 2013), irradiance (Hu et al., 2008) etc. They have high proliferation rate and photosynthetic performance (Chisti, 2007; Wu et al., 2013). Culture media could produce biochemical variations in microalgae (Ilavarasi et al., 2011; Lincymol et al., 2012; Naseera et al., 2014; Neethu et al., 2016; Prabha et al., 2017). The fatty acid profile can undergo variations with age, growth phase and nutritional value of the culture media used (Pratiwi et al., 2009; Huerlimann et al., 2010; Costard et al., 2012; Lidiya et al., 2018; Aswathy et al., 2020). Recent metabolic and epigenomic studies under different environmental

conditions also give convincing data regarding lipid metabolism alterations of microalgae (Rosenberg, 2014).

Nutrient stress in the culture media is a common strategy employed to stimulate large amount of lipid production in microalgae (Otsuka, 1960; Liu et al., 2008; Hu et al., 2008; Mujtaba et al., 2012; Sharma et al., 2012; Wei-Bao et al., 2013). Although widely investigated, the underlying mechanism of this strategy is still poorly understood. Nitrogen and phosphorus are the main growth limiting nutrients of phytoplankton in the native surroundings (Bergstrom et al., 2008; Davey et al., 2008; Persic et al., 2009).

Nitrogen can strongly influence the metabolism of lipids and fatty acids. Cell nitrogen is a constituent of proteins, amino acids and nucleic acids and phosphorus is mainly used to build phospholipids and nucleic acids (Geider, 2002; Griffiths et al., 2012; Hu, 2013). The acquisition apparatus (pigments) is rich in nitrogen while the assembly machinery is rich in nitrogen and phosphorus (Klausmeier et al., 2004). Smaller pools of phosphorus in Adenosine Triphosphate (ATP) and Nicotinamide Adenine Dinucleotide Phosphate (NADP) also take part in the energy producing processes (Gael et al., 2010). Ultimately these elements determine the lipid or carbohydrate productivity in them.

The main part played by nitrogen in enhancing the lipid productivity of numerous microalgal species is highlighted in several studies (Spoehr & Milner, 1949; Shifrin & Chisholm, 1981; Cobelas & Lechado, 1989; Roessler, 1990; Thompson, 1996; Illman et al., 2000; Li et al., 2008; Hu et al., 2008; Basova, 2005; Merzlyak et al., 2007; Zhu et al., 2015; Fakhry et al., 2015). Phosphorus limitation lead to enhanced lipid productivity in *Phaeodactylum tricornerutum* (Abida et al., 2015), *Monodus subterraneus* (Khozin et al., 2006), *Chaetoceros* sp. and *Diacronema lutheri*, but decreased lipid content in *Nannochloris atomus* and *Tetraselmis* sp. (Reitan et al., 1994). Additional studies unveiled a higher relative content of palmitic (C16:0) and oleic (C18:1n-9) acids and lower relative content of long chain omega-3 polyunsaturated fatty acids due to phosphorus paucity (Reitan et al., 1994).

The foremost aim in this study is to evaluate the fatty acid composition when the microalga, *I. galbana* exposed under nutrient deficient cultural conditions. The present study provides novel

insights that may facilitate the shaping of microalgal fatty acid profiles depending on the scope of application.

## Materials and Methods

Pure culture of microalgae was collected from Central Marine Fisheries Research Institute (CMFRI), Kochi and was maintained in standard f/2 medium (Table 1) so that maximum production of biomass could be ensured. In the present study standard f/2 medium in filtered and sterilized sea water of salinity 33 ppt was used for control ( $N_{100}$  cultures) (Table 1). The other treatments used were f/2 medium with 25% nitrogen source ( $N_{25}$ /nitrogen limited cultures) and f/2 medium with 25% phosphorus source ( $P_{25}$ /phosphorus limited cultures). The experiment was conducted in Completely Randomised Design (CRD) and each measurement was done with 3 replications.

