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Variations in lipid profile of of the marine microalga *Nannochloropsis salina* in four different culture media

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ABSTRACT

The present work envisages close monitoring of variations in lipid content and fatty acid profile of the marine microalga *Nannochloropsis salina*, by growing it in 4 different culture media. Pure cultures of microalga were collected from the marine hatchery complex of the ICAR-Central Marine Fisheries Research Institute, Kochi and cultured in four different media *viz*., f/2 medium (Guillard, 1975), Miquel's medium (Miquel, 1892), Walne's medium (Walne, 1974) and Chu#10 medium (Chu *et al*., 1942). Standard algal culture conditions *viz*, salinity 33 ppt, pH 8.0, culture room temperature of 25ºC, light intensity of 1500 lux with a photoperiod of 12 h and Light:Dark cycle of 12 h were maintained. Lipids extracted were subjected to gas chromatography to ascertain their fatty acid profile. Both Miquel's and f/2 media produced the maximum amount of lipid content (40.2 and 40% respectively). Total saturated fatty acids ranged from 39.17% (Miquel's medium) to 84.45% (Chu#10). For monounsaturated fatty acids, the highest value was obtained for Miquel's medium (42.79%) and minimum for Chu#10 (10.55%). Polyunsaturated fatty acids accounted for over 23.50% of the total fatty acids in Walne's medium followed by Miquel's (18.04%), f/2(12.76%) and Chu#10(5.00%) media. One way analysis of variance of the data brought to light that the medium composition can influence the fatty acid pattern of microalgae significantly.

Keywords: Fish culture, Live feed, Micronutrients, Omega-3, Omega-6

Introduction

The rapid growth of some microalgal cultures offers opportunities to continuously produce lipid, starch or protein-rich biomass and have been used as live feed in hatcheries of finfishes and shellfishes (Atalah *et al*., 2007; Ganuza *et al*., 2008; Hemaiswarya *et al*., 2011; Velasquez *et al*., 2016) which indirectly nourishes human diet (Bold, 1942; Canizares *et al.*, 1994). Nearly all microalgal biomass are rich sources of omega-3/ omega-6 fatty acids, essential amino acids (leucine, isoleucine and valine) and carotene.

Screening and optimisation of culture medium is among the foremost preconditions for photoautotrophic cultivation of microalgae. The growth performance of microalgae is mostly determined by the quality of the medium used for their cultivation (Lam and Lee, 2012; Li *et al*., 2012; Prathima *et al*., 2012). Manipulation of the nutritional content of the microalgae is possible by changing media composition and culture strategies (Otero *et al.*, 2006; Ilavarasi *et al*., 2011; Lincymol *et al*., 2012; Naseera *et al*., 2013; Neethuand Dhandapani, 2016; Praba *et al*., 2016). Even the essential fatty acid composition of the algae could be modulated using these strategies (Kaladharan *et al*., 1999; Martinez-Fernandez *et al.,* 2006; Rivero-Rodriguez *et al.*, 2007; Lidiya and Joseph, 2018; Aswathy *et al*., 2020). Since the composition and availability of macro and micronutrients in the media directly influence algal cultures, they should be supplied at an optimal level (Liu *et al*., 2008). Macronutrients such as nitrogen, potassium, magnesium, sulfur and sodium are non-toxic to algal cells and so can be added at higher concentrations. In contrast, essential micronutrients such as Fe, Cu, Mn, Zn, Co and Mo are growth-limiting at low concentrations and toxic at higher concentrations. These micronutrients play a major role in many metabolic channels that affect the growth of algal cultures (Sunda and Huntsman, 1998; Sunda *et al*., 2005).

The genus *Nannochloropsis* was first described by Hibberd (Hibberd, 1981) which is broadly well appreciated in aquaculture (Roncarati *et al*., 2004; Bentley *et al*., 2008) thanks to its comparatively high rate of growth, resistance to mixing and contamination along with high nutritional values and high lipid content (Rodolfi *et al*., 2003; Olofsson *et al*., 2012). The highlight of *Nannochloropsis* biomass for the aquaculture industry is attributed to its high content of omega-3 fatty acid, eicosapentaenoic acid or EPA (C20:5 n-3) (Watanabe, 1979; Watanabe *et al*., 1983; Koven *et al*., 1990; Seto *et al*., 1992; Sukenik

et al., 1993). *Nannochloropsis* species was found to support relatively high rates of rotifer reproduction which are living food capsules for fish larvae (Hirayama *et al*., 1979; Yamasaki *et al*., 1989; Ahmad, 1991).

