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ORIGINAL PAPER

Chemical characterization and nutritional evaluation of microalgal biomass from large-scale production: a comparative study of five species

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Abstract
Biomass from five microalgal species, *Porphyridium cruentum, Isochrysis galbana*, *Phaeodactylum tricornutum, Tetraselmis suecica* and *Nannochloropsis gaditana,* produced at an industrial plant in outdoor photobioreactors, was studied with the aim to evaluate their suitability to the food and nutraceutical sectors. Microalgal biomass was analyzed for proximates, nonprotein nitrogen, energy, fatty acids, minerals, trace elements and mercury contents. Proximate analyses showed wide differences among microalgal species, in accordance with their different taxonomic position, especially as regards protein (19.6–33.2% dry mass), carbohydrate (15.9–42.2% dry mass) and lipid (5.7–31.1% dry mass) contents. All species proved to be a good source of minerals and trace elements and of polyunsaturated fatty acids (47.4–59.1% of total fatty acids) with varying profiles. N-3 fatty acids were prevalent in *P. tricornutum*, *N. gaditana*, *I. galbana* and *T. suecica* (32.6–36.4% of total fatty acids), whereas n-6 fatty acids, mainly arachidonic (C20:4) and linoleic (C18:2) acids, were prevalent in *P. cruentum* (43.3% of total fatty acids). *N. gaditana*, *P. tricornutum*, and *P. cruentum* were rich in eicosapentaenoic acid (36.0, 29.3%, and 15.9% of total fatty acids, respectively), while *I. galbana* was a good source of stearidonic (C18:4, 12.2% of total fatty acids) and docosahexaenoic (9.0% of total fatty acids) acids, undetectable or present at low levels in the other species. *I galbana* and *T. suecica* showed also high percentages of α-linolenic acid (C18:3, 12.2%–15.7% of total fatty acids). All microalgae were characterized by good nutrient contents and confirmed to be potentially valuable ingredients for nutritional or nutraceutical purposes.

Keywords Microalgae · Chemical composition · Nutrients · Nutritional value · Fatty acids · Proximate composition · Minerals

Introduction

The growing need to produce safe and healthy food in a global context of limiting resources and climate changes suggests the opportunity to exploit natural and sustainable sources of nutrients and functional ingredients for the food sector. Microalgae are a heterogeneous group of microorganisms at the base of the aquatic food chain, rich sources of nutrients and bioactive molecules. Their mass cultivation has the advantage of a high sustainability, as they absorb CO_2 from the atmosphere, withstand extreme environmental conditions, have a high productivity and do not compete with terrestrial crops for agricultural land [1, 2].

Among the several thousands of microalgal species available in nature, only a limited number are known and much less are commercially exploited. With a biorefinery approach, microalgal biomass may have multiple industrial and biotechnological applications, from biofuel production to the extraction of high-value products for the pharmaceutical, nutraceutical, functional food and cosmeceutical markets [3]. Nonetheless, the content of high-value proteins, lipids, carbohydrates, vitamins, pigments and other health-promoting bioactive substances, together with their

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safety requisites, makes some microalgal species suitable for direct consumption, although nowadays their use in human nutrition is limited to the few species allowed by novel food regulations [4–6].

Up to now, most of the microalgal species commercially exploited find their main market in aquaculture as feed for larval and juvenile molluscs, crustaceans and fish and for the zooplankton needed in the sea-farm food chain [7]. A deep knowledge of the chemical composition of microalgae grown under controlled and standardized conditions is the first step to understand and promote the potentiality of the whole biomass as a nutritional supplement or ingredient in the food sector. The golden-brown flagellate *Isochrysis galbana*, the diatom *Phaeodactylum tricornutum*, the green alga *Tetraselmis suecica* and the Eustigmatophyta *Nannochloropsis gaditana* are marine microalgae finding a common application in aquaculture as feed for farmed aquatic species [8–10]. More recently, these species are also receiving interest as potential feedstock for biofuel and as sources of high-value molecules for the nutraceutical, pharmaceutical and cosmetic industries [11–13]. The unicellular red alga *Porphyridium cruentum* raises biotechnological interest as a source of valuable polyunsaturated fatty acids, pigments and other high-value compounds for cosmetic and pharmaceutic formulations [14, 15].

