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Biochemical Profile of Five Species of Cultured Marine Microalgae[1]

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Abstract

Biochemical profile of five marine microalgae *Isochrysis galbana, Chaetoceros calcitrans, Tetraselmis suecica, Nannochloropsis oculata* and *Aphanocapsa* sp. was studied to understand the changes of the biochemical profile under different culture conditions, i.e. Conway and f/2 media at 20 and 30ppt salinities (‰). The microalgae culture was undertaken in the indoor facility with 24±1° C temperature, P^H 8.7±1, 24-hour illumination. Biochemical constituents of five algal species showed variations, the highest protein (26.86%) was recorded in *C. calcitrans* in f/2 medium at 30‰, lipid (32.00%) in *N. oculata* in Conway medium at 30‰, carbohydrate (32.06%) in *T. suecica* in Conway medium at 30‰ and ash (54.71%) in *C. calcitrans* in f/2 medium at 30‰. Among macro minerals, a high concentration of sodium (1.05g/100g) was recorded in *C. calcitrans* in Conway medium-20‰, potassium (4.12g/100g) in *C. calcitrans* in f/2 medium-30‰, calcium (1.57g/100g) in *C. calcitrans* in Conway medium-20‰, phosphorus (8.53g/100g) in *C. calcitrans* in Conway medium-20‰, magnesium (4.10g/100g) in *N. oculata* in f/2 medium-20‰. Among micro minerals, a high concentration of iron (1.44g/100g) was recorded in *C. calcitrans* in f/2 medium-30‰, zinc(0.95g/100g) in *I. galbana* in Conway medium-20‰, manganese(0.02g/100g) in *N. oculata* in Conway medium-30‰, copper(0.04g/100g) in *Aphanocapsa* sp. in Conway medium-30‰. Among fatty acids, C16:0 become the dominant saturated fatty acid (SFA) found in *I. galbana*, C16:1 become the dominant monounsaturated fatty acid (MUFA) found in *N. oculate* and C18:2 become the dominant polyunsaturated fatty acid (PUFA) found in *I. galbana* cultured in Conway medium-20‰. The biochemical composition of five microalgae indicated that these are potential food sources for humans, cattle and aquaculture industries.

Keywords: Marine microalgae, Biochemical profile, Fatty acids, Culture conditions, Conway and f/2 media, Different salinities.

1. Introduction

Microalgae are considered a very interesting source for the preparation of new food products and can be used to improve the nutritional value of conventional foods because of their valued biochemical composition (Niccolai *et al*., 2019). Algal biomass is recognized as the most reliable raw source for balancing the ever-increasing global need for food, feed, biofuel and chemical production (Vandamme *et al*., 2013; Foley *et al*., 2011). Microalgae products that are commercially available appear to be valuable alternative food and feed products. However, because of the wide range of nutrient profiles, attention should be paid to product analytical characterization (Wild *et al*., 2018). The utilization of microalgae and cyanobacteria as a food source and food supplement is known for centuries (Wells *et al*., 2017). Microalgae are cultivated for human consumption in many Asian countries, Europe, USA, and Australia for several decades (Vigani *et al*., 2015). Microalgae are also commercialized in the cosmetics industry or as animal feed (Huntley *et al*., 2015; Ariede, 2017). Nowadays microalgal business sector is very dynamic with several new companies beginning every year; more than 150 companies of diverse scales, producing *Arthrospira* (*spirulina*), are running in Europe and France (Verdelho Vieira, 2015).

Microalgae, being the pioneers of the food chain, are contributing to the important additives of many habitats on the earth. Algae have the potential to considerably contribute to the future nutritional pool; consequently, it is necessary to understand the chemical composition of the algal biomass. Furthermore, they have been recognized as a promising and economically valuable natural source of high-value products like as fatty acids, carotenoids and steroids in the food and aquaculture industries. (Cardozo *et al*., 2007). In the last decade, research and the application of microalgae have increased significantly. Microalgae are a rich source of natural compounds that can be employed as functional components (Gouveia *et al*., 2010).