It should be noted that the optimal nutrient concentration varies depending on the microalgal strain, as well as the processing and cultivation parameters. Therefore, the medium should be optimised for each microalgal strain before use for mass cultivation. The current study was to inquire into the total fatty acid layout in *Nannochloropsis salina*, a unicellular marine microalga, when grown in four different standard culture media to identify and select the medium that increases unsaturation and thereby the nutritional status of microalga.

Materials and methods

Pure culture of *N. salina* was obtained from ICAR-Central Marine Fisheries Research Institute (ICAR-CMFRI), Kochi, Kerala, India and was maintained in standard f/2 medium so that maximum production of biomass could be ensured. Since no single medium will be suitable for higher growth and biochemical production in microalgal species, it was worth trying a few different formulations that are frequently used and found in the literature. The 4 different media *viz,* f/2 medium (Guillard, 1975); Miquel's Media (Miquel, 1892); Walne's medium (Walne, 1974) and Chu#10 medium (Chu, 1942) were prepared as per standard published methodologies.

The experiment was conducted in completely randomised design (CRD) and each measurement was done in 3 replications. About 600 ml (20% of the culture medium) of microalgal isolate having exponentially growing cells at an initial inoculum density of 20-30x10^4 cells ml^{-1} (Pavlo *et al*., 2016) was transferred into previously autoclaved, properly capped and aerated borosilicate 4 l culture flasks (a set of 3 for each treatment) under aseptic conditions. For the illumination of the cultures, fluorescent tubes having an intensity of 1500 lux were employed (Hoff and Snell, 1987). A light/dark (L/D) cycle of 12 h of light and 12 h of darkness was used for maintaining the stock as well as major cultures which could be controlled by an auto timer (Barsanti and Gualtieri, 2006). Air-conditioned rooms having a fixed temperature of 25ºC were used for keeping the stock cultures indoors. For cultures, seawater of salinity 33-34 ppt was used (Barsanti and Gualtieri, 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 and Snell, 1987), the fully grown culture was harvested during the late exponential phase using High-Speed Refrigerated Centrifuge (HIMAC CR 22G). For this purpose, the algal suspension was centrifuged at 10000 rpm for 1 min. The supernatant was discarded and the pellets were collected after multiple washing with seawater.

Total lipids were extracted as per Bligh and Dyer (1959). About 500 mg to 1 g of wet microalgal sample along with a pinch of butylated hydroxy toluene (to prevent oxidation) was homogenised well in 5–10 ml distilled water using pestle and mortar. The pulp was transferred to a 250 ml conical flask and mixed with a 20–30 ml chloroform-methanol (2:1 v/v) mixture and shaken well. The mixture was kept overnight at 4ºC in the dark for complete extraction and then 20 ml chloroform and 20 ml distilled water was added. The resulting solution was subjected to centrifugation and 3 layers were obtained. Then the mixture was transferred to a separating funnel and the lower chloroform layer was carefully collected free of the interface by filtering through sodium sulfate using a filter paper. It was concentrated in a pre-weighed round bottom flask at 40–45°C using a rotary vacuum evaporator. This was allowed to cool and the weight (w_1) was noted. Total lipid was calculated using the formula Lipid = (w_1-w_2/w_3) x 100 (where w_1 = weight of flask +lipid, w_2 = weight of flask, w_3 = weight of sample taken).

