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Morphological and chemical aspects of *Chlorella pyrenoidosa*, *Dunaliella tertiolecta*, *Isochrysis galbana* and *Tetraselmis gracilis* microalgae

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ABSTRACT

This study evaluates the growth and chemical composition of the following marine microalgae: *Dunaliella tertiolecta*, *Isochrysis galbana*, and *Tetraselmis gracilis* and the chemical composition of *Chlorella pyrenoidosa*. Microalgae can produce a number of compounds of high commercial value for the industry, mainly for the food industry. The growth kinetics, cell volume, pigments, carbohydrates, proteins, lipids, and fatty acid and amino acid composition were evaluated. *I. galbana* had the largest number of cells per mL^{-1} (107), concentration of carotenoids (6.33 μg·mL^{-1}), and carbohydrates (34.32%). *D. tertiolecta* and *T. gracilis* had the highest cell volume (560.6 and 592.7 μm^3, respectively), the highest amount of total dry biomass. *D. tertiolecta* had the highest chlorophyll concentration (9.05 μg·mL^{-1}), and *C. pyrenoidosa* had the highest protein (48.16%) and lipid (14.30%) content. The marine species *D. Tertiolecta*, *I. galbana*, and *T. gracilis* had high levels of monounsaturated fatty acids (C18:1 n9), and *C. pyrenoidosa* was high in polyunsaturated fatty acids (C18:2 n6 and C18:3 n3), indicating the present high nutritional value fatty acids. The microalgae studies showed a composition of amino acids that meet the nutritional requirements recommended by the FAO g·100 g^{-1} (FAO/WHO/UN, 1985) for adults and children (2 - 5 years), indicating that these proteins can be used in foods.

Keywords: Microalgae; Macronutrients; Chromatography

1. INTRODUCTION

Microalgae are photosynthetic microorganisms with simple growing requirements. Its biomass can be used to produce human dietary supplements, animal feed and others. The demand for health beneficial substances has been growing on the world market, and most species studied produce optimal concentrations of compounds with high commercial value and interest to the food industry such as proteins, carbohydrates, lipids, enzymes, antibiotics, vitamins, and pigments. The biomass, merchandized as powdered formulations, tablets, capsules, and extracts can be used in pastry, snacks, sweets, drinks, and nutritional supplements [1-9]. Some species can produce 15% - 40% oil and are a promising alternative due to their rapid growth and accumulation of triglycerides [10,11]. The carbohydrates produced affect the osmotic equilibrium of the cell or act as energy store (starch and crisolaminarin), thus being a source of energy for the consumers [12-14]. The Microalgae *D. tertiolecta*, *I. galbana* and *T. gracilis* are flagellated marine species. The *D. tertiolecta* has been cultivated for the extraction of carotenoids used as natural dyes. The *T. gracilis* presents rapid growth and high tolerance to growing conditions, being mainly produced to feed aquatic organisms. *I. galbana* is rich in chlorophyll, carotenoids (fucoxanthin, *β*-carotene, diadinoxantin, diatoxantin) and crisolaminarin (polysaccharide derived glucose glycosidic type *β*-1,3). The freshwater specie *C. pyrenoidosa*, not flagellated, is rich in chlorophyll and is already being marketed as vitamin supplement in human nutrition [15-18].

They are known for their high levels of protein and essential amino acid composition, which are not synthesized by the human body and have lower levels of phenylalanine; therefore, they can offer additional bene-

fits for people with Phenylketonuria (PKU). The aim of the present study is to investigate the morphological characteristics and chemical composition of some species of microalgae as well as their use as food.

2. MATERIALS AND METHODS

2.1. Microalgae

The three species of marine flagellate microalgae, *Dunaliella tertiolecta*, *Isochrysis galbana*, and *Tetraselmis gracilis* (**Figure 1**) were grown in duplicate at the Institute of Eco-development of Baía da Ilha Grande (POMAR/IED-BIG Project). The lyophilized microalgae *Chlorella pyrenoidosa* was obtained from the Galena® company (Campinas-SP, Brazil). The three marine species were selected due to their rapid growth in monoalgal culture and *Chlorella* also due to its chemical composition and by offering greater biomass for specific studies with protein hydrolysates.