There are numerous microalgae species available, but knowledge of their physicochemical characteristics and understanding their chemical composition is essential in screening because it allows researchers to target valuable compounds, pigments, antioxidants, polyunsaturated fatty acids (PUFAs) and other novel food compounds in the microalgae (Batista *et al*., 2013). Microalgae represent a valuable source of a diverse range of lipids with a variety of applications. Polyunsaturated fatty acids, as well as the essential fatty acids linoleic acid, a-linolenic acid (ALA), g-linolenic acid (GLA), arachidonic acid (AA), and

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eicosapentaenoic acid (EPA), are especially interesting. Essential fatty acids are precursors to prostaglandins and their role in the pharmaceutical sector is growing (Borowitzka, 1988; Becker, 1994). Fatty acids are essential for the growth of marine organisms; hence they play a vital role in aquaculture. As a result, some microalgal species that are high in necessary PUFAs may be suggested as ideal for feeding marine animals. Micro-algae are few of the most significant feed sources in aquaculture because of their nutritional value and their ability to synthesize and store up higher quantities of ω3-PUFAs (Patil *et al*., 2007), like 22:6n-3 (DHA) and 20:5n-3 (EPA), two of the most vital essential fatty acids necessary for gametogenesis (Ehteshami *et al*., 2011).

Over decades, enhancement of lipid content in microalgae by various means without altering the lipid productivity was targeted. Various changes in environmental, nutritional, and physiological conditions for the cultivation of microalgae, as well as genetic manipulations, have been attempted for enhanced lipid production (Kumar *et al*., 2020). The microalgae chemical composition can vary with culture age, as well as variations in ambient (Fernández-Reiriz *et al*., 1989) and culture conditions (Araújo and Garcia, 2005). The composition of the growth medium is a critical aspect in producing the biomass with specific desired features. It is well understood the relation between the nutrients added and biomass composition (Becker, 1994). The growth medium, on the other hand, has an impact on the specific growth rate and maximal level of biomass production. A nutrient deficiency in the medium can cause the algae to adapt its metabolism to new environmental conditions. A modification in the growth medium alters the biochemical profile of the biomass, primarily proteins, lipids, carbohydrates and pigments (Sánchez *et al*., 2000). In addition, a batch culture is under continuous chemical change because of the interaction of the organisms with the growth medium. These changes can impact cell metabolism and subsequently their chemical composition (Lourenço *et al*., 2002).

The present study was designed to evaluate the biochemical profile of five species of microalgae cultured in two different culture media at two different salinities.

2. Materials and methods

2.1. Culture conditions

The stock cultures of five marine microalgal species *I. galbana, C. calcitrans, T. suecica, N. oculata* and *Aphanocapsa* sp. used in this study were procured from the Central Marine Fisheries Research Institute (CMFRI), Visakhapatnam, Andhra Pradesh, India. The samples were cultured in Walne medium (Walne, 1970) and subjected to consecutive rounds of serial dilution and streaked on agar plates and test tube slants. The isolates were then maintained on agar plates and in cotton plugged test tube slants at 24 ± 1° C, 12 h light:12h dark photoperiod with an intensity of 60-80 µmol photons.$m^{-2}.s^{-1}$ light. The stock cultures of microalgae were maintained in 50ml conical flasks stoppered with cotton plugs and were sub-cultured in fresh medium for every 2 weeks. The microalgal culture was undertaken in Conway (Walne, 1970) and f/2 (Guillard and Ryther 1962) media at two different salinities (20ppt and 30ppt) to test the variations of biochemical composition under different culture conditions. The treatments (culture conditions) expressed in the table as conway-20ppt and 30ppt are for the algae cultured in Conway media at 20ppt and 30ppt salinity, respectively. Similarly, the treatments f/2-20ppt and 30ppt are for the algae cultured in f/2 media at 20ppt and 30ppt salinity, respectively. The algal biomass used for fatty acids was obtained from algae cultured in Conway medium at 20ppt salinity to test the fatty acid profile in fixed ambient conditions. The microalgae culture was undertaken in the indoor facility from initial culture volume of 100 ml, 250 ml and 2 L in conical sterilized glass conical flask then up to 20 L in a PET (Polyethylene terephthalate) jars at 24±1° C temperature, P^H 8.7±1 and 24-hours illumination with the intensity of 80 µmol photons.$m^{-2}.s^{-1}$ for about 4 days. Then indoor mass culture was carried out in 200 L round vertical Fiberglass Reinforced Plastic (FRP) transparent tank by using the 20 L culture volume as seed culture under the same conditions. The exponential growth phase of algal cultures was observed according to the cell counts using Neubauer haemocytometer under a bright-field contrast microscope (Olympus CH20*i* magnification 400-1000X).