Fatty acids were analysed as fatty acid methyl esters (FAMEs). For this 5 ml of 0.5N methanolic alkali was added to the lipid and reflexed for 5 min in a boiling water bath under a nitrogen atmosphere so that breakage of ester bonds (saponification of lipid) occurred. After cooling, 5 ml BF_3 Methanol solution was pipetted out and slowly added, refluxed for another 5 min in boiling water bath under nitrogen atmosphere and the mixture was kept for cooling so that FAMEs were formed (Metcalf *et al*., 1966). About 5-6 ml saturated NaCl was added to it and mixed well. FAMEs were mixed well with petroleum ether (double the volume of the solution) three times. Each time lower layer was discarded and the top layer of petroleum ether having fat was used. The process of extraction was repeated 3 more times with distilled water. Finally, the lower layer was filtered through anhydrous Na_2SO_4, rotary evaporated and the concentrate was then reconstituted in a minimum amount of petroleum ether and used for injecting in gas chromatograph (GC). For injection, 1 µl of the sample was used. The area of each component was obtained from computer-generated data. FAMEs were identified by comparison of retention times with the known standards (SupelcoTM 37 Component FAME Mix, Catalog No. 47885-U) and the results were expressed as % total fatty acid.

Statistical analysis

Statistical evaluation (to compare means) was carried out with the Statistical Program for Social Sciences 13.0

(SPSS Inc, Chicago, USA, ver. 22.0). The differences between treatments were analysed by one-way ANOVA, taking p=0.05 as significant, followed by Tukey's test.

Results and discussion

The amount of lipid produced (in percentage) from the harvested algal biomass of all experimental treatments is presented in Table 1. The highest lipid content was obtained from *N. salina* cultures in Miquel's as well as in f/2 media (41.0%, 40.2% respectively) followed by Walne's (32.54%) and Chu#10 medium (26.13%). Analysis using one-way ANOVA revealed that there was a notable difference ($p \leq 0.05$) in the total lipid content among cultures in the four different media.

Various authors have reported the lipid content of *Nannochloropsis* spp. under various experimental conditions. Chiu *et al*. (2009) reported that under optimal growth conditions *N. oculata* had a lipid content above 50%. Bondioli *et al*. (2012) obtained a lipid content of 39.1% in *Nannochloropsis* sp. (F&M-M24 strain) whereas Xu *et al*. (2004) evidenced 22-31%. Meng *et al*. (2015) stated a lipid content of 28-59% in *N. oceanica* IMET1. Mitra *et al*. (2015) observed a lipid content of 22.3 to 38.6% in *Nannochloropsis gaditana*. The above pre-stated reports regarding lipid content in *Nannochloropsis* spp. were in harmony with the results of the current study. The present result contradicts the result obtained by Kaladharan *et al*. (1999) where they got only 11.28% of lipid content in *N. salina* cultures grown in Walne's medium. This may be because of the difference in the analytical procedures adopted and variations in culture environments such as temperature or photoperiod selected.

Iron (Fe) is considered as one of the most important trace elements in microalgae growth media. It is expected that Fe increases overall lipid accumulation due to down-regulation of iron requiring fatty acid desaturase enzymes. A substantial increase in lipid radicals in the membranes of *Chlorella vulgaris* was noticed when iron was added up to 500 µM (Estevez *et al.*, 2001). Hence, the highest amount of Fe source (2 g 100 ml^{-1} stock solution) possibly resulted in the high lipid content in *N. salina* cultures in Miquel's medium.

Being a constituent of cytochromes, Fe plays an important role in nitrogen assimilation as a functional part of ferredoxin and affects the synthesis of phycocyanin and chlorophyll. The addition of Fe in the form of inorganic salts will tend to precipitate and become inaccessible to algae (Becker, 1994). Since Miquel's medium contains Fe in the form of ferric chloride, it may shorten N assimilation thereby leading to lipid induction.

The use of Fe in the form of chelated complex (Fe EDTA) is more useful (Becker, 1994). Both f/2 medium and Walne's medium contain Fe source in the form of chelated complex and proper uptake of macronutrients especially nitrogen happens to lead to the high performance of photosynthetic pathway instead of lipid synthetic pathway. The reason for having higher lipid content (41.00%) in the f/2 medium than that in Walne's (32.54%) may be due to the fact that f/2 contains a lower amount of Fe source than Walne's leading to lesser N assimilation and a substantial increase in lipid production. Omar (2002) observed that *Botryococcus sudeticus* accumulated more lipids (30%) at a low concentration of Zn. A close examination of the chemical composition of Miquel's medium reveals that it lacks the above trace element thereby increasing the lipid value.