2.2. Microalgal Culture

The microalgae were cultured in duplicate in 20 L polypropylene containers in Guillard (1975) medium prepared with filtered seawater [19]. The cultures were aerated with compressed air and were stored under a 24 h-photoperiod and fluorescent light (four 40 W fluorescent bulbs) at 20˚C (±2˚C). The pH equilibrium (7 to 8) was measured once daily and maintained using a TRIS buffer. The culture growth was evaluated by direct cell counting using Fuchs-Rosenthal chambers in the morning up to the eighth day, and was concentrated by tangential filtration and lyophilization. **Table 1** shows the initial cells' density (number of cells per ml).

2.3. Cell Volume Measurements

The cell volumes were determined [20]. The microalgae, fixed in a Lugol's solution, were photographed using an Olympus Microscope BX5 (S20) using a cell^B Image acquisition software (3.0) to obtain the biovolume measurements (m^3); the cell measurements were taken assuming an ellipsoid cell shape as shown in **Eq.1**.

$$V = (\pi/6) \cdot d2 \cdot h$$

where: V = cell volume; π = 3.14; d = cell diameter; h = cell average height.

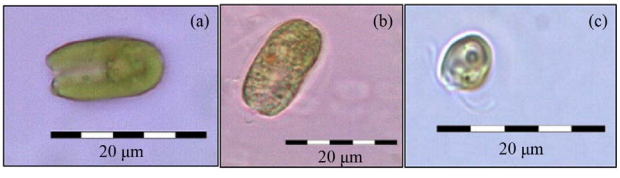


Figure 1. Marine microalgae *D. tertiolecta* (a) *T. gracilis* (b) and *I. galbana* (c) examined with a Olympus S20 microscope (100×).

Table 1. Average initial density of cells used in the culture.

Species	Initial Number of Cells*
Dunaliella tertiolecta	2.83×10^5
Isochrysis galbana	1.29×10^6
Tetraselmis gracilis	2.32×10^5

*Average result of growth in duplicate.

2.4. Pigments (Chlorophyll and Carotenoids)

Five ml-aliquots of the cultures were filtered through borosilicate filter and homogenized in 5 ml of 90% acetone in triplicate. The sample was stored in the dark under refrigeration for 20 hours at 4˚C for pigment extraction. The extract was centrifuged at 3000 rpm for 10 min, and the absorbance of the supernatant (3 mL) was determined by spectrophotometry in semi-darkness [21-23].

2.5. Biomass Dry Weight

An aliquot of 4 mL of concentrated cell culture was vacuum filtered using a Sartorius filtration system. The residue retained on the filter was dried in an oven at 50˚C and determined gravimetrically to constant weight [15].

2.6. Chemical Composition of Biomass

2.6.1. Total Carbohydrates Determination

Intracellular and extracellular carbohydrate analysis was performed using 2 mg of dry weight. The analysis was based on complex sugars, and derivatives from yellow-orange colored complexes with phenol and concentrated sulfuric acid that absorb light at 485 nm [24].

2.6.2. Total Protein Determination

The intracellular proteins were determined using 2 mg of dry weight of cells by the interaction between the dye "Coomassie blue" and macromolecules containing basic side chains or aromatic amino acids. The interaction between the high molecular weight protein and the dye changes the equilibrium of the dye to the anionic form, which absorbs light at 595 nm due to the pH [25,26].

2.6.3. Total Lipid Determination

The lipid extraction [27] was performed using 500 mg of dry cells. Total lipids were estimated gravimetrically based on the dry mass of lipids and cells.

2.7. Determination of the Lipid Composition by the Methyl Ester Profile

The extracted lipids were subjected to methylation [28]. The fatty acid methyl esters were separated by the addition of distilled water: PA hexane (1:1), and the upper phase (hexane) was collected and concentrated at

60˚C. The dried sample was resuspended in 300 µL of hexane, and 1 µL was analyzed by gas chromatography using a Shimadzu GC-2014 with Quadrex Carbowax 20 M column (30m × 0.32 mm × 0.25 µm) and oven at 200˚C, flow of 20 ml/min, and Split injector and FID at 250˚C [29]. The esters were identified, and the profile was obtained by the integrated peak areas.