2.2. Harvest of algal biomass and drying

Algal biomass was harvested when cells were at the end of their exponential phase (5days) of culture by adding 0.2g of aluminium sulphate for one litre of culture volume, based on a result obtained from the bench level experiment on this study and also the earlier experiments of Shelef *et al*. (1984) and Michael Babu *et al*. (2013). The alum added culture was aerated for 1hour. After the aeration, culture was left undisturbed for 30-60 min. for complete settlement of biomass.

The flocculated algal mass was collected, and the obtained wet biomass was rinsed thoroughly with distilled water to remove the salt and excess alum, then filtered through Whatman no.1 filter paper. The microalgal paste obtained was dried in a precision hot air oven (Kemi) at 45-50° C temperature for 48h to get the dry biomass. This dry biomass was stored in a desiccator in a cool place and later used for the estimation of biochemical analysis.

The known weight of dried algal mass was fine powdered using agate mortar and pestle. The required quantity of powder was taken for the quantitative estimation of protein, lipid, carbohydrate, ash, minerals and fatty acids. This study was undertaken from June 2017 to 2018 in the dept. of M.L.R., A.U., Andhra Pradesh and NIN, Hyderabad, Telangana, India.

2.3. Biochemical composition

Estimation of protein was followed by the Folin Ciocalteau method of Lowry *et al*. (1951). Protein was extracted with 1N NaOH using Bovine serum albumin as standard. Lipid extraction was performed by modified Bligh and Dyer (1959) by soaking the samples in chloroform: methanol (2:1). The carbohydrate estimation was by the Anthrone method of Carroll *et al*. (1956) using 5% trichloroacetic acid. Glucose was the standard for this analysis. Ash was determined by burning the tissue powder (1g) for 12h in a silica crucible in a muffle furnace at 550˚C according to the AOAC (2000) method. Then the sample residues were placed in a desiccator to cool and

then the weight was recorded. The results were expressed as a percentage of dry weight.

2.4. Sample preparation and estimation of minerals

Minerals were assayed using the Perkin Elmer ELAN 9000 ICP-MS (Inductively Coupled Plasma mass spectrometry) followed by the protocol of Rodenas de la Rocha *et al*. 2009. The microwave-assisted sample digestion was followed by Smith and Arsenault (1996).

The values were expressed in gm/100g.

2.5. FAME preparation and estimation of fatty acids

The lipid extraction was performed by the modified Bligh and Dyer method (1959). The preparation for FAME was followed by a procedure similar to that of D'Oca *et al*. (2011b). Fatty acid methyl ester (FAME) analysis was carried out by Gas Chromatography and Mass Spectrometry (GC-MS) on SUPELCO SP- 2330 chromatograph using flame ionization for detection. The fatty acid structures were validated by comparing their retention times and fragmentation patterns with gas chromatography and mass spectrometry (GC-MS) standards of their methyl esters (Figure. 1 and Table 4). The results were expressed in mg/g.

2.6. Statistical analysis:

All analyses were performed in triplicates. Protein, lipid, carbohydrate and ash data are expressed as mean ± SD. Statistical analysis for proximate composition data was carried out by using Origin Pro 8.

3. Results and discussion

There are numerous microalgal species available, and understanding their chemical composition is essential in screening because it allows researchers to target valuable compounds, pigments, antioxidants, polyunsaturated fatty acids (PUFAs) and other compounds in the microalgae (Batista *et al*., 2013). The synthesis and accumulation of cell constituents such as pigments, proteins, carbohydrates, amino acids, nucleic acids and lipids can be influenced by nitrogen availability (Rodriguez-Lopez *et al*., 1980; Utting, 1985). The majority of the nitrogen in cells is in the form of proteins. As a result, nitrogen intake by the medium has a direct impact on protein synthesis. In the exponential phase of cell growth, protein synthesis is predominant over neutral lipid accumulation (Sukenik and Wahnon, 1991).