Results of GC analysis of *N. salina* in 4 different culture media are depicted in Fig. 1 to 4. The fatty acids belonging to the family of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) were analysed in all experimental cultures and are expressed as percentage of total fatty acids (% TFA) (Table 2 to 4). Total saturated fatty acids ranged from 39.17% (Miquel's medium) to 84.45% (Chu#10). The results of the present study showed that among saturated fatty acids, lauric acid (C12) is the major SFA in cultures of almost all media followed by palmitic acid (C16:0) which ranged from 5.56% (f/2), 6.81% (Miquel's), 7.77% (Walne's) to 13.12% (Chu#10). The amount of myristic acid (C14) obtained was almost equal in all media (2.29 to 3.37%). The presence of 0.8% stearic acid in Miquel's medium is similar to the results by Alicia *et al*. (2015) in *N. gaditana* where the fatty acid profile evidenced the presence of 4.4% myristic acid and 0.8% stearic acid. Data analysis showed that there are remarkable variations in SFA content among the cultures.

Table 1. Variations in total lipid (%) in *N. salina* in 4 different culture media

Sl. No.	Culture media	Lipid	Mean±SD
1.	f/2	40	40 ± 2.08
2.	Miquel's	40.2	40.2 ± 3.40
3.	Waine's	32.54	32.54 ± 2.35
4.	Chu#10	26.13	26.13 ±3.56

Value of p=0.001

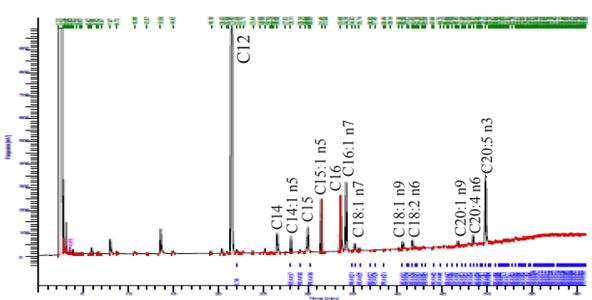

Fig. 1. Result of GC analysis of *N. salina* in f/12 medium

Higher content of monounsaturated fatty acids (MUFAs) was obtained for Miquel's medium (42.79%) followed by f/2 medium (20.11%), Chu#10 (10.55%) and lowest for Walne's (9.39%) medium. One-way ANOVA showed that there is a notable difference ($p \leq 0.05$) in the MUFA content among the four different treatments. The observations of Carrero *et al*. (2011) and Alicia *et al*. (2015) in *N. gaditana* regarding MUFA content (above 40 and 46.5% respectively) are in tune with the findings in the current study.

It is found that among the 9 classes of MUFA, oleic acid (18:1 n-9) is the major one present in *N. salina*, which attained maximum value in Miquel's medium (19.90%) and minimum in f/2 (1.27%). In Chu#10, both *cis* and

