

Chemical Characterization of Six Microalgae with Potential Utility for Food Application

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Abstract Microalgae contain high levels of proteins, carbohydrates, and lipids, and have found a useful application in enhancing the nutritional value of foods. These organisms can also synthesize long-chain fatty acids in the form of triacylglycerols, such as α -linolenic acid (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linolenic acid (LA), γ -linolenic acid (GLA) and arachidonic acid (AA). The aim of this study was to determine the chemical composition and measure protein, carbohydrates, fibers, lipids as well as the fatty acids composition of six microalgae species with potential application in the food industry. Two freshwater species, *Chlorella vulgaris* and *Spirulina platensis*, and four

marine species, *Nannochloropsis oculata*, *Nannochloropsis gaditana*, *Porphyridium cruentum*, and *Phaeodactylum tricornutum*, were used in the experiments. Intracellular protein was the most prominent algal component (42.8–35.4 %), followed by carbohydrate + fiber (32.3–28.6 %), and lipids (15.6–5.3 %). *N. gaditana* is rich in saturated fatty acids, mainly palmitic acid (5.1 g/100 g), while the cells of *S. platensis* and *C. vulgaris* algae are abundant in GLA (1.9 g/100 g) and ALA (2.8 g/100 g) acids, respectively. *P. cruentum* differs from other algae, because it contains a large amount of AA (3.7 g/100 g). The marine microorganisms *N. oculata* and *P. tricornutum* are also a source of essential long-chain polyunsaturated fatty acids (LC-PUFA- ω 3), mainly composed of EPA and DHA. Our results suggest that the freshwater species *C. vulgaris* and *S. platensis* are attractive nutritional supplements because of their low fiber and high protein/carbohydrate contents, while the marine species *P. tricornutum* and *N. oculata* can enrich foods with LC-PUFA- ω 3, because of their favorable ω 3/ ω 6 ratio.

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Introduction

Microalgae are a group of eukaryotic organisms and photosynthetic cyanobacteria able to accumulate sugars, carbohydrates, proteins, lipids, and other valuable organic substances by the efficient use of solar energy, CO₂, and nutrients. These microorganisms convert inorganic substances such as carbon, nitrogen, phosphorus, sulfur, iron, and trace elements into organic matter (green, blue-green, red, brown, and other colored biomass) [1].

Microalgae are considered one of the most promising feedstock materials for developing a sustainable supply of commodities, including both food and non-food products. Microalgae have also great potential, as they produce natural compounds that could be used as functional food ingredients [2, 3]. Currently, edible oils, proteins, and carbohydrates are consumed in a variety of food products, which contain ingredients from both plant and animal origin. In this regard, microalgae can be used to enhance the nutritional value of foods. When using screening methodologies to identify valuable compounds (pigments, antioxidants, polyunsaturated fatty acids, etc.) in microalgae, knowledge of the chemical composition is a first requirement [1, 4]. The microalgae are an extremely diverse collection of organisms with a large variation in chemical compositions, but this diversity is not yet fully explored [5]. For this reason, the nutritional content of algal biomass is sometimes poorly defined and for most species, including well-studied species like *Spirulina*, there is little consensus on their biochemical composition of different algal species.

Several fatty acids are synthesized by humans, but there is a group of essential fatty acids, the polyunsaturated fatty acids (PUFAs), which the human body cannot produce: omega-3 (ω -3) and omega-6 (ω -6). Therefore, both ω -3 and ω -6, which are necessary for human health, are entirely derived from the diet [5, 6] and nutrition experts have recommended that an ω 3/ ω 6 fatty acids ratio of $\leq 1:5$ is desirable [7]. Since the Western diet contains massive quantities of ω -6, ω 3/ ω 6 ratios of up 1:25 have been reported in the literature [8], which has been recognized as undesirable [9], and most people consume thus a PUFA-deficient diet. Therefore, nutritionists emphasize the need to consume seafood (notably fish) and green vegetables to prevent an array of disorders, especially cardiovascular diseases [8, 10]. As reported by Armenta and Valentine [7], single-cell oils (SCO) containing long-chain polyunsaturated fatty acids (LC-PUFA) such as EPA/DHA acids derived from algae are considered a promising oil alternative to oils from fish and land-based plant sources.

The development of novel foods based on microalgal biomass is an exciting tool for providing nutritional supplements with biologically active compounds (e.g., antioxidants, PUFAs- ω 3) [11]. Depending on species/strain, environmental conditions and harvesting/processing methods, algal biomass after oil extraction may be a highly attractive source of essential dietary amino acids, fatty acids, sugars, vitamins, minerals, carotenoids and other health-promoting nutrients that are well suited to be human food or feed additives for terrestrial livestock and aquatic animals [12, 13]. Although the potential for algal products/co-products for nutritional applications has long been recognized, so far it has had limited commercial success with only a few species (e.g., *Spirulina*, *Chlorella*) occupying niche markets

[14]. Advancing our knowledge of the biochemical composition of algae is a key requirement for realizing the potential of algal products/co-products.