The protein content of five microalgal species cultured under different mediums and salinities showed variations. The highest protein (26.85%) was reported in *C. calcitrans* cultured in f/2 medium at 30ppt salinity, whereas the lowest value (8.5%) was reported in *Apanocapsa* sp. cultured in Conway medium at 30ppt salinity (Table 2). The protein content of five species of microalgae in the current study was almost within the range of values found in earlier studies for the different microalgal species cultured under different conditions (Coutteau, 1996; *Sánchez et al*., 2000; Rebolloso-Fuentes *et al*., 2001; Becker, 2007; Chin Ming *et al*., 2012; Batista *et al*., 2013; Bi & He 2013; Eshak & Omar 2017; Niccolai *et al*., 2019). But relatively highest content of protein was reported in previous studies on various microalgal species cultured under different conditions (Vargas *et al*., 1998; Phatarpekar *et al*., 2000; Lourenço *et al*., 2002; Batista *et al*., 2013; Bi & He 2013; Wild *et al*., 2018; Niccolai *et al*., 2019). These variations may be attributed to the culture conditions and species specificity.

Carbohydrates are the first of the organic nutrients to be used to generate required energy (Heath, 1987). They act as precursors to the non-essential amino acids and nutrients, which are metabolic intermediates essential for growth (NRC, 1993). The carbohydrate content in five microalgal species varies from 16.44% to 32.06%. The highest carbohydrate content was reported in *T. suecica* cultured in Conway medium at 30ppt salinity, whereas the lowest was reported in *C. calcitrans* cultured in f/2 medium at 30ppt salinity (Table 2). The carbohydrate content of five microalgal species in the current study was more or less similar to that of the carbohydrate found in other microalgal species studied by earlier authors (Vargas *et al*., 1998; Phatarpekar *et al*., 2000; Rebolloso-Fuentes *et al*., 2001; Lourenço *et al*., 2002; Becker, 2007; Chin Ming *et al*., 2012; Batista *et al*., 2013; Bi & He 2013; Eshak & Omar 2017; Wild *et al*., 2018; Niccolai *et al*., 2019; Reitan *et al*., 2021).

Lipid composition and yield are affected by the growth variables such as growth phase (Xu *et al*., 2008), culture medium composition (Valenzuela-Espinoza *et al*., 2002), irradiance (Thompson *et al*., 1993), and temperature (Renaud *et al*., 2002). Under nitrogen limiting conditions, lipid content increases dramatically in all microalgal species (Hu *et al*., 2008). Neutral lipid production was high during the stationary phase (Doan *et al*., 2011). The lipid content of five microalgal species in the current study showed variations. The highest lipid (32.00%) was reported in *N. oculata* cultured in Conway medium at 30ppt salinity, whereas the lowest value (2.00%) was noticed in *C. calcitrans* cultured in f/2 medium at 30ppt salinity (Table 2). The values found in the present study are almost similar to that of earlier results on various cultured microalgal species (Coutteau, 1996; Vargas *et al*., 1998; Phatarpekar *et al*., 2000; Rebolloso-Fuentes *et al*., 2001; Lourenço *et al*., 2002; Becker, 2007; Huerlimann *et al*., 2010; Chin Ming *et al*., 2012; Batista *et al*., 2013; Wild *et al*., 2018; Niccolai *et al*., 2019; Reitan *et al*., 2021). But relatively highest lipid content was found in some microalgae when compared to values obtained in the present study (Phatarpekar *et al*., 2000; Batista *et al*., 2013). These variations may be attributed to the culture medium, salinity and species variations.

Ash content of photosynthetic microalgae is an important criterion for determining their cultivation efficiency (Liu *et al*., 2015). Ash indicated the mineral content of a plant/animal (Khawaja, 1966). The ash content of five microalgal species showed variations in this study. The highest ash (54.71%) was reported in *C. calcitrans* cultured in f/2 medium at 30ppt salinity, whereas the lowest (15.75%) was reported in *N. oculata* cultured in Conway medium at 30ppt salinity (Table 2). The values found in the present study almost equal values found in earlier publications (Coutteau, 1996; Batista *et al*., 2013; Bi & He 2013; Kent *et al*., 2015; Eshak & Omar 2017; Molino *et al*., 2018; Niccolai *et al*., 2019). On the contrary to the present study, relatively lower values were found in some of the microalgal species (Coutteau, 1996; Vargas *et al*., 1998; Rebolloso-Fuentes *et al*., 2001; Kent *et al*., 2015; Zheng *et al*., 2017; Wild *et al*., 2018; Molino *et al*., 2018; Metsoviti *et al*., 2019; Niccolai *et al*., 2019).