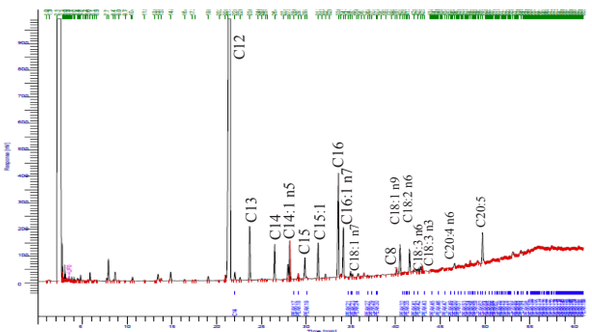

Fig. 3. Result of GC analysis of *N. salina* in Walne's medium

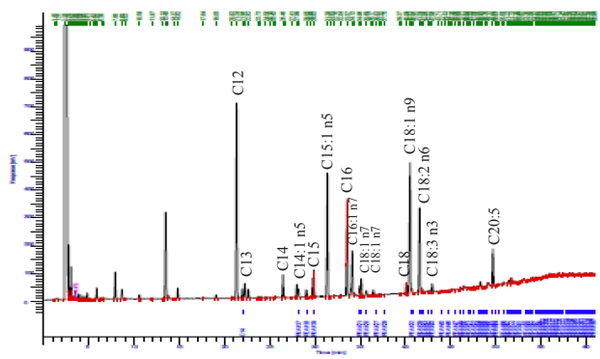

Fig. 2. Result of GC analysis of *N. salina* in Miquel's medium

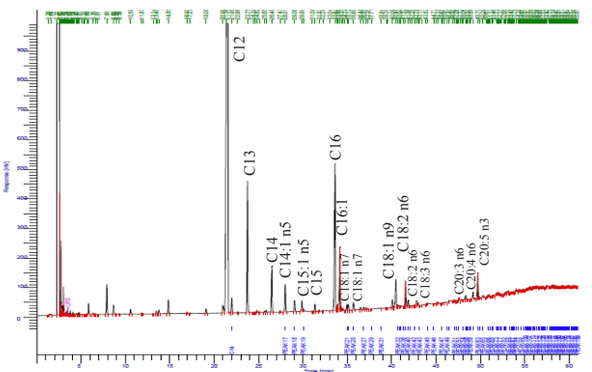

Fig. 4. Result of GC analysis of *N. salina* in Chu#10 medium

Table 2. Variations in saturated fatty acid components (%) in *N. salina* in 4 different culture media

Sl. No.	Fatty acids		f/2 medium	Miquel's medium	Walne's medium	Chu#10 medium
1	Lauric acid	C12	54.29 ±0.01	25.21±0.00	52.09 ±0.01	58.3 ±0.01
2	Tridecanoic acid	C13	nd	1.05 ±0.01	3.38 ±0.03	8.95 ±0.10
3	Myristic acid	C14	3.36 ±0.08	2.90 ±0.01	2.29 ±0.02	3.37 ±0.01
4	Pentadecanoic acid	C15	3.95 ±0.01	2.42 ±0.00	1.57 ±0.02	0.70 ±0.20
5	Palmitic acid	C16	5.56 ±0.32	6.81±0.71	7.77 ±0.09	13.12 ±0.21
6	Stearic acid	C18	nd	0.78 ±0.02	nd	nd
Total SFA (%)			67.13 ±0.40	39.17 ±0.75	67.10 ±0.15	84.45 ±0.53

Value of p=0.000. nd: not detected

Table 3. Variations in monounsaturated fatty acid components (%) in *N. salina* in 4 different culture media

Sl. No.	Fattyacids		f/2 medium	Miquel's medium	Walne's medium	Chu#10 medium
1	Oleolauric acid	C12:1 n-5	nd	nd	nd	1.07±0.01
2	Oleomyristic acid	C14:1 n-5	1.99±0.03	1.75±0.03	0.96±0.01	2.05±0.01
3	Penta decenoic acid	C15:1 n-5	4.281±0.09	17.50±0.01	2.40±0.10	0.56±0.40
4	Palmitoleic acid	C16:1 n-7	10.80±0.02	2.37±0.41	3.26±0.20	3.08±0.21
5	Vaccenic acid	C18:1 n-7 *cis*	1.23±0.01	1.26±0.01	0.40±0.02	0.48±0.01
6	Vaccenic acid	C18:1 n-7 *trans*	nd	nd	nd	0.45±0.03
7	Oleic acid	C18:1 n-9 *cis*	1.27±0.70	19.90 ±0.80	2.06±0.05	0.61±0.01
8	Oleic acid	C18:1 n-9 *trans*	nd	nd	nd	2.26±1.50
9	Eicosaenoic acid	C20:1 n-9	0.54±0.03	nd	0.33±0.04	nd
Total MUFA (%)			20.11±0.87	42.79 ±1.25	9.39±0.42	10.55±2.26

Value of p=0.000. nd: not detected

Table 4. Variations in polyunsaturated fatty acid components (%) in *N. salina* in 4 different culture media