The main nutritional interest of microalgae raises especially from their valuable lipid profile due to the high polyunsaturated fatty acid (PUFA) content, although they are also reported to be good sources of proteins, carbohydrates, pigments and antioxidants [16–20].

This study was designed to gather a comprehensive characterization of the nutritional profile, minerals and trace elements of *I. galbana*, *P. tricornutum*, *T. suecica*, *N. gaditana* and *P. cruentum* biomass produced at an industrial plant in outdoor photobioreactors with the aim to evaluate their potential value and suitability to food applications.

Materials and methods

Microalgal biomass

The commercial microalgal biomass from *Porphyridium cruentum* (Phylum Rhodophyta, Class Porphyridiophyceae), *Isochrysis galbana* (Phylum Haptophyta, Class Coccolythophyceae), *Phaeodactylum tricornutum* (Phylum Bacillariophyta, Class Bacillariophyceae), *Tetraselmis suecica* (Phylum Chlorophyta, Class Chlorodendrophyceae) and *Nannochloropsis gaditana* (Phylum Ochrophyta, Class Eustigmatophyceae) was produced at the industrial plant of Archimede Ricerche Srl (Camporosso, Imperia, Italy; http://www.archimedericerche.com). Microalgae were grown in Green Wall Panel photobioreactors (3000–10,000 L), provided with automated controls to maintain optimal growth conditions. Growth occurred on natural sunlight in enriched artificial seawater, the F/2 medium, containing a mixture of nutrients, microelements and vitamins [21]. The supply of food-grade carbon dioxide as the carbon source was automatically regulated by a pH-control system. Before the onset of the stationary phase, cultures were stopped and cells harvested by centrifugation (Westfalia Separator, Dűsseldorf, Germany). Biomass was provided to our laboratory as homogeneous freeze-dried samples packed under vacuum (3–7% moisture values). Samples were stored at − 80 °C at our laboratory until analyses.

Proximate analysis

Moisture, ash, and crude protein (Kjeldahl N x 4.78) were determined according to AOAC methods [22]. Moisture and ash contents were determined gravimetrically by oven drying at 105 °C, up to a constant weight, and incineration in a muffle furnace at 550 °C, respectively. Nonprotein nitrogen (NPN) was determined by the Kjeldahl method after protein precipitation with 10% (w/v) trichloroacetic acid. Total carbohydrates were determined spectrophotometrically by the phenol–sulphuric acid method using glucose for the calibration curve [23]. Total lipids were determined according to a chloroform:methanol:water extraction protocol specific for microalgae developed at our laboratory and partly derived by previous methods [24, 25]. Briefly, 1–2 g dry algal powder was mixed with 10 mL methanol containing 500 ppm tert-butylhydroquinone (TBHQ) in a centrifuge tube and vortex-mixed for 30 s (Reax 2000, Heidolph, Schwabach, Germany). Chloroform (10 mL) was added and the mixture was vortexed again (30 s) and sonicated (37 kHz, 10 min, room temperature) in an ultrasonic bath (Ultrasonic S60 H, Elma Schmidbauer GmbH, Singen, Germany). After centrifugation (1300×*g*, 15 min at 5 °C), the extract was recovered and the residual biomass was extracted and sonicated repeating the procedure once more. The two extracts were kept separated, each added 5 mL of 1% w/v sodium chloride in water, vortexed and centrifuged (1300×*g*, 10 min, 5 °C). After centrifugation, the upper phases in each tube were discarded, while the lower phases were combined and filtered through a Whatman no. 2 filter paper funnel containing anhydrous sodium sulfate to remove any water residue. Solvent in the lipid extract was removed by rotary evaporation (R-210 Rotavapor®, Bűchi, Switzerland) at 40 °C and the lipid content was determined gravimetrically. Results are expressed as weight percentages on a dry matter basis.