2.8. Determination of Amino Acid Composition

The total amino acids were determined by High Performance Liquid Chromatography (HPLC) using pre-column derivatization [30] by 6-Aminoquinolyl-N-Hydroxysuccinimidyl Carbamate (AQC), which enabled the detection of sulforated, acid hydrolysis, and tryptophan amino acids using fluorescence detection. The separation of the derivatized amino acids was optimized using an AccQ·TagTM C18 column 3.9 × 150 mm, 4 mm particle size, and their quantitation was performed using external standardization with a standard solution containing a mixture of free amino acids. The results were expressed as mg/100g of sample (AOAC 982.30).

2.9. Statistical Analysis

The Statistica software package (for Windows version 4.0 Statsoft, Inc., 1993) was used, and the means were subjected to the Tukey test at 5% significance level.

3. RESULTS AND DISCUSSION

3.1. Microalgae Culture

According to **Figure 2**, *I. galbana* (8.56×10^7) had the highest average number of cells from the beginning of the culture up to the 8th day. *D. tertiolecta* (0.94×10^6) and *T. gracilis* (1.31×10^6) showed similar growth curve and a smaller number of cells. However, the number of cells must be evaluated together with the cell volume (cell size) and the biomass dry-weight because a greater number of cells do not necessarily mean larger biomass. Other authors have also found maximum cell density of 10^6 cells/mL for the *D. tertiolecta* and *T. gracilis* in the stationary phase after the 8th day of culture varying aeration, the culture medium and its nutrients, temperature, and lighting [3,14,31-33]. Most of these studies have reported curve in the order 10^6 - 10^7 cells/mL for *I. galbana* [3,34-36]. In the present study, a total of 10^7 cel/mL was found for *I. galbana* and 10^6 cells/mL for *D. tertiolecta* and *T. gracilis*.

3.2. Cell Volume Measurements

According to **Table 2**, the length and width of *D. tertiolecta* and *T. gracilis* was very similar. Their volume was about 7 times larger than that of *I. galbana*. According to the literature, the length and width of *D. tertiolecta* and *T. gracilis* was 8:13 and 6.36 µm and 9.59 µm and 14.1, respectively; these values are smaller than those found in the present study, which may be due to the nitrogen content used in the culture [32]. Other authors found a cell volume range from 127 to 215 µm^3, 57.3 to 58.3 µm^3, and 152 to 391 µm^3 for *D. tertiolecta*, *I. galbana*, and *T. gracilis*, respectively [3,32]. The average cell volume (µm^3) found in this study was higher than that reported in the literature, and it represents an increase of 185.35% and 74% when compared to the highest volumes found. *I. galbana* would have greater advantage over the other two species in terms of cell number, but it has smaller cell volume and cellular dry weight when compared to these other species.

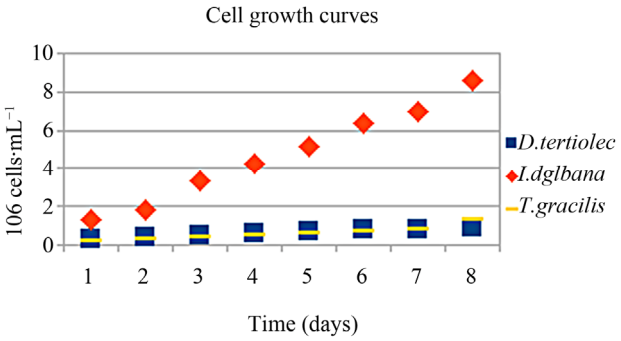


Figure 2. Growth curves of the average number of cells (mL^{-1}) of the three marine microalgae studied.

Table 2. Average cell size and volume.

Species	Measurments (µm)		Average cellular volume
	Lenght (SD)	Width (SD)	(µm^3)
D. tertiolecta	15.41^a (±1.11)	9.72^b (±0.97)	614.03 (±166.66)
I. galbana	5.43^c (±0.72)	5.17^c (±0.72)	78.48 (±26.35)
T. gracilis	15.67^a (±1.59)	9.02^b (±1.26)	680.99 (±237.85)

SD = Standard deviation; n = Measurements of 30 cells per sample Different superscript letters indicate significant differences by the Tukey test (P ≥ 0.05).