Sl. No.	Fatty acids		f/2 medium	Miquel's medium	Walne's medium	Chu#10 medium
1	Linoleic acid	C18:2 n-6 cis	1.13 ±0.01	11.99 ±0.03	1.54 ±0.01	0.78 ±0.01
2	Linoleic acid	C18:2 n-6 trans	nd	nd	nd	1.15 ±0.00
3	α linolenic acid	C18:3 n-3	nd	1.05 ±0.00	0.36 ±0.02	nd
4	Gamma linolenic acid	C18:3 n-6	nd	nd	0.24 ±0.01	0.43 ±0.03
5	Dihomogamma linolenic acid	C20:3 n-6	nd	nd	nd	0.46 ±0.00
6	Arachidonic acid	C20:4 n-6	1.20 ±0.02	nd	0.14 ±0.01	1.14 ±0.01
7	Eicosapentaenoic acid	C20:5 n-3	10.43 ±0.80	5.00 ±0.60	21.22 ±0.09	1.04 ±0.02
Total PUFA (%)			12.76±0.83	18.04±0.63	23.50±0.11	5.00±0.06

Value of p= 0.000. nd: not detected

trans forms were obtained (0.61 and 2.26% respectively) and in Walne's medium value was 2.06%. The fatty acid profile revealed the presence of oleomyristic acid as 0.96% (Walne's medium) followed by 1.75% (Miquel's), 2% (f/2) and 2.05% (Chu#10). This finding is in agreement with that of Alicia *et al*. (2015) in *N. gaditana*.

Polyunsaturated fatty acids (PUFAs) accounted for over 23.50% of the total fatty acids in Walne's medium followed by Miquel's (18.04%), f/2 (12.76%) and Chu#10 (5.00%). The total PUFA obtained in Walne's medium is in agreement with those obtained by Carrero *et al*. (2011) (above 20%) and Alicia *et al*. (2015), (23.8%) in *N. gaditana.* In the current study, a marked difference ($p \leq 0.05$) is evident in the polyunsaturated fatty acid radicals among cultures in different media.

A close examination of the chemical combination of Walne's and Miquel's media revealed that they have sufficient amount of macronutrients like N, P, K and Ca. Usually, under conditions of nitrogen sufficiency, microalgae synthesise membrane glycerolipids which reside in the plasma membrane and endoplasmic membrane systems (Piorreck and Pohl, 1984). These glycerol-based membrane lipids are constituted mainly of long-chain unsaturated fatty acids containing various kinds of PUFA and play a structural role in the cell (Hu *et al*., 2008). Thus, PUFA is accumulated more at the growth phase in which cell division actively progressed (Hu *et al*., 2008). The presence of a large amount of PUFA in *N.salina* cultures grown in Walne's as well as in Miquel's media further affirms the above-mentioned findings.

The nitrogen source in f/2 medium is sodium nitrate, in Chu#10 medium it is calcium nitrate while in Walne's and in Miquel's it is potassium nitrate. *N. salina* cultures in Chu#10 medium had a minimum value of total lipid, MUFA, PUFA and EPA. This is because the calcium nitrate in this medium is actually responsible for protein biosynthesis and thereby it accounts for algal growth and not for lipid synthesis (Ilavarasi *et al*., 2011). Another aspect is that the PO_4 source (K_2HPO_4) enhances algal growth, not lipid synthesis (Turpin, 1986).

The present experiment exhibited 6 classes of PUFAs, among them C18:2 n-6 attained a maximum in Miquel's medium (11.99%) and for the other 3 media, values were less. The amount of arachidonic acid is more or less equal in f/2 (1.2%) and in Chu#10 (1.14%) media. DGLA (C20:3 n-6) was spotted only in Chu#10 medium. Hoffmann *et al*. (2010) observed that *N. salina* contains 2.5 to 4.5% PUFA content as well as 1.1-3.5% EPA content.

The value of palmitoleic acid (C16:1 n-7) obtained from *N. salina* cultures grown in Walne's and Chu#10 media in the present study is similar with that obtained for *N. limnetica* cultures (Krienitz and Wirth, 2006). Similarly, Volkman *et al*. (1993) reported 2.18% palmitoleic acid (C16:1 n-7) and 0.63% oleic acid (C18:1 n-9) in *N. oculata* CS 216 which is equivalent to 2.37 and 0.61% obtained from *N. salina* cultures grown in Miquel's and Chu#10 media respectively. Cultures in Walne's medium produced the maximum amount of EPA (21.22%) and minimum by those in Chu#10 (1.04%) medium. When f/2 medium produced 10.43%, it was 5% for Miquel's medium.