The main objective of the present study was to compile information on the biochemical composition of microalgae, which is needed for selecting the optimal species for specific food applications (rich in protein, carbohydrates, PUFA, etc.). The microalgae species *Chlorella vulgaris*, *Spirulina platensis*, *Nannochloropsis gaditana*, *Nannochloropsis oculata*, *Phaeodactylum tricornutum*, and *Porphyridium cruentum* were mass cultivated in artificially illuminated photobioreactors to produce sufficient biomass quantities to be able to determine their chemical composition and nutritional value.

Experimental Procedures

Acquisition of Algal Biomass

Freshwater species *Chlorella vulgaris* and *Spirulina platensis* were obtained from the Laboratory of Biochemistry Engineering, Federal University of Rio Grande. Marine species *Nannochloropsis gaditana* (clone 130) was kindly supplied by Banco de Micro-organismos Marinhas Aidar & Kutner (BMA&K), Oceanographic Institute at the University of São Paulo. Microalgae *C. vulgaris*, *S. platensis*, and *N. gaditana* were cultured in the Laboratory of Food Biotechnology, Federal University of Santa Catarina. Microalgae were cultivated through the inoculation of microalgal cultures (monospecific algal cultures) in appropriate growth media: for *C. vulgaris*, Bold Basal Medium (BBM) was used [15], *S. platensis* cells were grown in Paoletti Synthetic Medium (PSM) [16], and *N. gaditana* cells were cultured in previously autoclaved (121 °C/15 min) seawater enriched with F/2 media [17] with salinity of about 34.0 ‰. Cultures of *C. vulgaris*, *S. platensis*, and *N. gaditana* were developed in inverted conical photobioreactors (5 L capacity) with the respective growth media and scaled-up to 100-L capacity fiber photobioreactors (0.50 m diameter, 0.90 m length). The photobioreactors were placed under a photoperiod of 12:12 h light/dark at room temperature (25 ± 2 °C), at a light intensity of $216 \mu\text{mol photons m}^{-2} \text{s}^{-1}$ provided by cool-white fluorescent lamps. Air saturated with CO₂ was continuously pumped into the photobioreactors. When the microalgal culture reached the stationary growth phase, the entire culture broth (100 L) was harvested by continuous centrifugation at 4000 rpm for approximately 1 h. The concentrated microalgal pellet was then transferred to a dish and dried in a dehydrator at 45 ± 5 °C for 24 h before analysis.

Marine algal species *N. oculata*, *P. tricornutum*, and *P. cruentum* were cultured in the Laboratory of Algae

Cultivation, Federal University of Santa Catarina. The cultures were developed in two open tanks and a glass fiber cylinder that we custom designed and built. After cultures were grown in 5 L capacity Erlenmeyer flasks, mass cultures of *N. oculata* and *P. tricornutum* were scaled-up in 500-L capacity open tanks (1.60 m diameter and 2.75 m length) containing F/2 media [17]. Cool-white fluorescent lamps were placed over the tanks under a constant light intensity of 100- $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. The alga *P. cruentum* mass culture was scaled up in a 180-L capacity glass fiber cylinder (0.50-m inner diameter and 1.00-m length) containing F/2 media [17]. The surface of the cylinder was exposed continuously to 10 cool-white light lamps (100- $\mu\text{mol photons m}^{-2} \text{s}^{-1}$). The tanks and cylinder were constantly CO_2 -aerated (40 L min^{-1}) and maintained at room temperature (22 ± 1 °C) using air conditioners. Salinity in the experiments was 35.0 ‰. As soon as the cultures reached the stationary growth phase, the entire culture broth was harvested by continuous centrifugation and concentrated to a biomass slurry, which was washed with isotonic ammonium formate [18] and centrifuged at 3500 rpm for 35 min. Finally, the wet biomass was transferred to a dish and dried in a dehydrator (45 ± 5 °C, 24 h) before further analysis.

Biomass Composition Analysis

For each of the six microalgae species, triplicate samples of dried biomass were analyzed to determine moisture, ash, dietary fiber, carbohydrate, protein, lipid contents, and fatty acids composition.

Moisture

Moisture was determined by drying the sample in an oven at 105 °C for 3–4 h (until constant weight) [19]. The average moisture values were used to calculate the chemical composition as a percentage of total dry matter.

Mineral content

Total ash content was determined by heating the samples to 550 °C for 5 h using a carbolite muffle furnace [20].

Dietary Fiber

Total dietary fiber (TDF) content was determined with a total dietary fiber analysis kit (Megazyme International Ireland Ltd, Wicklow, Ireland) [21], which includes enzymatic hydrolysis with α -amylase, protease, and amyloglucosidase and is approved by the AACC (Method 32-05-01) and the AOAC (Official Method 985.29). Duplicate samples (approximately 1 g) were suspended in 50 mL phosphate buffer and submitted to enzymatic hydrolysis by

incubating with 50 μL of α -amylase at 100 °C for 30 min. The pH was adjusted to 7.5, 100 μL of protease was added, and samples were incubated at 60 °C for 30 min. Next, the pH was adjusted to 4.5, 200 μL of amyloglucosidase was added, and the samples were incubated at 60 °C for 30 min. Finally, fiber was precipitated with 95 % ethanol at 60 °C, filtered through fritted glass crucibles with a Celite filter and the residue in the crucible was dried in an oven at 105 °C, cooled in a desiccator, and weighed.