3.3. Pigments (Chlorophyll and Carotenoids)

According to **Table 3**, chlorophyll *a* was the non-degraded pigment found in the largest amount in the three microalgae species studied. *D. tertiolecta* showed a higher content of chlorophyll a and b (52.98 and 31.87 µg·L^{-1}, respectively). The second pigment found in the largest amount in *D. tertiolecta* and in *T. gracilis* was chlorophyll b, and in *I. galbana* it was chlorophyll c. Chlorophyll a accounted for the highest levels of pigment found since it is the main photosynthetic pigment and the other chlo-

Table 3. Average number of pigments present in the microalgae *D. tertiolecta*, *I. galbana*, and *T. gracilis*.

	Pigments (μg·mL^{-1} culture)				
Species	**Clorophyll a**	**Clorophyll b**	**Clorophyll c**	**Feophyita a**[1]	**Carotenoid**
D.tertiolecta (SD)	5.30^{a} (±3.16)	3.19^{a} (±0.32)	0.56^{b} (±0.64)	1.79^{b} (±1.12)	2.29^{b} (±0.27)
I.galbana (SD)	3.27^{c} (±0.83)	0.31^{c} (±0.50)	1.08^{a} (±0.74)	0.66^{c} (±0.71)	5.86^{a} (±0.19)
T.gracilis (SD)	3.83^{b} (±5.34)	2.54^{b} (±6.32)	0.65^{b} (±0.83)	3.38^{a} (±2.64)	2.41^{b} (±0.44)

SD = Standard deviation. [1]Lorenzen, 1967; Other analyses: Jeffrey & Humphrey, 1975; Different superscript letters indicate significant differences by the Tukey test ($P \geq 0.05$).

rophyll pigments are accessories which may or may not be in combined with chlorophyll a [32]. The degradation product of chlorophyll (pheophytin a) was found mainly in *T. gracilis*, and *I. galbana* showed higher levels of carotenoids, specially observed in the yellow colored culture.

Chlorophyll c is an intermediate link in the process of energy transfer from the carotenoids and chlorophyll pathway, and it is the pathway of chlorophyll biosynthesis of chlorophyll a and b [37]. *I. galbana* was the species with the most carotenoids and chlorophyll c, confirming this theory. Therefore, combining the cell dry weight and culture density, the average sum of chlorophylls values found was from 2:46 to 5:48 μg·g^{-1} and 5:58 to 0.98 μg·g^{-1} of carotenoids in the microalgae studied. When comparing with traditional foods rich in chlorophyll (**Table 3**), it is clear that the content of these pigments in these microalgae is small; however, it is significant in large-scale cultivation. In other studies, the values of 1.12, 0.32, and 0.93 μg/mL chlorophyll a were found in *D. tertiolecta*, *I. galbana*, and *T. gracilis*, respectively. The amount of 142.3 μg/L of chlorophyll a has been found in *I. galbana*, and 0.66, 0:10 and 0.56 μg/mL of carotenoids in *D. tertiolecta*, *I. galbana*, and *T. gracilis*, respectively. The microalgae investigated in the present study are chlorophyll sources with potential for modulation of xenobiotic metabolism and antioxidant and antimutagenic activity, which are effective in preventing gastrointestinal cancer [3,38]. However, it is non that chlorophylls are unstable pigments and they can be better preserved by HTST (High Temperature Short Time) treatment; moreover, their effectiveness is improved with the intake of fresh food. Carotenoids can be used as food or food additive since they can reduce atherosclerosis progression and enhance immune response preventing the formation of free radicals and reactive oxygen species that can damage mitochondrial enzymes, plasma membrane, and DNA [39-42].

3.4. Biomass Chemical Composition

Meats and soybean are rich in protein; meat is the richest available source of protein (more than 43%). Rice contains more than 77% carbohydrate. Milk and soybeans contain balanced proportions of protein, carbohydrates, and lipids [9,43].