About 600 ml (20% of the culture medium) of microalgal isolate having exponentially growing cells at an initial inoculum density of  $20\text{-}30 \times 10^4$  cells  $\text{ml}^{-1}$  (Pavlo et al., 2016) was transferred into previously autoclaved, properly capped and aerated

Table 1. Composition of standard F/2 medium (Guillard, 1975)

<b>Solution A</b>	
Sodium nitrate	7.5 g 100 ml <sup>-1</sup>
Sodium ortho phosphate	500 mg /100 ml <sup>-1</sup>
Sodium silicate	3 g 100 ml <sup>-1</sup>
<b>Solution B</b>	
Ferric chloride	0.3 gm 9.5 ml <sup>-1</sup>
Sodium EDTA	0.44 gm 9.5 ml <sup>-1</sup>
Manganese chloride	18 gm 100 ml <sup>-1</sup>
Zinc sulphate	2.2 g 100 ml <sup>-1</sup>
Cobalt chloride	1 g 100 ml <sup>-1</sup>
Copper sulphate	0.98 g 100 ml <sup>-1</sup>
Sodium molybdate	0.63 g 100 ml <sup>-1</sup>
<b>Solution C</b>	
Thiamine	200 mg L <sup>-1</sup>
Biotin	1 mg L <sup>-1</sup>
Cyanocobalamin	1 mg L <sup>-1</sup>

1ml each from solution A, 1 ml of solution B and 1 ml solution C were added to 1L of filtered and boiled sea water.

borosilicate 4 l culture flasks (a set of 3 for each treatment) under aseptic conditions. For illumination of the cultures, fluorescent tubes having intensity 1500 lux were employed (Hoff et al., 1987). A light/dark (L/D) cycle of twelve hours of lightness and 12 h of darkness was used for maintaining the stock as well as major cultures which could be controlled by auto timer (Barsanti et al., 2006). Air conditioned rooms having a fixed temperature of 25°C were used for keeping the stock cultures as well as indoor. The initial pH was set as 8-8.5 (Becker, 1994). For cultures, sea water of salinity 33-34ppt was used (Barsanti et al., 2006).

The duration of lag phase, log phase and stationary phases of the microalga could be monitored based on cell count, following the method of Andersen et al. (2005). After determining cell concentration (Hoff et al., 1987), the fully grown culture was harvested during the exponential phase (10<sup>th</sup> day of culturing) using High Speed Refrigerated Centrifuge (himac CR 22G). For this purpose, algal suspension was centrifuged at 10000 rpm for one minute. The supernatant was removed and the pellets were collected after multiple washing with sea water.

Extraction of total lipids was done as per Bligh & Dyer, 1959. About 500 mg to 1 gm of wet microalgal sample along with a pinch of Butylated Hydroxy Toluene (BHT-to prevent oxidation) was homogenized well in 5-10 ml distilled water using pestle and mortar. The pulp was transferred to a 250 ml conical flask and mixed with 20-30 ml chloroform methanol (2:1 v/v) mixture and shaken well. The mixture was kept overnight at 4°C preferably in the dark for complete extraction. At the end of this period, a further addition of 20ml chloroform and 20ml distilled water was made. The resulting solution was subjected to centrifugation and 3 layers were obtained. Then the blend was passed on to a separating funnel and the lower chloroform layer was carefully collected free of interface by filtering through sodium sulphate using a filter paper. It was concentrated in a pre-weighed round bottom flask at 40-45°C with the aid of a rotary vacuum evaporator. The weight ( $w_1$ ) was recorded after cooling.

Estimation of total lipid was done using the formula,  $\text{Lipid} = (w_1 - w_2 / w_3) \times 100$  (where  $w_1$  = weight of flask + lipid,  $w_2$  = weight of flask,  $w_3$  = weight of sample taken).

Fatty acid methyl esters (FAMES) were prepared following the methods of Metcalf et al. (1966). For this 5 ml of 0.5 N methanolic alkali was added to the extracted lipid and refluxed for 5 min in boiling water bath under nitrogen atmosphere so that breakage of ester bond (saponification of lipid) occurred. After cooling, 5 ml  $\text{BF}_3$  methanol solution was pipetted out and slowly added into it, refluxed for another 5 min in boiling water bath under nitrogen atmosphere and the mixture was kept for cooling so that FAMES were formed.