Minerals and trace elements

Mineral (Ca, Mg, Na, K, P) and trace element (Fe, Zn, Cu, Mn) contents were quantified by Inductively Coupled

Plasma Optical Emission Spectrometry (Optima 8000™ ICP-OES, Perkin-Elmer, Waltham, MA, USA) after liquid ashing (6 mL HNO_3 + 1 mL H_2O_2) of the samples in a microwave digestion system (1200 Mega, Milestone Srl, Italy). Standard Reference Materials, Cabbage (IAEA-359, International Atomic Energy Agency Reference Materials Group) and Haricots vert (BCR 383, Community Bureau of Reference, Brussels), were analyzed as a check on the accuracy of the analysis.

Mercury

Total mercury levels were evaluated by Thermal Decomposition–Amalgamation–Atomic Absorption Spectrophotometry (DMA-80 Direct Mercury Analyzer, Milestone Srl, Italy) as previously described [26].

Fatty acids

Fatty acids were methylated using boron trifluoride in methanol as esterification reagent [27]. The esterified fatty acids were quantified by GC–MS-FID (7890A Series-Agilent Technologies Santa Clara, CA, USA). Separations were accomplished on a Mega-wax column (30 m × 0.32 mm i.d., 0.25 µm film thickness). The Certified Reference Material Supelco® 37 Component FAME Mix C4-C24 (Supelco, Bellefonte PA, USA) was analyzed as a control of the accuracy of the analysis. Fatty acids were identified comparing retention times with known authentic standards and using the NIST08 Mass Spectral Library (National Institute of Standards and Technology, Gaithersburg, MD, USA). Some minor fatty acids were identified only by matching their mass spectrum with the mass spectral database. Relative quantities are expressed as weight percent of total fatty acids. For nutritional evaluation purposes, percent of total fatty acids data have been converted to amounts per 100 g biomass according to Exler and Kinsella [28].

Statistical analysis

Data are reported as the average ± standard deviation of triplicate analyses on microalgal biomass. Analysis of variance (One-Way ANOVA) and Multiple-Range LSD test were applied to find out significant differences ($P \leq 0.05$) among species. The software used for statistical analyses was Statgraphics® Centurion, Version XV (StatPoint Technologies Inc., Warrenton, VA, USA).

Results and discussion

Proximate composition

The macronutrient profile of algal biomass showed wide differences among species (Table 1) in accordance with their different taxonomic position. Crude protein, total lipid, total carbohydrate, energy and ash contents differed significantly ($P \leq 0.05$) among species. The crude protein content ranged from 19.6 to 33.2 g 100 g^{-1} dw, with *P. cruentum* showing the lowest and *N. gaditana* the highest value (Table 1). Intermediate levels (26–29 g 100 g^{-1}dw) were shown by *I. galbana*, *P. tricornutum* and *T. suecica*. Such values confirm these microalgae as a protein-rich biomass, although values found in the literature are variable and highly dependent on species, culturing conditions and growth stages [18, 19, 29]. In addition, the analytical method used and the N-to-protein conversion factor adopted may highly affect the final value. We adopted 4.78 as a N-to protein conversion factor according to Lourenço et al. [30] as this factor, in place of the conventional 6.25, takes into account the relevant

Table 1 Chemical composition and energy content of biomass from the five microalgal species objects of this study

	P. cruentum		*I. galbana*		*P. tricornutum*		*T. suecica*		*N. gaditana*	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Crude protein (Nx4.78), (g)	19.57[a]	0.12	28.98[d]	0.26	26.95[c]	0.05	26.05[b]	0.22	33.17[e]	0.58
Total lipid (g)	5.69[a]	0.26	31.09[e]	0.10	12.73[b]	0.13	14.68[c]	0.20	27.89[d]	0.30
Total carbohydrate (g)	42.17[c]	1.40	17.67[a]	0.82	16.91[a]	1.61	24.01[b]	0.43	15.90[a]	2.28
Ash (g)	23.59[d]	0.09	15.16[b]	0.07	27.95[e]	0.08	17.99[c]	0.06	11.52[a]	0.04
Total N (g)	4.09[a]	0.02	6.06[d]	0.05	5.64[c]	0.01	5.45[b]	0.04	6.94[e]	0.12
NPN (g)	0.99[a]	0.03	3.18[d]	0.07	2.02[c]	0.18	1.66[b]	0.06	1.04[a]	0.02
NPN (% of total N)	24.29[b]	0.58	52.48[e]	0.98	35.77[d]	3.20	30.33[c]	1.02	15.07[a]	0.02
Energy value										
kcal	296[a]	4	465[d]	1	294[a]	1	332[b]	4	442[c]	2
kJ	1237	16	1946	2	1228	9	1387	17	1848	10