The macronutrient composite-on of the microalgae studied was compared with that of commodities.

According to **Table 4**, the content of these macronutrients in these microalgae can reachlevels close to those found in soybeans and milk. The highest carbohydrate content was found in *C. pyrenoidosa* (34.34%), *I. galbana* (34.32%), and *T. gracilis* (29.96%). The highest lipid (14:30%) and protein content (48.16%) was found in *C. pyrenoidosa*. The amount of carbohydrates and proteins increased or decreased according to the species, and the lipid content in *D. Tertiolecta* and *I. galbana* showed no significant difference.

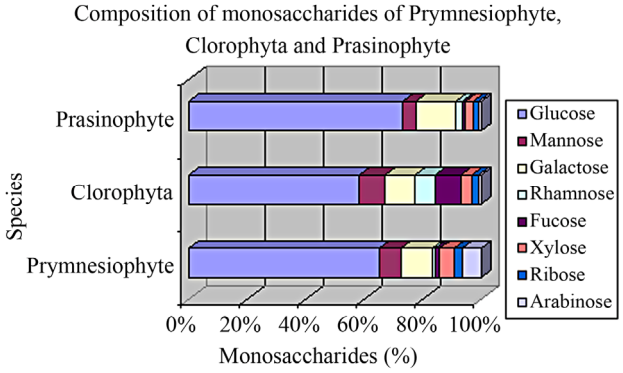
According to the literature, the content of protein in *D. Tertiolecta and C. pyrenoidosa* is higher (50% to 57% and 26% to 70%, respectively) [3,5,26,31,33,4-46]. Researcher have found that monosaccharides present in microalgae contain carbohydrates, mainly glucose (21% - 87%), galactose (1% - 20%), and mannose (2% - 46%) and varying amounts (0% - 17%) of arabinose, fucose, rhamnose, ribose, and xylose. According to one of these authors, species rich in mannose causes low digestibility in animals, and the composition of carbohydrates may be different depending on the phase of the cell cycle [12]. The composition of monosaccharides present in *Prymnesiophyte* (*I. galbana*), *Clorophyta* (*C. pyrenoidosa* and *D. tertiolecta*) and *Prasinophyte* (*T. gracilis*) can be seen in **Figure 3**. Some authors found 9.0 21.69% carbohydrates, 63.6% protein, and 2% to 20.5% lipids in *D. tertiolecta* [7,12,25,47]. Other authors found 12% to 50.8% protein, 21.7% to 21.9% lipids, and 7.6% to 14.2% carbohydrates in *I. galbana* [34,47]. In a study conducted in 2010, it was found more proteins in *T. gracilis* (33.6%) compared to that of *I. galbana* (29.4%) and *D. tertiolecta* (26.0%) in Conway medium [3]. Under temperatures between 25˚C and 35˚C, the content of protein may decrease and that of carbohydrates may increase, showing influence of temperature on the chemical composition [48].

The seawater used in the preparation of the culture medium can contain varied amounts of non-conservative nutrients such as phosphate and nitrate. The nutrient composition of the culture medium and the differences between species can also explain variations in the content of protein, carbohydrates, and lipids.

Table 4. Chemical composition of microalgae macronutrients and commodities.

Species (%)	Macronutrient Composition* (SD)		
dry weight	Proteins*	Carbohydrate**	Lipids
C. pyrenoidosa	48.16[a] (±2,02)	34.34[a] (±1.70)	14.30[a] (±0.40)
D. tertiolecta	38.52[b] (±0.28)	24.61[b] (±2.61)	11.64[b] (±1.37)
I. galbana	27.10[d] (±1.01)	34.32[a] (±2.58)	10.54[b] (±0.84)
T. gracilis	33.02[c] (±0.43)	29.96[a] (±1.99)	7.95[c] (±0.42)
Commodities			
Bread[a,b]	25 - 39	30 - 38	0.2 - 1
Meat[a,b]	43 - 74	0 - 1	12 - 34
Milk[a,b]	22 - 26	35 - 38	28 - 29
Rice[a,b]	7 - 8	77 - 80	0.6 - 2
Soybeans[a,b]	36 - 37	30	18 - 20

SD = standard deviation; *Analyses performed in triplicate; [a]SPOLAORE et al., 2006 (%); [b]FRANCO, 2002 (%); different superscript letters in the column indicate significant differences by the Tukey test ($P \geq 0.05$); ** Values calculated based on the amount of dry sample used in the Bradford and Dubois assays.