Camacho-Rodriguez *et al*. (2014) found that culture media with low amount of zinc element would be having a lesser value of EPA content in *N. gaditana*. This finding is in tune with the present result because both Miquel's and Chu#10 media are lacking the said trace element and are having low values of EPA (5 and 1.04% respectively). A higher quantity of Zn accounts for increased EPA content in Walne's medium cultures (21.22%) than those in f/2 medium (10.43%). They also stated that biotin (Vitamin B7) was important for EPA production while thiamine (Vitamin B1) and cyanocobalamin (Vitamin B12) were not. Another reason for the production of EPA in f/2 medium cultures is the presence of biotin in it. Chen *et al*. (2013) also showed that micronutrients are important for the growth and EPA content of *N. oceanica* CY2 by comparing two different culture media where one has more trace elements than the other. A close perusal of the chemical composition of f/2 medium and Walne's medium indicates that they have a fair balance of micronutrients regarding both their presence and concentration. Many of these micronutrients are lacking in Miquel's and Chu#10

media. Therefore, the optimum balance of micronutrients in f/2 and Walne's media for the investigated algal species could be one of the reasons for the higher value of EPA.

Different authors have reported the value of EPA content in different experimental setups and in different species of *Nannochloropsis* which are in agreement with the results of the present study. Zou *et al*. (2000) reported 2.3-5.7% EPA content in *Nannochloropsis* spp. Chaturvedi and Fujita (2006) observed 2.4% EPA content in *Nannochloropsis oculata* ST-6 (wild type) having a PUFA content of 3%. Meng *et al*. (2015) obtained an EPA value as 2.7-5.2% for *Nannochloropsis oceanica* IMET1 where total lipid content was 28-59%. Krienitz and Wirth (2006) estimated an EPA value of 0.22-5.6 for *Nannochloropsis limnetica* SAG18.99 where total PUFA was 0.84-12.25%. Molino *et al*. (2019) and Mitra *et al*. (2015) published an EPA value of 4.4 to 11% in *N. gaditana* cultures. In their experiment, *N. gaditana* cultures displayed 1.6-3.5% linoleic acid (18:2 n-6), 0.3-1.1% ALA (18:3 n-3) and 0.4 -3.4% AA (20:4 n-6). The findings of the present study are in agreement with these previous reports (Table 4).

There are reports about high concentrations of Zn, negatively affecting cell division, total chlorophyll content and ATPase activity in microalgae (Omar, 2002). In addition, a high concentration of Zn was found to increase lipid peroxides in microalgae like *Pavlova viridis* (Li and Zhu, 2006), resulting in lipid depravity. The effects of molybdenum (Mo) in microalgae lipid accumulation are still unclear and require further investigation. However, it is known that Mo is essential for the assimilation of nitrate (Raven, 1988) and for the conservation of homeostasis, while certain enzymes carry Mo cofactors conserved in eukaryotes. It was documented that Manganese (Mn) limitation suppresses photosynthetic activities in algae (Constantopoulos, 1970) and thereby enhances lipid synthetic activity. The present result regarding lipid production is at par with the above findings since Miquel's medium is lacking the above microelements. The reasons for the anomalous results are unknown. Differences probably arise from the culture conditions, analytical methods, or in the growth phase sampled.

The main goal of this work was to describe the lipid classes of *N. salina* and their composition in terms of esterified fatty acids when grown in different standard microalgal culture media. Since Walne's medium enriches polyunsaturated fatty acid content, cultures from the same medium are suitable for optimised larval rearing in aquaculture . As *N. salina* is rich in neutral lipids it can be used as a potential source of biofuel which is a promising industry in the future for which Chu#10 medium can also be used. A detailed understanding of biochemical productivities and fatty acid expression patterns in different algal species can help to estimate the dietary potential and it can open possibilities for the manipulation of culture conditions.

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