Values refer to 100 g dry biomass

Different superscript letters in the same row correspond to significant differences ($P \leq 0.05$)

presence of nonprotein nitrogen (NPN) in microalgae. This nitrogen fraction, mainly represented by nucleic acids, free amino acids and inorganic nitrogen, is present at variable levels in microalgae, depending on the species and growth phase. The NPN levels observed in our study, ranging from about 1 g 100 g^{-1} dw (*P. cruentum* and *N. gaditana)* to 3.2 g 100 g^{-1} dw (*I. galbana*), indicate a not negligible and highly variable contribution of this fraction to total nitrogen, accounting for a minimum of 15% (*N. gaditana*) to over 50% (*I. galbana*) of total nitrogen (Table 1). These values are in accordance with the NPN ranges reported in the literature [30, 31].

Microalgal biomass from the five species under study was characterized by sharply different lipid levels (Table 1). The lowest total lipid content was shown by *P. cruentum* (5.69 g 100 g^{-1} dw), whereas the highest levels were attained by *N. gaditana* (27.89 g 100 g^{-1} dw) and *I. galbana* (31.1 g 100 g^{-1} dw) (Table 1). These values are in accordance with the literature, reporting *P. cruentum* as a species accumulating carbohydrates and *Nannochloropsis* sp. and *Isochrysis* sp. as oleaginous species receiving interest as sources of n-3 fatty acids in aquaculture and as potential feedstock for biofuel production [18, 32–34]. Although the lipid content of microalgae is species specific, its level is also highly dependent on culture conditions and time of harvest. This fact explains the variable levels found in the literature.

Carbohydrates are nutrients highly represented in microalgae, both as intracellular storage carbohydrates and cell wall structural polysaccharides. Total carbohydrate contents (Table 1) were comparable in the three species *N. gaditana*, *P. tricornutum* and *I. galbana* (15.9–17.7 g 100 g^{-1} dw), whereas *T. suecica* (24.0 g 100 g^{-1} dw) and *P. cruentum* (42.2 g 100 g^{-1} dw) showed significantly higher values ($P \leq 0.05$). These data are in general accordance with literature values [18, 32, 35, 36]. The high carbohydrate content of *P. cruentum* is peculiar of this unicellular red alga, encapsulated within a sulphated polysaccharide-rich cell wall [18, 32]. The accumulation of substantial amounts of carbohydrates in *P. cruentum*, under selected conditions, has been object of studies and represents one of the elements raising biotechnological interest towards this species [14, 15]. Sulphated polysaccharides, also released in the growth medium as exopolysaccharides, have multiple interests because of demonstrated antiviral, antioxidant and anti-inflammatory activities valuable in the pharma sector and rheological properties useful for food applications as thickening agents [37, 38]. The second species most abundant in carbohydrates, *T. suecica* (24.01 g 100 g^{-1} dw), is known for its richness in intracellular starch so as to be considered a potential feedstock for bioethanol production [12]. In addition, the peculiar cell wall polysaccharide of *T. suecica* has been reported to contain an interesting monomer, 3-deoxy-D-manno-oct-2-ulosonic acid, with precious pharmacological properties and a high economic value [36]. In *Nannochloropsis* sp., cellulose is the main component of its tough and highly recalcitrant cell wall, protected by an outer hydrophobic algaenan layer, whereas in *P. tricornutum,* the cell wall is mainly composed of a sulphated glucuronomannan [39, 40]. Beta-glucans, sulphated polysaccharides, cellulose and other microalgal polysaccharides may also act as dietary fiber within the gastrointestinal tract thus helping regulate energy intake, and serum glucose and lipid levels.