Protein Content

Total nitrogen was determined by the Kjeldahl method after acid digestion (AOAC 991.20), ammonium addition, steam distillation, and titration with 0.1 N HCl [19]. Protein content was calculated using a nitrogen-to-protein conversion factor of $\text{N} \times 4.78$ [22].

Lipid content

After acid digestion with 4.0 N HCl for 6 h, intracellular lipids were extracted with petroleum ether by the Soxhlet method (AOAC 963.15), concentrated in a rotary evaporator, dried in an oven, and weighed [19].

Carbohydrates

The total carbohydrate content of each sample was calculated according to: $(100 \% - (\text{moisture} + \text{ash} + \text{protein} + \text{lipid} + \text{fiber}))$ [23].

Fatty Acids Composition

Fatty acids composition was determined after converting the fatty acids to their corresponding fatty acids methyl esters (FAME), which were determined by gas chromatograph using a GC-2014 (Shimadzu, Kyoto, Japan), equipped with split-injection port, flame-ionization detector and 105 m-long Restek capillary column (ID = 0.25 mm) coated with 0.25 μm of 10 % cyanopropylphenyl and 90 % biscyanopropylsiloxane. The injector and detector temperatures were both 260 °C. The oven temperature was initially set at 140 °C for 5 min, programmed to increase at 2.5 °C min^{-1} , and held at 260 °C for 30 min. The injection volume was 1 μL , and the split ratio was 10:1. Nitrogen was used as the carrier gas (flow rate was 2.2 mL min^{-1}) at a constant pressure of 130.3 pKa. Fatty acid methyl esters were identified by comparison with the retention time of individual standards (Sigma, St. Louis, USA). For long-chain fatty acids (>C19) a correction factor for the quantification was used [24]. The proportions of the individual acids were calculated by the ratio of their peak area to the total area of all observed acids and expressed as mass percentage.

Table 1 Chemical composition of six microalgal biomass

Compositions (%)	<i>Chlorella vulgaris</i>	<i>Spirulina platensis</i>	<i>Nannochloropsis gaditana</i>	<i>Nannochloropsis oculata</i>	<i>Phaeodactylum tricorutum</i>	<i>Porphyridium cruentum</i>
Dry matter	93.8 ± 0.3 ^a	89.9 ± 0.5 ^d	94.7 ± 0.4 ^{ab}	95.9 ± 0.1 ^b	98.6 ± 0.2 ^e	87.4 ± 0.3 ^c
Moisture	6.2 ± 0.5 ^b	10.1 ± 0.4 ^d	5.3 ± 0.3 ^{ab}	4.1 ± 0.2 ^a	1.4 ± 0.3 ^c	12.6 ± 0.4 ^e
Total ash	7.3 ± 0.2 ^a	11.6 ± 0.3 ^b	12.3 ± 0.2 ^{ac}	8.5 ± 1.7 ^{bc}	16.1 ± 0.2 ^c	15.9 ± 0.3 ^c
Total fiber	5.6 ± 0.4 ^a	8.5 ± 0.5 ^b	14.1 ± 0.3 ^c	13.0 ± 0.4 ^c	13.2 ± 0.3 ^c	18.3 ± 0.2 ^d
Total protein	41.4 ± 0.4 ^a	42.8 ± 0.1 ^a	41.6 ± 0.3 ^a	42.1 ± 0.1 ^a	39.0 ± 0.1 ^b	35.4 ± 0.9 ^c
Total lipid	12.8 ± 0.1 ^a	5.5 ± 1.2 ^b	8.1 ± 0.1 ^b	15.6 ± 1.1 ^c	14.9 ± 0.4 ^c	5.3 ± 0.3 ^b
Carbohydrates	26.7 ± 1.2 ^a	21.5 ± 0.5 ^b	18.6 ± 0.3 ^{bc}	16.7 ± 0.6 ^{bc}	15.4 ± 0.5 ^{cd}	12.5 ± 0.6 ^d

Data represent the mean % ±SD ($n = 3$)

Values in the same row with different superscript letters are significantly different ($p < 0.05$)

Lipids Nutritional Quality Indexes (IQN)

The nutritional quality of the lipid fraction can be assessed by three separate indexes that are calculated based on the concentration of saturated fatty acids (lauric C12:0, myristic C14:0, palmitic C16:0, and stearic C18:0), monounsaturated fatty acids (MUFA, oleic C18:1 ω 9), and polyunsaturated fatty acids (linoleic C18:2 ω 6, linolenic C18:3 ω 3, arachidonic C20:4 ω 6 and eicosapentaenoic C20:5 ω 3) according to:

- (1) Atherogenicity index (AI) = [(C12:0 + (4 × C14:0) + C16:0)]/(Σ MUFA + $\Sigma\omega$ 6 + $\Sigma\omega$ 3) [25].
- (2) Thrombogenicity index (TI) = (C14:0 + C16:0 + C18:0)/[(0.5 × Σ MUFA) + (0.5 × $\Sigma\omega$ 6 + (3 × $\Sigma\omega$ 3) + ($\Sigma\omega$ 3/ $\Sigma\omega$ 6))] [26].
- (3) Fatty acids hypocholesterolemic/hypercholesterolemic ratios (H/H) = (C18:1 ω 9 + C18:2 ω 6 + C20:4 ω 6 + C18:3 ω 3 + C20:5 ω 3)/(C14:0 + C16:0) [26].

Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using STATISTICA Software (version 7.0) from StatSoft Inc. [27]. A P value of <0.05 was considered statistically significant, and if significant differences were observed, treatment means were pairwise compared with the Tukey test.

Results and Discussion

Composition of Algal Biomass

The chemical compositions of the six microalgae species are shown in Table 1. The moisture content in dried microalgae ranged from 1.4 to 12.6 %, with *P. cruentum* and *S. platensis* having relatively high moisture values (12.6 and

10.0 %, respectively). Ash contents were higher in biomass of the marine species *P. tricorutum* and *P. cruentum* (16.1 and 15.9 %, respectively). Zhu and Lee [18] have shown that washing the wet biomass with a 0.5 M ammonium formate solution greatly reduces the salt content of dry matter (DM) of these marine algal species. In fact, ash levels in non-washed biomass of marine *P. tricorutum* and *P. cruentum* were 40.7 and 33.2 %, respectively. The diatom *P. tricorutum* has a high inorganic content due to the fact that its cell wall is covered by silica. The species *N. gaditana* and *S. platensis* had intermediate total ash contents (12.3–11.6 %), while *N. oculata* and *C. vulgaris* were found to have the lowest ash contents (8.5–7.3 %, respectively).

Dry matter (protein, carbohydrate + fiber, and lipids) is the major component in all the algae studied and differences among species were small. Protein is the most abundant component followed by carbohydrate + fiber, and lipids. Algal biomass from the species studied contains on average 40 g protein (range 35.4–42.8 %), 18 g carbohydrate (range 12.5–26.7 %), 12 g fiber (range 5.6–18.3 %), and 10 g lipid (range 5.3–15.6 %) per 100 g of biomass DM. The total dry matter accounted for an average of 93.3 % of total DM (range 87.4–98.6 %), in good agreement with Matos et al. [28], who reported a total biomass DM of 81.3–94.5 % for *C. vulgaris*, using similar methods. In general it is common that total dry matter of microalgae is found to be less than 100 % [12].

Using a nitrogen-to-protein (N-to-P) conversion factor of N × 4.78 [22], the protein content in *S. platensis* (42.8 %) was calculated to be significantly higher than in *N. oculata* (42.1 %), *N. gaditana* (41.6 %), and *C. vulgaris* (41.4 %), whereas the lowest levels were found in *P. tricorutum* and *P. cruentum* (39.0 and 35.4 %, respectively, $P < 0.05$). Among the six microalgae, *Spirulina* has been most extensively used as a source of single-cell protein (SCP) and was even carried by astronauts during space travel. Many microorganisms (algae, bacteria, fungi yeast/filamentous) can be used as a source of SCP, but due

to their low nucleic acid content and high level of essential amino acids, algae are preferred over fungi and bacteria as a source of SCP for human consumption [28]. The non-protein nitrogen (NPN) content in microalgae has been reported to range from 4 to 40 % depending upon species, season and growth phase [12, 22, 29]. The N-to-P factor assumes that the protein source contains 16 % N and does not take into account the often high content of NPN found in microalgae. Our findings on protein content in the microalgal biomass are in accordance with data from Tibbetts et al. [12], who reported that the N-to-P factor of $N \times 6.25$, which has been historically applied for microalgae, is incorrect and should be avoided [13, 30]. It should be noted that the estimates for the crude protein include other nitrogen compounds, e.g., nucleic acids, amines, glucosamides, and cell wall materials, which in general are expected to account for around 10 % of the total nitrogen found in microalgae [31].

The carbohydrate and dietary fiber contents in the algal biomass samples were found to be very diverse, varying between 12.5–26.7 % and 5.6–18.3 %, respectively. *P. cruentum* has the highest and well-balanced carbohydrate (12.5 %) and dietary fiber (18.3 %) content (Table 1). Actually, *P. cruentum* showed the highest carbohydrate + fiber content (30.8 %), which could be associated with its composition in sulfated polysaccharides (exopolysaccharide) [32, 33]. Of the species studied, *Chlorella* and *Spirulina* were found to contain the lowest amounts of fiber (5.6 and 8.5 %, respectively), which is important for human use, since low fiber values suggest an easily digestible biomass [31, 32]. It is important to note that, while the fiber fraction in most terrestrial plants is generally comprised of cellulose, hemicellulose and lignin, the fiber in microalgae contain no lignin and has low hemicellulose levels. This makes it easier to digest, and *Chlorella* and *Spirulina* are in fact widely used as a dietary supplement for human consumption. Moreover, *Chlorella* is recognized as safe food ingredient, with GRAS (Generally Recognized as Safe) status by the US FDA (Food and Drug Administration) [29]. The other microalgae (*N. gaditana*, *N. oculata*, *P. cruentum*, and *P. tricornutum*) have high fiber contents, which has been an argument against the use of marine microalgae in human nutrition, such as SCP [29, 31]. While all microalgae produce hydrocarbons as energy and carbon stores, some microalgae have a preference for carbohydrate rather than lipid accumulation and these species are gaining attention as potential feedstocks for bioethanol production [34]. We found that *C. vulgaris* biomass contains the highest carbohydrate content (~26.7 %), in agreement with Tibbetts et al [12]. These authors reported that *Chlorella* sp. (and similar species like *Scenedesmus*, *Chlamydomonas*, and *Tetraselmis*) typically produce large amounts of carbohydrate as energy and carbon reserves. As a result, it has