These variations may be attributed to culture conditions and species variations.

3.1. Statistical analysis:

Analysis of variance (two-way) of biochemical constituents was statistically significant ($p<0.05$) between the species, but not significant ($p>0.05$) within the species.

Table 2. Percentage composition of biochemical constituents in five microalgae under different culture conditions

Protein

Treatments	*I. galbana*	*C. Calcitrans*	*T. suecica*	*N. oculata*	*Aphanocapsa* sp.
Conway-20 ppt	20.53±0.8632	20.86±0.1665	8.90±0.8326	20.22±0.7400	17.94±0.2203
f/2-20 ppt	16.25±0.3780	23.52±0.5594	13.61±0.4969	18.14±0.1400	21.42±0.4046
Conway-30 ppt	17.16±0.3601	24.24±0.7076	11.88±0.5556	22.68±0.5011	8.15±0.5507
f/2-30 ppt	22.70±0.7270	26.85±0.7335	12.13±0.2203	20.80±0.5768	20.08±0.4454

Lipid

Treatments	*I. galbana*	*C. Calcitrans*	*T. suecica*	*N. oculata*	*Aphanocapsa* sp.
Conway-20 ppt	22.50±0.6132	7.00±0.800	22.5±0.5204	24.00±0.7000	13.28±0.6050
f/2-20 ppt	19.00±0.4500	5.17±0.2052	21.67±0.72	26.00±0.4473	11.13±0.1401
Conway-30 ppt	21.85±0.9765	9.22±0.3500	25.28±0.53	32.00±0.5766	12.00±0.8500
f/2-30 ppt	18.00±0.5550	2.00±0.4895	24±0.4358	29.00±0.7211	9.00±0.8660

Carbohydrate

Treatments	*I. galbana*	*C. Calcitrans*	*T. suecica*	*N. oculata*	*Aphanocapsa* sp.
Conway-20 ppt	29.94±0.1305	17.65±0.9073	32.02±0.0550	31.43±0.0450	29.62±0.7965
f/2-20 ppt	32.05±0.2514	23.81±0.1101	31.84±0.0251	30.52±0.7597	31.11±0.2311
Conway-30 ppt	30.73±0.0152	17.84±0.4794	32.06±0.0400	29.57±0.9814	31.56±0.10583
f/2-30 ppt	29.15±0.9901	16.44±0.4557	31.72±0.0208	31.42±0.0763	31.56±0.0300

Ash

Treatments	*I. galbana*	*C. Calcitrans*	*T. suecica*	*N. oculata*	*Aphanocapsa* sp.
Conway-20 ppt	27.03±0.7211	54.49±0.5507	36.58±0.7402	24.35±0.7272	39.16±0.0252
f/2-20 ppt	32.70±0.5507	47.50±0.7271	32.88±0.1302	25.34±0.5507	36.34±0.7211
Conway-30 ppt	30.26±0.2313	48.70±0.8200	30.78±0.0762	15.75±0.2312	48.29±0.5507
f/2-30 ppt	30.15±0.1410	54.71±0.8632	32.15±0.5507	18.78±0.4960	39.36±0.8300