After cooling, 5-6 ml saturated NaCl was added to it and mixed well. FAMES were mixed well with petroleum ether (having double volume of the solution) for three times. Each time lower layer was discarded. The upper petroleum ether layer containing FAMES was washed thrice with distilled water, filtered through anhydrous  $\text{Na}_2\text{SO}_4$ , rotary evaporated and the concentrate was then reconstituted in minimum amount of petroleum ether and used for injecting in GC. For injection, 1 $\mu$ l of sample was used. FAMES were identified by comparison of retention times with the known standards (Supelco<sup>TM</sup> 37 Component FAME Mix, Catalog No. 47885-U), and the results were expressed as %Total Fatty Acid (TFA). The area of each component was obtained from computer generated data.

The experiment was conducted in Completely Randomised Design (CRD). Each measurement was taken with triplicates and the mean value of the experimental results was calculated using MS-Excel. Statistical evaluation (to compare means) was carried out with the Statistical Program for Social Sciences (SPSS Inc, Chicago, USA, ver. 22.0). The differences between treatments were analysed by one-way ANOVA, taking at  $p \leq 0.05$  as significant according to Duncan's post hoc tests.

## Results and Discussions

The fatty acid composition in *Isochrysis galbana* under control as well as nutrient deficient conditions is shown in Table 2. Cultures of *I. galbana* in triplicates were analysed under normal lab situations for lipid content and fatty acid profile. Fig. 1 shows the percentage of total lipid content in *I. galbana* when grown in control and nutrient limited media. Table 3 reveals that there is notable difference ( $p \leq 0.05$ ) in the total lipid content between control and nutrient deficient cultures. Same trend was found in *I. galbana* CASA CC 101 when grown

in nitrogen limited cultures (Jeyakumar et al., 2020). In the same way, total lipid content was foremost in *Chaetoceros muelleri* and *Dunaliella salina* when they were grown in a culture medium lacking nitrogen (Gao et al., 2013). It was observed that phosphorus starvation induces lipid accumulation in microalgae. The current experiment elucidated that the total lipid content in phosphorus deficient *I. galbana* cultures showed variations from control cultures significantly. This could be settled on with a previous finding on *Scenedesmus* LX1 in which littlest amount of phosphorus (intercellular phosphate) supported 30% hike in lipid content (Wu et al., 2013).

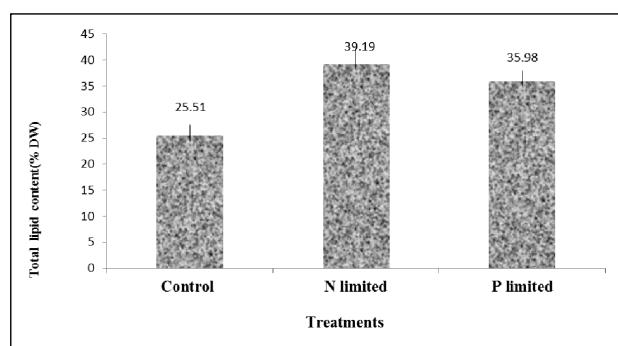


Fig. 1. % of total Lipid Content in *I. galbana* cultures when grown in control and nutrient deficient media. (Values are given as means of 3 independent experiments with standard error as error bars).

The fatty acids belonging to the family of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) were analyzed in all experimental cultures and are presented as percentage of total fatty acids (%TFA) (Fig. 2). Saturated fatty acids (SFAs) accounted for about 70% of the total fatty acids in control whereas it is 63% in nitrogen limited cultures and 60.17% for phosphorus limited one. Their values are significantly different in nutrient stressed conditions when compared with that of control.

The present experiment elucidated that nutrient limitation or a stressed state in *I. galbana* cultures found to produce a statistically significant amount of SFA, MUFA and PUFA. This observation is on par with that of Sabretin, 2012 who noticed that *I. galbana* promoted high molecular weight lipid production at low temperatures between 15°C to 22.5°C which is a stressed condition. During prolonged starvation period it is reported that carbohydrate would first be produced to reserve

energy, and then triacylglyceride (TAG) would be synthesized as a longstanding storage purpose (Siaut et al., 2011).