been proposed that the use of carbohydrate-rich algal biomass (for example *C. vulgaris*) as feedstock for bioethanol production may be advantageous over conventional feedstocks by providing increased hydrolysis efficiency, higher fermentable yields, and reduced production costs [35, 36].

With regard to the algal intracellular lipids, total lipid contents vary from 5.3 to 15.6 % of dry matter. Although this can be considered a narrow range, statistical differences were observed between lipid contents among species ($P < 0.05$). The marine algae *P. cruentum* and *S. platensis* have relatively low, statistically equal values for lipid contents (5.3 and 5.5 %, respectively) and similar lipid contents values were reported by Reboloso-Fuentes et al. [32] for *P. cruentum* (6.3 %) and by Henrikson et al. [4] for *S. platensis* (4.5–7.0 %). The algae *N. gaditana* and *C. vulgaris* had low to moderate lipid content (8.1 and 12.8 %, respectively), while *P. tricornutum* and *N. oculata* showed the highest lipid content (14.9 and 15.6 %, respectively) (Table 1), with an interesting composition in terms of PUFA- $\omega 3$ (Table 2). Our findings are consistent with those of Franz et al. [37], who reported a lipid content for *P. tricornutum* and *N. oculata* of about 15.6 and 16.1 %, respectively. Since the algal cultures studied were not subjected to nutrient starvation (N-deficient) or any other mechanism that can induce high lipid productivity, it is not surprising that the lipid content was relatively low compared to protein and carbohydrate + fiber. If the fatty acids profile of that lipid would be nutritionally attractive, these products could potentially be marketed as single-cell oil (SCO). However, modified cultivation/processing protocols could easily be employed to enhance lipid accumulation [38, 39].

Fatty Acids Composition

The microalgal lipid fraction was analyzed in terms of its fatty acids composition by identifying the main fatty acids, as well as the proportion of total saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) $\omega 3$ and $\omega 6$ fatty acids. Fifteen fatty acids, ranging from C12:0 to C22:6 $\omega 3$, were identified and quantified as percentage of the total fatty acid content of the algal samples (Table 2).

The results showed that the marine species (*P. tricornutum* and *N. oculata*) contain high concentrations of PUFAs- $\omega 3$, predominantly C20:5 $\omega 3$ (EPA) and C22:6 $\omega 3$ (DHA) along with substantial amounts of C16:1 (monounsaturated fatty acid) and C16:0 (saturated fatty acid). In contrast, marine *P. cruentum* has relatively high concentrations of PUFAs- $\omega 6$, predominantly C20:4 $\omega 6$ (AA), whereas the freshwater algae species *C. vulgaris* and *S. platensis* contain high concentrations of C18:3 $\omega 3$ (ALA) and C18 $\omega 6$ (GLA) PUFAs, respectively. *N. gaditana* contains a high concentration of saturated fatty acids (SFA), predominantly C16:0