(Adapted from Brown *et al*. [12])

Figure 3. Composition of monosaccharides present in *Prymnesiophyte*, *Clorophyta*, and *Prasinophyte*.

3.4.1. Lipid Composition

Table 5 shows the fatty acid profile of the species studied and of some traditional sources of lipids. Palm stands out for its high content of C16:0 (palmitic acid: 59.12%) and C18:1 (oleic acid: 29.69%); beef tallow for C16:0 (21.83%), C18:0 (stearic acid - 23.99%), and C18:1 (40.84%); soybean for C18:1 (23:31%) and C18:2 (linoleic acid: 55.31%); and olive oil for C18:1 (67.99%) and C18:2 (2.15%). Similar results were found in the literature [49]. All marine species had high content of monounsaturated fatty acids (MUFA), and freshwater *C. pyrenoidosa* had the highest PUFA content (61.17%), indicating good nutritional lipid profile. The amount of C18:1 n-9 was high in all marine species; but only *I. galbana* had high C14:0 (myristic acid). Researchers have found 41.8%, 22.0%, and 32.9% of SAFA, MUFA, and PUFA in *D. tertiolecta* [3]. High content of MUFA (54.59%), mainly C18:1 and SAFA (33.75%), was found in the present study. Some authors found the following composition of fatty acids in *I. galbana*: 11.1% C14:0, 15.5% C16:0, 1.1% C16:1, 2.0% C18:0, 28.6% C18:1, 7.4% of C18:2, and 3.6% C18:3 on the 8th day of culture; on the 4th day, however, the levels of C14:0, C16:1 and C18:3 were higher (13.5%, 1.4% and 4.6%, respectively), showing increased concentration of higher unsaturated acids in this microalgae. Other authors found 11.4% and 38.9% C14:0, 14.5% to 25.2% of C16:0, 16.1% to 19.6% C18:1, 8.6% C18:2, and 15.4% C18:4 [8,42,48], as well as 48.06% SAFA, 39.16% MUFA, and 12.72% PUFA in *I. galbana* [44], which are different from the levels found in the present study. The amount of 25.4% of C18:1, 15.2% C16:0, 14.6% C18:3, 9.0% C18:4, 6.4% C18:2 were found in *T. gracilis* [50]. In the present study, on the other hand, the levels of C16:0, C18:1 were higher and C18:3 was lower. In 2010, 52.26%, 23.16%, and 24.51% of SAFA, MUFA and PUFA. Higher amounts of MUFA (54.70%) and lower amounts of SAFA (35.06%) were found in the present study. The microalgae showed high contents of C16:0, 21:47% to 30.90%, characteristic of palm and beef tallow, and 37.11% to 41.32% of C18:1. It is worth mentioning that the fatty acids eicosapentaenoic acid (C20:5 n3) and docosahexaenoic (C22:6 n3) were not found in the present study; however, other authors have found these fatty acids in these species [8,11].

3.4.2. Amino Acid Composition

According to **Table 6**, the most abundant amino acids in all species were glutamic acid and aspartic acid, and histidine was found in the smallest amount. These results are similar to those found in Conway culture medium. [3]. Larger amounts of arginine were found in *D. Tertiolecta* and *T. gracilis* (g/100g 19.34 and 16.09, respectively), which can occasionally be regarded as essential. Some studies have reported that two species of *Tetraselmis* (*T. suecica and T. Chuii*) contained higher amount of arginine compared to that of other species [44]. Another study on types and amount of amino acids in microalgae has reported similar compositions between the species studied, suggesting proteins of similar quality. Aspartate and glutamine were found in higher concentrations (7.1% to 12.9%); cysteine, methionine, tryptophan, and histidine were found in lower amounts (0.4% to 3.2%), and other amino acids were found in amounts between 3.2% to 13.5% suggesting that the protein contains essential amino acids [12]. *C. pyrenoidosa* had the highest levels of tryptophan. The essential amino acid profile was similar to that of some legumes such as beans, peas, soybeans, and lentils [52-55]. All species showed levels of essential amino acids that meet the nutritional requirement recommended by FAO g/100g (FAO/WHO/UN, 1985) for adults and children (2 - 5 years).