A set of 13 fatty acids were identified in the control cultures (Fig. 3). Among the 6 identified saturated fatty acids, maximum presence was that of lauric acid (C12:0) (44.90%) followed by myristic acid (C14:0) 8.73%, tridecylic acid (C13:0) 6.87%, palmitic acid (C16:0) 5.45%, stearic acid (C18:0) 4.72% and pentadecylic acid (C15:0) 1.63%. When we consider nitrogen limited cultures, 14 different fatty acids could be identified (Fig. 4). Major SFAs obtained were lauric acid (28.59%) followed by palmitic acid (13.50%). The amount of tridecylic acid and myristic acid were 10.26% and 10.60%. Data analysis showed that there is remarkable difference ( $p < 0.05$ ) in the SFA content in  $N_{25}$  cultures. A total of 20 different fatty acids could be isolated in phosphorus limited cultures (Fig. 5). Among them the saturated fatty acids obtained were lauric acid (C12:0) (33.41%) followed by myristic acid (C14:0) 10.92%, palmitic acid (C16:0) 8.46% and pentadecylic acid (C15:0) 1.15%. The SFA content in  $P_{25}$  cultures showed a marked difference ( $p \leq 0.05$ ) with that of control.

In control cultures, monounsaturated fatty acids included oleomyristic acid (C14:1 n-5), pentadecenoic acid (C15:1n-5) palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9) whereas in nitrogen deficient cultures, oleic acid (C18:1n-9) was present in maximum amount (9.96%) followed by oleomyristic acid (C14:1n-5) (8.58%), which dominated over oleopalmitic acid (C16:1n-7) (1.67%), erusic acid (C22:1n-9) (0.90%), eicosenoic acid (C20:1n-9) (0.70%) and nervonic acid (C24:1n-9) (0.41%). The prevalent monounsaturated fatty acids in  $P_{25}$  were oleic acid cis (C18:1n-9, 7.25%), oleopalmitic acid (C16:1n-7, 3.71%), pentadecenoic acid (C15:1n-5, 2.35%) and oleomyristic acid (C14:1n-5, 2.23%). Other MUFAs include vaccenic acid cis and trans (C18:1 n-7, 0.73 and 1.69% respectively), oleic acid trans (C18:1 n-9, 0.74%), eicosenoic acid (C20:1 n-9, 0.53%), erusic acid (C22:1 n-9, 0.30%) and nervonic acid (C24:1 n-9, 0.09%). In the present study, a notable difference ( $p \leq 0.05$ ) is evident in the monounsaturated fatty acid content in nutrient deficient cultures when compared with control cultures.

The major MUFA identified in the current nutrient limited cultures is the oleic acid. It is noted that in

Table 2. Fatty acid composition in *I. galbana* under control and nutrient limited cultures