Table 2 Fatty acids composition of biomass of six microalgae

	<i>Chlorella vulgaris</i>	<i>Spirulina platensis</i>	<i>Nannochloropsis gaditana</i>	<i>Nannochloropsis oculata</i>	<i>Porphyridium cruentum</i>	<i>Phaeodactylum tricornutum</i>
C12:0	–	90 ± 6	89 ± 6	108 ± 8	43 ± 3	77 ± 4
C14:0	34 ± 2	95 ± 5	890 ± 11	540 ± 12	22 ± 1	560 ± 23
C16:0	1930 ± 28	3420 ± 34	5130 ± 35	1940 ± 36	2950 ± 21	1360 ± 41
C18:0	67 ± 10	35 ± 5	160 ± 8	26 ± 1	78 ± 2	24 ± 2
Other SFA	125 ± 17	340 ± 13	344 ± 12	227 ± 9	344 ± 12	400 ± 11
Σ SFA	2156 ± 54	3930 ± 47	6613 ± 41	2840 ± 15	3437 ± 26	2420 ± 25
C15:1	280 ± 13	390 ± 9	69 ± 2	85 ± 2	150 ± 8	280 ± 6
C16:1	150 ± 4	560 ± 12	1900 ± 11	2240 ± 18	140 ± 4	1550 ± 13
C18:1	128 ± 6	45 ± 1	445 ± 8	280 ± 11	210 ± 10	129 ± 5
Other MUFA	131 ± 2	110 ± 5	192 ± 7	600 ± 6	17 ± 2	260 ± 6
Σ MUFA	690 ± 16	1105 ± 16	2606 ± 21	3205 ± 37	517 ± 11	2219 ± 17
C16:4 ω3	–	30 ± 2	–	–	50 ± 1	20 ± 1
C18:3 ω3 (ALA)	2820 ± 41	130 ± 8	34 ± 1	15 ± 2	20 ± 1	92 ± 2
C20:5 ω3 (EPA)	–	38 ± 1	–	2973 ± 24	697 ± 21	2753 ± 16
C22:6 ω3 (DHA)	–	62 ± 1	–	43 ± 5	–	80 ± 5
Σ PUFA-ω3	2820 ± 41	260 ± 7	34 ± 1	3031 ± 10	767 ± 12	2945 ± 34
C18:2 ω6 (LA)	1030 ± 22	150 ± 14	300 ± 10	290 ± 7	1040 ± 54	250 ± 8
C18:3 ω6 (GLA)	280 ± 10	1920 ± 42	120 ± 4	150 ± 3	18 ± 2	34 ± 2
C20:4 ω6 (AA)	28 ± 2	38 ± 2	–	–	3705 ± 26	83 ± 4
C22:5 ω6	–	38 ± 3	–	–	182 ± 7	115 ± 7
Σ PUFA-ω6	1338 ± 36	2146 ± 41	420 ± 8	440 ± 10	4945 ± 21	482 ± 12
SFA + MUFA + PUFAs	7004 ± 55	7441 ± 64	9673 ± 78	9516 ± 102	9666 ± 89	8066 ± 82

Data are expressed as mg/100 g ± SD ($n = 3$)

Numbers in bold indicate contents of the major fatty acid in the microalgal biomass

SFAs not shown in the table: *Tridecanoic acid* C13:0, *Pentadecanoic acid* C15:0, *Behenic acid* C22:0. MUFAs not shown in the table: *Margaroleic acid* C17:1, *Erucic acid* C22:1, *Nervonic acid* C24:1

with a low level of PUFAs. These results indicate that freshwater algae *C. vulgaris* and marine species *N. oculata*, *P. tricornutum* and *P. cruentum* followed the same PUFA > SFA relative pattern, while *S. platensis* and *N. gaditana* show the opposite pattern, SFA > PUFA.

Saturated Fatty Acids

Palmitic acid (C16:0) is the most abundant SFA (1.3–5.1 g/100 g) followed by myristic acid (C14:0, 22–890 mg/100 g) and stearic acid (C18:0, 24–160 mg/100 g) (Table 2). The sum of all identified SFAs ranged from 29 to 68 % of the total fatty acid content. We found that *N. gaditana* contains 68 % SFA, mainly comprised of palmitic acid (C16:0), followed by *S. platensis* with 52 % SFA, also mainly C16:0, which is in agreement with values reported in the literature for *N. gaditana* [40] and *S. platensis* [4]. *P. cruentum* and *C. vulgaris* have intermediate SFA contents (35 and 30 %, respectively), while *N. oculata* and *P. tricornutum* have the lowest SFA content.

Monounsaturated Fatty Acids

The highest monounsaturated fatty acids (MUFA) content was found in *N. oculata*, representing ~34 % of total fatty acid content, followed by *P. tricornutum* (28 %) and *N. gaditana* (27 %), whereas *S. platensis*, *C. vulgaris* and *P. cruentum* contain very low concentrations of MUFAs, on average about 10 %. The main MUFAs detected in all species studied are: C15:1, C16:1 and C18:1 ω-9, with a high variation in composition among the different species. Palmitoleic acid (C16:1) is the main MUFA found in all marine species studied, ranging from 1.9 to 2.2 g/100 g, except for *P. cruentum* that has a very low content of 0.14 g/100 g (Table 2). These results are consistent with those obtained by Oh et al. [41], who demonstrated that *P. cruentum* contains low levels of palmitoleic acid and higher levels of arachidonic acid (AA, C20:4ω6). It has been reported that the marine *Nannochloropsis* species [42–44] and *P. tricornutum* [45] have a high concentration of palmitoleic acid, suggesting that these algae have a tendency to produce unsaturated fatty acids.

Regarding the WHO/FAO recommendation about the amount of MUFAs in the human diet, the Expert Consultation [8] stated that there is convincing evidence that replacing SFA (C12:0-C16:0) as well as carbohydrates with MUFA reduces LDL and increases HDL cholesterol concentrations. Our data suggest that the marine species *N. oculata* and *P. tricorutum* have optimal compositions for the above purposes because these two species have the lowest SFA content (28–29 %), a low carbohydrate content (15.4–16.7 %), and a high MUFA content (28–34 %).