Minerals are necessary nutrients that are found in various enzymes and metabolism processes. Minerals aid as structural elements of soft tissue. The minerals are important for maintaining osmotic P^H, osmotic pressure, water balance and glucose/amino acid active transportation (Glover *et al*., 2002). Minerals showed variations in the five species of microalgae cultured in the present study. The highest values of sodium, calcium, phosphorous and potassium were reported in *C. calcitrans* cultured in Conway medium at 20ppt salinity and f/2 medium at 20ppt salinity respectively, whereas the highest value of magnesium was recorded in *N. oculata* in f/2 medium at 20 ppt salinity (Table 3). For the micro minerals, the highest value of Iron was noticed in *C. calcitrans* cultured in f/2 medium at 30ppt salinity. Zinc in *I. galbana* cultured in Conway medium at 20ppt salinity, Manganese in *N. oculata* cultured in Conway medium at 30ppt salinity, copper in *Aphanocapsa* sp. cultured in Conway medium at 30ppt salinity (Table 3). The results agree with the earlier works on various microalgal species (Fabregas & Herrero, 1986; Rebolloso-Fuentes *et al*., 2001; Batista *et al*., 2013; Bi & He 2013; Wild *et al*., 2018). On contrary to the present results, low values were noticed in some microalgal species (Moura-Junior *et al*., 2007). These variations may be attributed to the mineral composition of media and species specificity.

Table 3. Minerals in five microalgae under different culture conditions (g/100g)

a. I. galbana

Treatments	Macro minerals					Micro minerals			
	Sodium	Potassium	Calcium	Phosphorus	Magnesium	Iron	Zinc	Manganese	Copper
Conway 20 ppt	0.61	2.75	0.75	6.14	0.60	0.37	0.95	0.007	0.01
f/2 20 ppt	0.28	1.33	1.46	0.65	0.23	0.49	0.04	0.002	0.01
Conway 30 ppt	0.44	2.50	0.96	2.47	0.55	0.24	0.44	0.009	0.01
f/2 30 ppt	0.43	2.10	0.43	1.15	0.54	0.81	0.08	0.006	0.01

b. C. calcitrans

Treatments	Macro minerals					Micro minerals			
	Sodium	Potassium	Calcium	Phosphorus	Magnesium	Iron	Zinc	Manganese	Copper
Conway 20 ppt	1.05	3.97	1.57	8.53	1.60	0.47	0.06	0.01	0.04
f/2 20 ppt	1.00	1.60	0.86	0.60	0.29	0.59	0.14	0.004	0.01
Conway 30 ppt	0.27	1.57	0.88	7.33	0.46	0.23	0.03	0.01	0.01
f/2 30 ppt	0.50	4.12	0.78	1.03	1.29	1.44	0.05	0.009	0.01

c. T. suecica

Treatments	Macro minerals					Micro minerals			
	Sodium	Potassium	Calcium	Phosphorus	Magnesium	Iron	Zinc	Manganese	Copper
Conway 20 ppt	0.83	1.49	1.51	1.90	1.54	0.15	0.03	0.004	0.009
f/2 20 ppt	0.41	2.29	0.92	1.00	0.19	1.15	0.10	0.007	0.02
Conway 30 ppt	0.10	3.29	1.08	4.61	0.46	0.30	0.32	0.008	0.01
f/2 30 ppt	0.46	1.22	1.01	0.84	0.20	0.83	0.07	0.002	0.02

d. N. oculata

Treatments	Macro minerals					Micro minerals			
	Sodium	Potassium	Calcium	Phosphorus	Magnesium	Iron	Zinc	Manganese	Copper
Conway 20 ppt	0.28	3.24	0.92	7.49	0.51	0.36	0.46	0.01	0.01
f/2 20 ppt	0.58	1.50	0.36	0.61	4.10	0.48	0.08	0.005	0.01
Conway 30 ppt	0.28	1.32	1.24	6.90	1.01	0.44	0.63	0.02	0.007
f/2 30 ppt	0.21	1.73	0.47	0.86	2.12	0.64	0.07	0.008	0.01

e. *Aphanocapsa* sp.

Treatments	Macro minerals					Micro minerals			
	Sodium	Potassium	Calcium	Phosphorus	Magnesium	Iron	Zinc	Manganese	Copper
Conway 20 ppt	0.11	2.67	1.14	2.80	0.94	0.54	0.29	0.01	0.03
f/2 20 ppt	0.24	3.16	0.34	0.98	1.98	1.19	0.08	0.01	0.01
Conway 30 ppt	0.17	1.90	0.26	1.21	0.59	0.06	0.07	0.001	0.04
f/2 30 ppt	0.30	2.13	0.60	0.73	0.91	0.67	0.04	0.006	0.02