Sl. No.	Lipid Number	Common Name	% of Total Fatty acids (Control) Mean $\pm$ SD	% of Total Fatty acids ( $N_{25}$ ) Mean $\pm$ SD	% of Total Fatty acids ( $P_{25}$ ) Mean $\pm$ SD	$N_{25}$ vs. Control Sig.	$P_{25}$ vs. Control Sig.
1	C12:0	Lauric acid	44.90 $\pm$ 0.001	28.593 $\pm$ 0.003	33.41 $\pm$ 0.01	.000	.000
2	C13:0	Tridecylic acid	6.87 $\pm$ 0.001	10.263 $\pm$ 0.0	6.23 $\pm$ 0.01	.000	.000
3	C14:0	Myristic acid	8.73 $\pm$ 0.1	10.599 $\pm$ 0.001	10.92 $\pm$ 0.02	.000	.000
4	C14:1 (n 5)	Oleomyristic acid	11.11 $\pm$ 0.001	8.579 $\pm$ 0.002	2.23 $\pm$ 0.0	.000	.000
5	C15:0	Pentadecylic acid	1.63 $\pm$ 0.002	nd	1.15 $\pm$ 0.0	.000	.000
6	C15:1 (n 5)	Pentadecenoic acid	0.82 $\pm$ 0.001	nd	2.35 $\pm$ 0.001	.000	.000
7	C16:0	Palmitic acid	5.45 $\pm$ 0.0	13.500 $\pm$ 0.06	8.46 $\pm$ 0.01	.000	.000
8	C16:1 (n 7)	Oleopalmitic acid	2.42 $\pm$ 0.0	1.671 $\pm$ 0.001	3.71 $\pm$ 0.01	.000	.000
9	C18:0	Stearic acid	4.76 $\pm$ 0.001	nd	nd	-	-
10	C18:1 (n7 cis)	Vaccenic acid (cis 11)	nd	nd	0.73 $\pm$ 0.0	.000	.000
11	C18:1 (n7 trans)	Vaccenic acid (trans 11)	nd	nd	1.69 $\pm$ 0.01	.000	.000
12	C18:1 (n 9 cis)	Oleic acid (cis)	0.83 $\pm$ 0.001	9.962 $\pm$ 0.0	7.25 $\pm$ 0.03	.000	.000
13	C18:1 (n9 trans)	Oleic acid (trans)	nd	nd	0.74 $\pm$ 0.01	-	.000
14	C18:2 (n 6 cis)	Linoleic acid (cis)	1.34 $\pm$ 0.001	4.777 $\pm$ 0.0	0.97 $\pm$ 0.01	.000	.000
15	C18:2 (n6 trans)	Linoleic acid (trans)	nd	nd	0.87 $\pm$ 0.01	-	.000
16	C18:3 (n 6)	Gamma linolenic acid	nd	1.988 $\pm$ 0.001	4.15 $\pm$ 0.01	.000	.000
17	C18:4 (n 3)	Stearidonic acid	nd	5.438 $\pm$ 0.001	0.03 $\pm$ 0.0	.000	.000
18	C18:3 (n 3)	Alpha linolenic acid	8.32 $\pm$ 0.01	nd	10.84 $\pm$ 0.01	.000	.000
19	C20:1 (n 9)	Eicosenoic acid/Gondoic acid	nd	0.677 $\pm$ 0.001	0.53 $\pm$ 0.01	.000	.000
20	C20:5 (n 3)	EPA	nd	nd	0.56 $\pm$ 0.01	.000	.000
21	C22:1 (n 9)	Erusic acid/Brassicidic acid	nd	0.899 $\pm$ 0.0	0.3 $\pm$ 0.0	.000	.000
22	C22:5 (n 3)	DPA	nd	nd	0.53 $\pm$ 0.0	.000	.000
23	C24:1 (n 9)	Nervonic acid	nd	0.410 $\pm$ 0.0	0.09 $\pm$ 0.0	.000	.000
24	C22:6 (n 3)	Docosahexaenoic acid/DHA	2.86 $\pm$ 0.001	2.64 $\pm$ 0.04	2.26 $\pm$ 0.04	.001	.000

nd – not detected.

the present study amount of oleic acid (C18:1n9) increased from 0.83% (control), 7.25% ( $P_{25}$ ) to 9.96% ( $N_{25}$ ). This result is in consistent with those of Huerlimann et al. (2014) who recorded that in nitrogen starved cultures of *Isochrysis* aff. *galbana* (TISO) there is general increase in MUFAs and is primarily because of increased production of oleic acid(C18:1n-9). MUFAs are formed by plastidial de novo fatty acid synthesis which becomes the substructure for biosynthesis of PUFA in the endoplasmic reticum (Chen et al., 2013; Huerlimann et al., 2014). This report is in correlation with the findings of the present study ie. oleic acid (18:1 n-9) is the dominant MUFA produced in the nutrient limited cultures which functioned as substrate for the synthesis of PUFAs and thereby accounted for the production of more PUFAs in nutrient deficient cultures than control cultures.

Control cultures produced only 3 classes of PUFAs, among them the maximum amount was that of alpha linolenic acid (C18:3 n-3, 8.32%) followed by docosahexaenoic acid, DHA (C22:6 n-3, 2.86%) and linoleic acid *cis* (C18:2 n-6 *cis*, 1.34%). In N limited cultures, 4 classes of PUFAs obtained. These included stearidonic acid (C18:4 n-3), linoleic acid *cis* (C18:2 n-6), docosahexaenoic acid, DHA (C22:6 n-3) and gamma linolenic acid, GLA (C18:3 n-6).