Polyunsaturated Fatty Acids

Among the polyunsaturated ω -3 and ω -6 fatty acids (PUFAs), α -linolenic (ALA, C18:3 ω 3) and eicosapentaenoic (EPA, C20:5 ω 3) acids are the predominant PUFAs- ω 3, while γ -linolenic (GLA C18:3 ω 6) and arachidonic (AA, C20:4 ω 6) acids made up most of PUFAs- ω 6. High concentrations of PUFAs- ω 3 were observed in *C. vulgaris* (40 %), *P. tricorutum* (~37 %) and *N. oculata* (~32 %) of the total fatty acids, while high concentrations of PUFAs- ω 6 were found in *S. platensis* (29 %) and *P. cruentum* (51 %) of the total fatty acids. *N. gaditana* cells contain very low concentrations of PUFA (5 %) and has the least favorable ω 3/ ω 6 ratio (0.08) among all algae (Table 3).

Chlorella vulgaris contained 60 % PUFA, with a high proportion of ω 3 acids. In fact, except for *S. platensis*, *P. cruentum* and *N. gaditana*, all other microalgae showed a ω 3/ ω 6 ratio of ≥ 2.0 (Table 3). Indeed, in cyanobacteria (e.g., *S. platensis*) the unsaturated double bonds are preferentially in the ω 6 position while in Chlorophyceae they are mainly in the ω 3 position [1]. *S. platensis* is rich in γ -linolenic acid (GLA, C18:3 ω 6) (1.9 g/100 g). Gamma linolenic acid is as a precursor of C₂₀ eicosanoids (prostaglandins, leukotrienes, and thromboxanes) and has been associated with beneficial health effects, such as a reduction in LDL (low-density lipoproteins), anti-inflammatory

effects, stimulation of the apoptosis of cancer cells, and reduction in pain and inflammation associated with rheumatoid arthritis [9]. The species *S. platensis* is a well-known source of GLA, since in cyanobacteria this fatty acid plays the same role as α -linolenic acid (ALA, C18:3 ω 3) in algae and higher plants [4]. Although PUFA levels are relatively high for all species (>25 % of total fatty acid), except for *N. gaditana* cells, it consists of medium-chain PUFA (e.g., C₁₆ and C₁₈) and is devoid of long-chain (LC) PUFA (e.g., C₂₀ and C₂₂). This is generally typical for freshwater microalgae/cyanobacteria and makes them poor sources of nutritionally-essential LC-PUFA, arachidonic acid (AA, C20:4 ω 6), eicosapentaenoic acid (EPA, C20:5 ω 3) and docosahexaenoic acid (DHA, C22:6 ω 3). For this reason, *N. oculata* and *P. tricorutum* are more interesting for nutrition applications, because they are able to synthesize high amounts of EPA and DHA acids, and thus can be used to enrich functional foods with ω -3 fatty acids. Algal oils and SCO sources of LC-PUFAs are now becoming available (to provide EPA + DHA + AA) [2, 7]. In addition, an advantage of an SCO from algae is that it usually contains a significant amount of natural antioxidants (e.g., carotenoids and tocopherols), which can protect ω -3 fatty acids from oxidation, hence making this oil less prone to oxidation than oil derived from plants and marine animals [46].

As shown in Table 2, *C. vulgaris* and *P. cruentum* have the same PUFA content (~60 %). However, α -linolenic acid (ALA, C18:3 ω 3) in *C. vulgaris* contributes to an increased ω 3/ ω 6 ratio of 2.10, while arachidonic acid (AA, C20:4 ω 6) in *P. cruentum* causes a low ω 3/ ω 6 ratio of 0.15. Consequently, *C. vulgaris* has a favorable ω 3/ ω 6 ratio that is about 15-fold higher than that of *P. cruentum*, but the large amount of AA (3.7 g/100 g) in *P. cruentum* makes this marine alga a potential source for the production of arachidonic acid. Since the metabolic breakdown of AA leads to an increased production of prostaglandin E₂, which belongs to a class of hormone-like substances that participate in a wide range of bodily functions, thromboxane and leukotriene [47], the importance of this fatty acid to human-cell functioning is evident.

The marine microalgae *N. oculata* and *P. tricorutum* contain 37 and 43 % PUFA, respectively, with a high ω 3/ ω 6 ratio of 6.50. These microalgae are also rich in EPA and contain a small quantity of DHA. The species *N. oculata* contains 3.0 g EPA and 43 mg DHA, and *P. tricorutum* has 2.7 g EPA and 80 mg DHA per 100 g microalgal biomass. According to Borowitzka [5] the main market of these oils is infant formula and an oil rich in both DHA and EPA from a strain of *Schizochytrium* has recently reached the market [5]. Currently, there is no other commercial production of EPA-rich oils from microalgae, but Aurora Algae has announced a product from marine

Table 3 Nutritional quality indexes of the lipid fraction in the biomass of six microalgae

Species	P/S	ω 3/ ω 6	H/H	AI	TI
<i>C. vulgaris</i>	2.25	2.10	2.04	0.42	0.21
<i>S. platensis</i>	0.89	0.12	0.66	1.10	1.46
<i>N. gaditana</i>	0.46	0.08	0.12	1.70	3.82
<i>N. oculata</i>	2.33	6.88	1.44	0.63	0.22
<i>P. cruentum</i>	1.79	0.15	1.90	0.49	0.60
<i>P. tricorutum</i>	2.35	6.10	1.72	0.65	0.19