Fatty acids are essential for the growth of marine organisms; hence they play a vital role in aquaculture. As a result, some microalgal species that are high in essential PUFAs may be suggested as suitable for feeding marine animals. Among the saturated fatty acids, C16:0 was high in all the five microalgal species. Of the monounsaturated fatty acids, C18:1 was high in *I. galbana, T. suecica* and *Aphanocapsa* sp, whereas C16:1 was found in high content in *C.calcitrans* and *N. oculata*. The high content of polyunsaturated fatty acid C18:2 was found in all the five microalgal species in the present study (Table 5). Relatively low values of fatty acids were found in five microalgal species cultured in different conditions compared to earlier reports on various microalgal species cultured in different conditions (Lourenço *et al.*, 1997; Vargas *et al*., 1998; *Sánchez et al*., 2000; Rebolloso-Fuentes *et al*., 2001; Lourenço *et al*., 2002; Durmaz *et al*., 2009; Huerlimann *et al*., 2010; *Costard et al*., 2012; Chin Ming *et al*., 2012; Batista *et al*., 2013; Bi & He 2013; Selvakumar and Umadevi 2014; Eshak & Omar 2017; Wild *et al*., 2018; Niccolai *et al*., 2019; Reitan *et al*., 2021). These variations may be attributed to culture

conditions, extraction procedure and analysis. The decrease in cellular biochemical components with increasing growth rate could be due to nutritional exhaustion and a gradual metabolites accumulation in the media (Moal *et al*., 1987; Fernandez-Reiriz *et al*., 1989).

The difference in chemical composition of the culture medium and the difference in salinity in the experiments induced distinct biochemical profiles of five microalgal species used in the present study. The biochemical profile of microalgae might also vary greatly depending on the measuring methods followed (Barbarino & Lourenco, 2005), physiological condition of microalgae (Fernández-Reiriz *et al*., 1989), the experimental conditions used (Lourenço *et al*., 2002), like temperature (Durmaz *et al*., 2009), light intensity (Lourenço *et al*., 2008), medium of cultivation (Huerlimann *et al*., 2010) or in outdoor conditions (Banerjee *et al*., 2011).

63B Standard

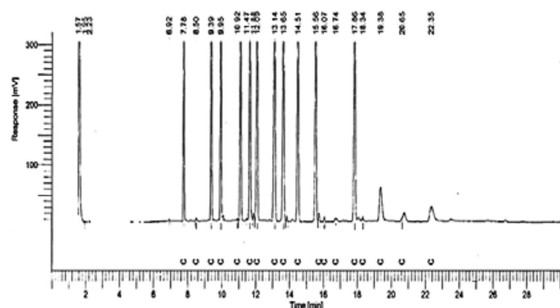
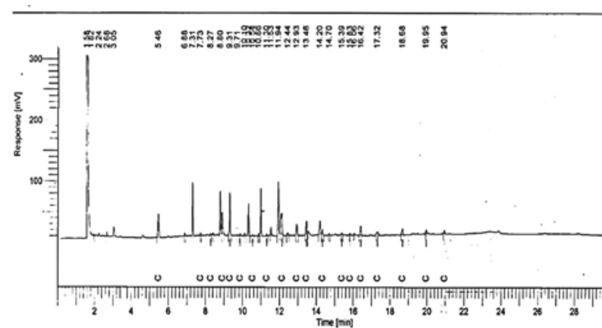