Under phosphorus stress, a significant increase in the level of polyunsaturated fatty acid synthesis is noticed. Among the 8 different classes of PUFAs, the highest value was obtained for alpha linolenic acid (C18:3 n-3, 10.84%) and the lowest was for stearidonic acid (C 18:4 n-3, 0.03%). The other PUFAs were linoleic acid *cis*(C18:2 n-6, 0.97%), linoleic acid *trans* (C18:2 n-6, 0.87%), gamma

Table 3. Comparison of transformed mean values of percentage of total lipid content and fatty acid classes of *I.galbana* in control and nutrient limited cultures based on Post-Hoc Test (Duncan) and their significance in Analysis of Variance.

Parameters	Control Cultures	N <sub>25</sub> Cultures	P <sub>25</sub> Cultures	Sig.
Lipid%	0.5293 <sup>a</sup>	0.6763 <sup>b</sup>	0.6432 <sup>b</sup>	0.001
SFA%	0.9984 <sup>c</sup>	0.9099 <sup>b</sup>	0.8878 <sup>a</sup>	0.000
MUFA%	0.4225 <sup>a</sup>	0.4906 <sup>c</sup>	0.4595 <sup>b</sup>	0.000
PUFA%	0.3617 <sup>a</sup>	0.3955 <sup>b</sup>	0.4663 <sup>c</sup>	0.000

Transformed Mean values having same superscripts in the same row belong to same homogeneous subsets.

linolenic acid (C18:3 n-6, 4.15%), eicosapentaenoic acid, EPA (C20:5 n-3, 0.56%) and docosapentaenoic acid, DPA (C22:5 n-3, 0.53%). Total PUFA content in both nitrogen and phosphorus limited cultures exhibited statistically notable difference ( $p \leq 0.05$ ).

Among the three treatments studied there is no significant variations in the value of docosahexaenoic acid (C22:6 n-3). This is in agreement with the observations of Zheng et al. (2019) that the value of DHA production in 18 strains of *Isochrysis* varied between 0.99% to 1.8%. Further the upshots of the present study envisaged an elevation of polyunsaturated fatty acids from 12.52% for control cultures to 14.84% for N<sub>25</sub> cultures to 20.21% for P<sub>25</sub> cultures under nutrient limited conditions.

In our work, nutrient limitations are associated with significant elevation in palmitic, C16:0 and oleic, C18:1 acids. This result is in accordance with the results published by Neha et al. (2019). They reported that nitrogen starvation in *I. galbana* changes the metabolic channel from protein synthesis to energy reserve (carbohydrate and lipid) and assemblage of saturated fatty acid at the cost of

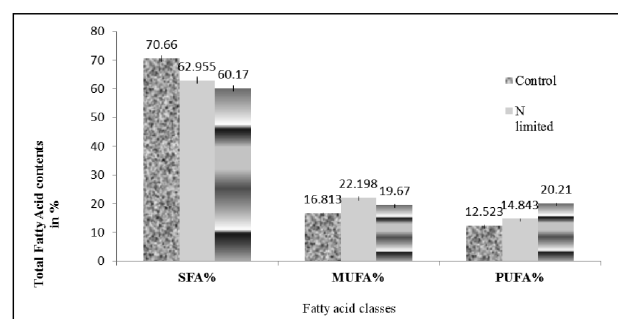


Fig. 2. % of Total fatty acid classes in *I. galbana* cultures when grown in control and nutrient deficient media. (Values are given as means of 3 independent experiments with standard error as error bars.

unsaturated fatty acid excluding docosahexaenoic acid. The findings of the current study are identical to some descriptions in literary works (Siaut et al., 2011; Ho et al., 2012) which stated that, under N starved cultural conditions, the foremost hikes in fatty acids were that of saturated (palmitic acid; setaric acid) and monounsaturated (palmitoleic acid; oleic acid) ones. Other experimenters have documented the primacy of palmitic acid, oleic acid and linoleic acid in many microalgae (Yeesang & Cheirsilp, 2011). Another supporting aspect is that many studies under nitrogen limited conditions, reported accumulation of SFAs and MUFAs as the main component of the lipid content in microalgae (Hu, 2004; Siaut et al., 2011; Ho et al., 2012).