P/S polyunsaturated/saturated, ω 3/ ω 6 Σ of the Omega 3 series/ Σ of the Omega 3 series, H/H Σ hypercholesterolemic/ Σ hypocholesterolemic, AI atherogenicity index, TI thrombogenicity index

eustigmatophyte *Nannochloropsis*. According to American Heart Association a daily intake of 500 mg EPA + DHA and 800–1000 mg of ALA per day are recommended for the primary prevention of coronary heart disease [48]. Our results indicate that the marine species *N. oculata* and *P. tricornutum* are potential sources of EPA, with an average of 2.8 g/100 g whereas *C. vulgaris* has a robust ALA (2.8 g/100 g) producing profile. These microalgae have therefore an enormous potential for application in the development of health food products, such as SCO.

Lipids Nutritional Quality Indexes (IQN)

The nutritional quality of lipid profiles observed in the algae species was evaluated by different indexes as shown in Table 3.

Foods with polyunsaturated and saturated fatty acids (P/S) ratios below 0.45 are considered by the FAO/WHO to be undesirable in the human diet [8], because of their potential to induce increases in blood cholesterol. The P/S ratios in all six algae are above 0.45, ranging from 0.46 in *N. gaditana* to 2.35 in *P. tricornutum*.

An additional approach to the nutritional evaluation of lipid profiles is the calculation of an index based on functional effects of fatty acids, e.g., the ratio hypocholesterolemic fatty acids/hypercholesterolemic fatty acids (H/H) index, which is based on current knowledge of the effects of individual fatty acids on cholesterol metabolism [26, 50]. Nutritionally, higher H/H values are considered more beneficial for human health, because a higher H/H ratio is directly proportional to a high PUFA content. Fatty acids from microalgae that are highly polyunsaturated are thought to have beneficial effects on cholesterol. The highest H/H value (2.04) was found in *C. vulgaris*, followed by *P. cruentum* species (H/H = 1.90). These results are in excellent agreement with H/H values for marine fish such as sardine and mackerel (H/H = 2.46) reported by Fernandes et al [6]. In addition, Testi et al. [51] reported H/H values for fish filets of sea bass and rainbow trout of 2.18–2.40.

Two other indexes are used to evaluate the potential for stimulating platelet aggregation, the atherogenicity index (AI) and thrombogenicity index (TI) according to Turan et al [49]. Lower AI and TI values indicate a greater potential to protect against coronary artery disease. In our study, AI values ranged between from 0.42 to 1.70, with *C. vulgaris* (0.42) and *P. cruentum* (0.49) showing the lowest values (Table 3). It is noteworthy that these two species also have the highest PUFA content (~ 60 %). Simat et al. [50] reported values of 0.59–0.92 for the omnivorous fish bogue (*Boops boops* Linnaeus). The lowest thrombogenicity index (TI) value of 0.19 was observed in the marine *P. tricornutum*, which is comparable to TI values for the marine fish sardine of 0.20 reported by Fernandes et al [6].

With regard to the $\omega 3/\omega 6$ ratio, typical Western diets have $\omega 3/\omega 6$ ratios that are profoundly skewed toward omega-6, which is believed to promote or cause several diseases [48]. This is mainly due to the disproportionately greater consumption of ω -6 rich vegetable oils (e.g., sunflower, peanut, corn) in comparison with the intake of ω -3 rich food sources, such as seafood, nuts, etc. Our data indicate that $\omega 3/\omega 6$ ratios of the algae species decreased in the order of *N. oculata* (6.88) > *P. tricornutum* (6.10) > *C. vulgaris* (2.10) > *P. cruentum* (0.15) > *S. platensis* (0.12) > *N. gaditana* (0.08), (Table 3). These results are in accordance with findings of other authors, for example Batista et al. [1], who reported higher $\omega 3/\omega 6$ ratios in marine algae species than freshwater algae species, suggesting that the marine species studied here (i.e., *N. oculata* and *P. tricornutum*) could be categorized as beneficial to human health consumption.

Conclusions

This study determined the biochemical compositions of the six microalgae strains. Algal biomass from these species contain on average 40 g protein, 18 g carbohydrate, 12 g fiber, and 10 g lipid per 100 g of biomass DM. The species *C. vulgaris* and *S. platensis* are rich in ALA (2.8 g/100 g) and GLA (1.9 g/100 g), respectively. The marine algae *P. tricornutum* and *N. oculata* contain 42 % and 37 % PUFA, respectively, with a favorable $\omega 3/\omega 6$ ratio of around 6.5, and are rich in EPA and DHA acids. The alga *P. cruentum* contains high PUFA- $\omega 6$ levels, due its high concentration of AA (3.7 g/100 g). Taken together, the results show that microalgae are excellent candidates as sources of high protein (*Spirulina*), high carbohydrate/low fiber (*Chlorella*) and high LC-PUFA- $\omega 3$ (*N. oculata* and *P. tricornutum*) contents with a high nutritional value, similar to fish oil.

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