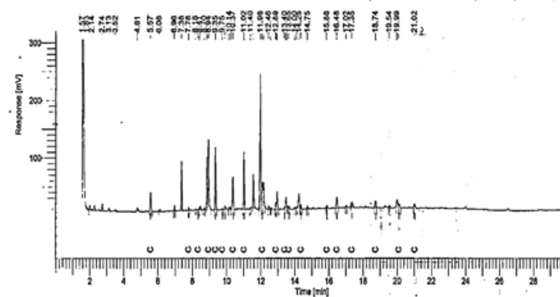
Figure 1. Chromatogram of 63B Standard

Table 4. Fatty acids of 63B Standard

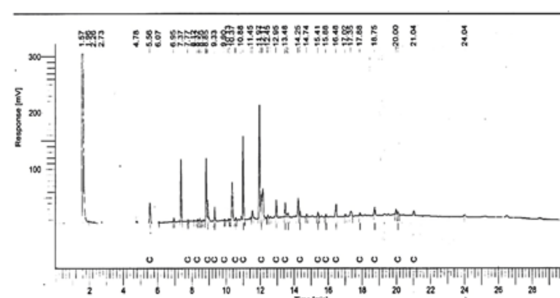
Fatty acids		63B Standard	
Common name	Empirical formula	Retention time (min)	Peak area (µV.s)
Saturated Fatty acids			
Lauric acid – methyl ester	C 12:0	7.778	2196493.55
Myristic acid - methyl ester	C 14:0	9.387	2287731.91
Palmitic acid - methyl ester	C 16:0	11.115	2381384.38
Margaric acid - methyl ester	C 17:0	12.090	2364453.53
Stearic acid - methyl ester	C 18:0	13.138	2421943.21
Arachidic acid - methyl ester	C 20:0	17.862	2348151.41
Unsaturated Fatty acids			
Palmitoleic acid - methyl ester	C 16:1n-7	11.666	2198859.12
Oleic acid -methyl ester	C 18:1n-9	13.649	240166.16
Linoleic acid - methyl ester	C 18:2n-6	14.508	2386692.37
α - Linolenic acid - methyl ester	C 18:3n-3	15.565	2344356.67



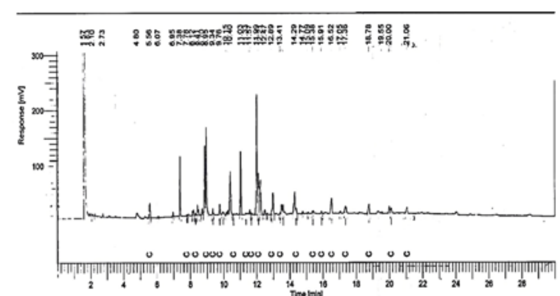
a. *I. galbana*



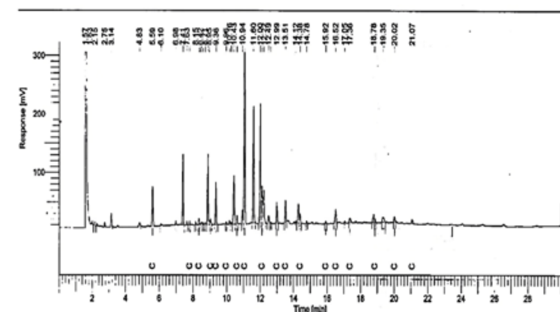
b. *C. calcitrans*



c. *T. suecica*



d. *N. oculata*



e. ***Aphanocapsa sp.***

Figure 2: GC-MS Chromatograms of fatty acids in five microalgae

Table 5. Fatty acids present in five microalgae (mg/g)

	SFAs				*MUFAs*			*PUFAs*	
	C12:0	*C14:0*	*C16:0*	*C18:0*	*C14:1*	*C16:1*	*C18:1*	*C18:2*	*C18:3*
I. galbana	0.12	2.46	3.17	0.86	0	0.52	0.89	1.20	0.12
C. calcitrans	0.05	1.27	1.35	0.52	0	0.89	0.25	0.46	0
T. suecica	0.04	0.36	2.44	0.54	0	0.21	0.51	0.75	0.14
N. oculata	0.08	1.12	6.20	0.64	0	3.64	0.66	0.80	0
Aphanocapsa sp.	0.02	0.13	1.67	0.69	0	0.1	0.27	0.93	0

SFAs- Saturated fatty acids; MUFAs- Monounsaturated fatty acids; PUFAs- Polyunsaturated fatty acids

4. Conclusions

Microalgae are one of the most capable sources for novel functional food products and still need evaluation for usage as food and feed. They can be employed to improve the nutritional value of foods because of their well-balanced chemical composition; and it is also possible to enhance the required biochemical component of microalgae by altering the culture conditions like culture medium and salinity. The research is further needed to develop novel foods for humans as well as aquaculture industry with these microalgae.

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Conflicts of Interest

The authors declare no conflict of interests.

Declaration of Author contributions

PYKR: study design.

GT: microalgae cultivation, biochemical composition analyses.

PYKR, GT, IRS: analysis of data, discussion of results, writing and revising the manuscript.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

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