Goncalves et al., 2013 found that membrane lipid acyl groups remodeled into TAG during nitrogen starvation in *Chlorella* UTEX29. Ultrastructural studies unveiled abundance of lipid bodies coupled with shortage of starch granules in nitrogen deficient cells. To scrutinize the happenstance of lipids after

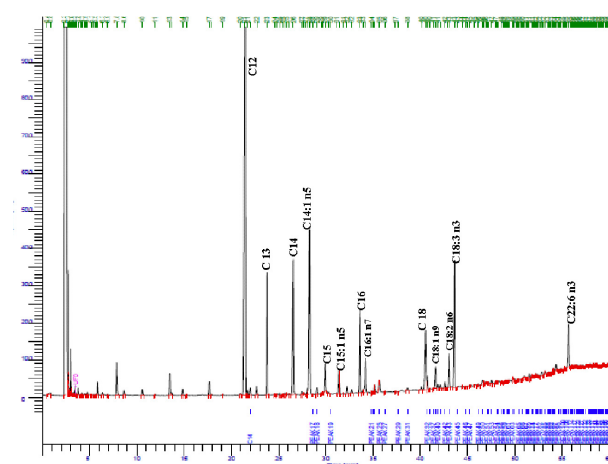


Fig. 3. Gas chromatogram of fatty acid profile of *Isochrysis galbana* in f/2 medium control

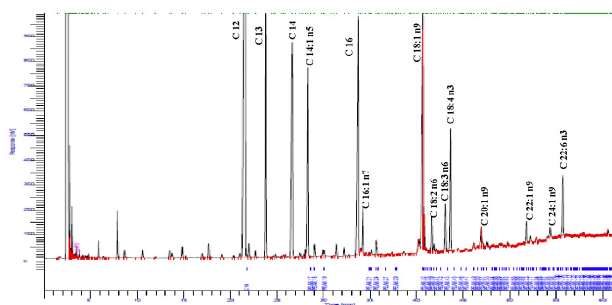


Fig. 4. Gas chromatogram of fatty acid profile of *Isochrysis galbana* in Nitrogen limited medium

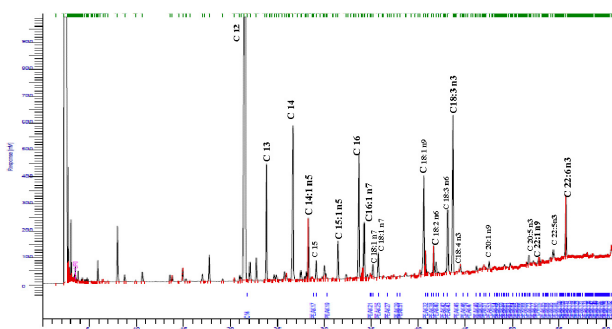


Fig. 5. Gas chromatogram of fatty acid profile of *Isochrysis galbana* in Phosphorus limited medium

nitrogen starvation,  $^{14}\text{C}$ -acetate was used. A notable drop off in  $^{14}\text{C}$ -galactolipids and phospholipids matched the surge in  $^{14}\text{C}$ -TAG starting at nitrogen scarcity, consonant with acyl groups from structural lipids as sources for TAG under nitrogen paucity. TAG might enact a safeguarding role to protect microalgae from oxidative injury under nutrient stress conditions (Hu et al., 2008). Under nitrogen starvation many microalgae are able to adapt their metabolic pathways towards the accumulation of high amounts of storage lipids (Ikarán et al., 2015). Nutrient scarcity can intensify fatty acid production and lipid synthesis (Litao et al., 2016). Their findings suggested that nitrogen deprivation when coupled with inhibition of Alternative Oxidase pathway (AOX pathway) caused the over production of reducing equivalents, over reduction of chloroplasts, leading to decreased evolution of photosynthetic oxygen and further increased assembling of fatty acids in *I. galbana*. These reports in the literature are in correlation with the findings of the current study.

In the present findings nitrogen limitation resulted in relative rise in lipid content of *I.galbana* is in consonant with some of the previous works (Li et al., 2012; Simionato et al., 2013; Chokshi et al., 2017).