The high energy content of microalgal biomass (294–465 kcal 100 g^{-1} dw) was correlated with lipid levels, except that in the case of *P. cruentum*, where the high energy contribution of carbohydrates counter-balanced its low lipid content. Values within this range are reported in the literature, with the lower levels referred to *P. cruentum* and the higher ones to the lipid-rich *N. gaditana* and *I. galbana* [18, 19, 32, 41]. However, as the microalgal biomass composition may be modulated through nutrient starvation or control of environmental conditions to increase the lipid or carbohydrate content of cells, variable energy values may be attained [35, 42].

Phaeodactylum tricornutum and *P. cruentum* were the species with the highest ash content (27.9 g and 23.6 g 100 g^{-1} dw, respectively) compared to the other species (11.5–18.0 g 100 g^{-1} dw) (Table 1). Comparable values are reported in the literature [32, 43]. However, highly variable ash contents and mineral profiles are reported in the literature, due to the strong influences of culturing and environmental conditions even within the same species.

Mineral composition

The elemental composition of algal biomass is reported in Table 2. Sodium (2.0–4.5 g 100^{-1} dw) and calcium (0.6–2.9 g 100 g^{-1} dw) were the most abundant minerals, followed by potassium (1.1–2.4 g 100^{-1} dw), phosphorus (0.3–1.2 g 100 g^{-1} dw) and magnesium (0.37–1.4 g 100 g^{-1} dw). *P. tricornutum* showed levels of potassium (2.4 g 100 g^{-1} dw), magnesium (1.4 g 100 g^{-1} dw) and of trace minerals such as iron (87.7 mg 100 g^{-1} dw), manganese (14.2 mg 100 g^{-1} dw) and copper (5.5 mg 100 g^{-1} dw) much higher than those of the other species ($P \leq 0.05$). *P. cruentum* was the species with the highest Ca:P ratio (6.53), largely surpassing *P. tricornutum* (2.45), *I. galbana* and *T. suecica* (1.84–1.86) and *N. gaditana* (0.71). As regards safety for human consumption, in the studied species, mercury levels (0.05–0.55 µg 100 g^{-1} dw) were well below the maximum value of 0.1 mg kg^{-1} set by the European legislation for food supplements [44]. Taking into consideration the levels of tolerable weekly intake (TWI) established by EFSA for mercury (1.3 and 4.0 µg kg^{-1} body weight for methylmercury and inorganic mercury, respectively), the total mercury levels detected are of no toxicological concern [45].

Table 2 Mineral composition of microalgal biomass from the five microalgal species objects of this study

	P. cruentum		*I. galbana*		*P. tricornutum*		*T. suecica*		*N. gaditana*	
	Mean	sd	Mean	sd	Mean	sd	Mean	sd	Mean	sd
Na (mg)	4452.6^{d}	117.3	2735.0^{b}	187.9	4328.9^{d}	136.7	3223.1^{c}	4.3	2022.6^{a}	183.2
Ca (mg)	2067.8^{d}	25.2	1145.5^{b}	87.1	2905.1^{e}	70.4	1790.7^{c}	56.6	631.3^{a}	7.9
K (mg)	1102.0^{a}	9.6	1314.4^{b}	55.9	2408.9^{c}	32.7	1349.4^{b}	74.3	1260.7^{b}	32.0
Mg (mg)	473.7^{b}	19.7	400.4^{a}	12.4	1448.1^{d}	41.4	601.4^{c}	8.2	370.3^{a}	13.6
P (mg)	316.7^{a}	8.0	625.3^{b}	23.3	1189.1^{e}	41.4	964.6^{d}	28.5	887.1^{c}	24.1
Ca:P ratio	6.53^{d}	0.09	1.84^{b}	0.21	2.45^{c}	0.14	1.86^{b}	0.11	0.71^{a}	0.03
Fe (mg)	37.7^{b}	0.9	31.0^{a}	0.7	87.7^{d}	5.1	30.9^{a}	0.2	48.9^{c}	2.2
Zn (mg)	11.5^{c}	0.2	13.4^{d}	0.2	10.3^{b}	0.5	6.6^{a}	0.2	10.1^{b}	0.3
Mn (mg)	2.5^{a}	0.1	3.6^{b}	0.1	14.2^{d}	0.6	3.5^{b}	0.2	9.3^{c}	0.2
Cu (mg)	1.2^{a}	0.0	2.8^{b}	0.0	5.5^{c}	0.2	1.1^{a}	0.0	2.9^{b}	0.1
Hg (µg)	0.14^{b}	0.01	0.07^{a}	0.02	0.46^{c}	0.03	0.05^{a}	0.00	0.55^{d}	0.03