Table 5. Composition of lipids present in the microalgae *D. tertiolecta*, *I. galbana*, and *T. gracilis* and traditional sources of lipids.

Fatty Acids	Microalgae				Oilseeds			
(%)	*C. pyrenoidosa*	*D. tertiolecta*[*]	*I. galbana*[*]	*T. gracilis*[*]	Palm[1]	Beef tallow[1]	Soybean[1]	Olive[2]
SAFA								
12:0	0.14[a] (±0.01)	ND	0.07[b] (±0.02)	ND	0.22	0.08	0.02	-
14:0	0.46[c] (±0.03)	0.45[c] (±0.02)	20.46[a] (±0.23)	0.58[b] (±0.06)	1.14	2.40	0.06	0.00
16:0	27.53[b] (±0.21)	30.48[a] (±0.95)	21.47[c] (±0.47)	30.90[a] (±0.40)	59.12	21.83	10.01	10.32
17:0	0.45[a] (±0.12)	1.04[a] (±0.05)	0.18[b] (±0.03)	0.72[a] (±0.40)	ND	1.54	ND	0.05
18:0	3.22[a] (±0.05)	1.52[b] (±0.41)	1.45[b] (±0.08)	1.89[b] (±0.75)	2.98	23.99	2.81	3.32
20:0	0.11[b] (±0.07)	0.21[b] (±0.10)	0.90[a] (±0.13)	0.26[b] (±0.08)	0.07	0.17	0.25	0.33
22:0	0.13[c] (±0.02)	0.05[d] (±0.01)	3.03[a] (±0.23)	0.76[b] (±0.03)	0.12	0.04	0.37	0.17
SUM	32.04	33.36	47.55	35.11	63.65	50.05	13.52	14.19
MUFA								
16:1	2.45[b] (±0.25)	9.36[a] (±0.42)	10.84[a] (±0.39)	9.31[a] (±0.92)	0.26	6.50	2.44	0.64
18:01 n9	4.22[c] (±0.13)	41.32[a] (±0.18)	37.11[b] (±0.54)	40.99[a] (±1.77)	29.69	40.84	23.31	67.99
20:1	0.10[c] (±0.02)	3.87[a] (±0.08)	0.43[b] (±0.06)	4.33[a] (±0.39)	0.05	0.34	0.14	0.21
22:1	0.04[c] (±0.01)	0.04[c] (±0.01)	0.52[a] (±0.02)	0.07[b] (±0.01)	0.03	0.004	0.09	-
SUM	6.81	54.59	48.90	54.70	30.03	47.68	25.97	68.84
PUFA								
18:02 n6	29.75[a] (±0.32)	3.89[b] (±0.44)	2.24[d] (±0.06)	2.69[c] (±1.10)	6.15	2.07	55.31	15.02
18:03 n3	31.42[a] (±0.40)	7.81[b] (±0.18)	1.35[c] (±0.07)	7.51[b] (±1.78)	0.17	0.21	5.2	0.98
SUM	61.17	11.70	3.59	10.20	6.32	2.27	60.42	16.00

SAFA: Saturated Fatty Acid; MUFA: Monoinsaturated Fatty Acid; PUFA: Poliinsaturated Fatty Acid. ND: Not Detected, SD: Standard deviation [*]Average of cultures in duplicate; Different letters in the same row indicate statistical difference by Tukey test ($P \geq 0.05$) [1]Profile obtained with the methylation reaction (topic 2.7); [2]AUED-PIMENTEL *et al*., 2008.

Table 6. Composition of total amino acids present in the microalgae *D. tertiolecta*, *I. galbana* and *T. gracilis* (g/100g).