They have documented that nitrogen scarcity resulted in low growth rate and protein synthesis in green algae *Microactinium pusillum*, *Nannochloropsis gaditana* and *Acutodesmus dimorphus* on the contrary, enhanced production of high energy molecules. It is well known that, under favorable culture conditions, many microalgae possess a common default photosynthetic carbon partitioning mechanism which may result in 8–15% lipid, 20–40% carbohydrate, 30–50% protein on as per total organic matter basis (Hu, 2005). During adverse culture conditions, microalgae undergo programmatic transitions in photosynthetic carbon partitioning particularly in the relative amounts of crude protein (Dean et al., 2010). Under environmental stress, such as nutrient paucity, the flow of carbon synthetic channel is diverted from the path of protein synthesis to that of lipid and/or carbohydrate synthesis. It could be the reasons of nitrogen denial raising the lipid or carbohydrate content.

Under stressed cultural conditions microalgae channelizes the photosynthetically fixed carbon molecules into the formation of non nitrogen compounds like carotenoids and high energy compounds like carbohydrate and lipid (Markou et al., 2012). Reduced and hydrophobic character enables them to be efficiently allocated into the cellular partitions which are used to sustain the longstanding stressful conditions (Courchesne et al., 2009). However, alterations in the cumulation setup of high energy molecules depend on microalgal carbon metabolism which is species-specific (Becker, 1994). Generally, the lipid content of microalgae multiplied up to 2- to 3-fold due to nitrogen scarcity for 4–9 days, whereas Shifrin et al. (1981) documented both increase and decrease in diatoms, depending on the species (Shifrin et al., 1981). Knuckey et al. (2002) evaluated 10 strains of diatoms for being used as feed for juvenile pacific oysters. Similarly Hassan et al. (2013) reported increased lipid production in *Nitzschia palea* when grown under low concentrations of nitrogen in the Chu medium. Same trend was observed when Chagoya et al. (2014) cultured 2 species of *Nitzschia* in f/2 medium containing different molar volumes of N,P and Si. The results revealed a decrease in lipid formation in both species when the N,P and Silicon concentration in the medium got increased.

Phosphorus shortage was found to trigger multiple results, including variations in cell lipid and

pigment content (Ota et al., 2016). Phosphorus scarcity produces striking alterations in the biosynthetic processes. In the present study, there is significant variation regarding the amount of monounsaturated and polyunsaturated fatty acids in nitrogen and phosphorus deprived cultures. Phosphorus has notable influence on lipid content and also in fatty acid composition of *Monodus subterraneus* (Khozin et al., 2006). This was supported by earlier findings in history (El-Sheek et al., 1995; Spijkerman et al., 2011) as they documented a remarkable elevation in the level of unsaturated fatty acids when green algae *Chlorella kessleri* as well as *Chlamydomonas acidophila* were exposed to phosphorus limited culture conditions.

As discussed already, the present findings are in alignment with earlier studies (Li et al., 2006; Guschina et al., 2009; Liang et al., 2013) since there is no reduction in TAG under nutrient deficient conditions. Liang et al. (2013) reported an effective phosphorus conserving mechanism in *Chlorella* sps. i.e. when grown under phosphorus paucity, a severe depletion in membrane phospholipids happened and were found to be replaced by non phosphorus glycolipids and sulpholipids. Earlier studies suggested that cell division rates as well as photosynthetic rates were slightly decreased under phosphorus starvation and it resulted in carbon accumulation in the form of triacyl glycerols which are abundantly present in saturated and monounsaturated fatty acids.

Based on the findings of the current study, it can be deduced that nitrogen as well as phosphorus depletion enhances unsaturation (oleic acid, linoleic acid, GLA, STA, ALA, eicosenoic acid, EPA, erusic acid, DPA, nervonic acid etc.) in *I. galbana*. The present observations suggest that low nutrient availability, especially nitrogen and phosphorus along with high levels of other macro and micro-nutrients, enriched the feed quality of microalgae by shifting the fatty acid picture from saturated to unsaturated. A comprehensive grasping of biochemical productivities and fatty acid expression patterns in different algal species can help to analyze their dietary value and it can open chances for the modifications of cultivating environmental conditions.

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