Aminoacids	Microalgae				FAO (1985)
(g/100g)	*C. pyrenoidosa*	*D. tertiolecta*[2]	*I. galbana*[2]	*T. gracilis*[2]	*Adults/Children*
Aspartic Acid	8.98[b] (±0.10)	9.02[b] (±0.09)	10.13[a] (±0.01)	8.84[c] (±0.03)	-
Serine	6.87[a] (±0.08)	4.90[c] (±0.04)	5.58[b] (±0.05)	4.50[d] (±0.08)	-
Glutamic Acid	11.83[c] (±0.06)	12.98[b] (±0.09)	12.92[b] (±0.11)	13.93[a] (±0.05)	-
Glycine	6.58[a] (±0.03)	5.96[b] (±0.08)	5.95[b] (±0.06)	5.97[b] (±0.05)	-
Histidine[1]	2.21[a] (±0.08)	1.29[c] (±0.01)	1.64[b] (±0.05)	1.27[c] (±0.02)	1.6/1.9
Arginine	8.17[d] (±0.02)	16.09[b] (±0.06)	9.41[c] (±0.10)	19.34[a] (±0.02)	-
Threonine	4.84[b] (±0.07)	4.94[b] (±0.07)	5.35[a] (±0.10)	4.51[c] (±0.02)	0.5/3.4
Alanine	8.19[b] (±0.08)	7.27[c] (±0.03)	9.39[a] (±0.01)	6.90[d] (±0.07)	-
Proline	4.32[a] (±0.05)	4.35[a] (±0.01)	4.47[a] (±0.04)	3.63[b] (±0.03)	-
Tyrosine	3.80[a] (±0.03)	3.08[b] (±0.03)	2.88[c] (±0.03)	2.87[c] (±0.01)	-
Valine	5.32[b] (±0.04)	5.02[c] (±0.04)	5.69[a] (±0.03)	4.75[d] (±0.01)	0.3/3.5
Lysine	7.61[a] (±0.04)	4.66[c] (±0.08)	4.91[b] (±0.04)	4.44[d] (±0.04)	1.6/5.8
Isoleucine	3.45[b] (±0.06)	3.15[c] (±0.05)	4.30[a] (±0.01)	3.30[b] (±0.10)	1.3/2.8
Leucine	8.20[b] (±0.09)	7.59[c] (±0.05)	8.39[a] (±0.02)	6.94[d] (±0.04)	1.9/6.6
Phenylalanine	4.63[b] (±0.05)	5.00[a] (±0.04)	4.97[a] (±0.01)	4.52[c] (±0.01)	1.9[*]/6.3[*]
Methionine	0.98[b] (±0.01)	1.50[a] (±0.02)	1.42[a] (±0.10)	1.36[a] (±0.02)	1.7[**]/2.5[**]
Cysteic Acido	1.53[a] (±0.03)	1.59[a] (±0.04)	1.09[c] (±0.08)	1.29[b] (±0.01)	-
Tryptophan	2.50[a] (±0.07)	1.64[b] (±0.05)	1.53[b] (±0.06)	1.67[b] (±0.09)	0.9/1.1

Aminoacid requirement by FAO g/100g (FAO/WHO/UN, 1985) for adults and children 2 - 5 years. [*]Phe + Tyr; [**]Met + Cys; [1]condicionally essential. [2] Refers to the average of two different cultures (duplicate). Values in parenthesis refer to standard deviation (n = 2). Different letters in the same row indicate statistical difference by Tukey test ($P \geq 0.05$).

4. CONCLUSION

D. tertiolecta and *T. gracilis* had the highest amount of biomass. *D. tertiolecta* had the highest content of chlorophyll, and *I. galbana* had the highest content of carotenoids. Higher levels of PUFA were found in *C. pyrenoidosa* showing better nutritional quality of this oil. The marine species stand out for the presence of MUFA, especially oleic acid (C18:1 n9). The concentration of essential amino acids meets the nutritional guidelines for children and adults (FAO/1985) and suggests that the microalgae proteins can be used in the production of food as well as combined with other protein sources. The bioavailability of nutrients and the composition of nitrogenous and carbohydrate compounds in the microalgae are issues that have been little studied but should be further investigated.

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