Values refer to 100 g dry biomass

Different superscript letters in the same row correspond to significant differences ($P \leq 0.05$)

Fatty acid profile

The total saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acid compositions of microalgal biomass are reported in Table 3. All microalgae showed a prevalence of PUFA (47.4–59.1% of total fatty acids) over SFA (23.0–35.7% of total fatty acids) and MUFA (6.1–30.5% of total fatty acids).

Fatty acid profiles differed widely, both qualitatively and quantitatively, among species. SFA were mainly represented by palmitic acid (C16:0), prevalent in *P. cruentum* (34.1% of total fatty acids), *N. gaditana* (21.6% of total fatty acids), *T. suecica* (18.3% of total fatty acids) and *P. tricornutum* (14.4% of total fatty acids), and by myristic acid (C14:0), prevalent in *I. galbana* (16.1% of total fatty acids). MUFA were mainly represented by palmitoleic acid (C16:1 n-7), prevalent in *N. gaditana* (22.25% of total fatty acids) and *P. tricornutum* (18.65% of total fatty acids) and by oleic acid (C18:1 n-9) prevalent in *T. suecica* (19.6% of total fatty acids) and *I. galbana* (16.5% of total fatty acids). *P. cruentum* distinguished for the very low MUFA percentage (6.1% of total fatty acids), a feature reported in the literature for this species [18] and consistent with its low lipid content.

Among PUFA, n-3 fatty acids were prevalent in *N. gaditana* and *I. galbana* (36.2–36.4% of total fatty acids), *T. suecica* (34.6% of total fatty acids) and *P. tricornutum* (32.6% of total fatty acids), whereas n-6 fatty acids, mainly arachidonic (C20:4) and linoleic (C18:2) acids, were prevalent in *P. cruentum* (43.3% of total fatty acids).

As regards single n-3 fatty acids, *P. cruentum, P. tricornutum*, and *N. gaditana* were rich in eicosapentaenoic acid (C20:5, 15.9%, 29.3% and 36.0%, respectively). *I. galbana* was a good source of stearidonic acid (C18:4, 12.2% of total fatty acids), a precursor of eicosapentaenoic acid, and of its elongation–desaturation product, docosahexaenoic acid (C22:6, 9.0% of total fatty acids), undetectable or present at low levels in the other species. *I galbana* and *T. suecica* showed also consistent percentages of α-linolenic acid (C18:3, 12.2% and 15.7% of total fatty acids, respectively).

A high long-chain polyunsaturated fatty acid (LC-PUFA) productivity of *Porphyridium*, *Nannochloropsis*, *Phaeodactylum, Tetraselmis* and *Isochrysis* spp. is reported in the literature, although values of single fatty acids may largely vary as their synthesis and accumulation depend highly on cultivation conditions and growth stage [11, 41–43, 46–50].

As primary producers of n-3 PUFA, essential nutrients for the growth and development of aquatic animals, as well as for humans at any life stages, the nutritional value of marine microalgae is mostly linked to their fatty acid profile [43].

n-3 LC-PUFA are important components of human cells, playing beneficial effects on human health, particularly for their protective effects towards cardiovascular and inflammatory diseases. In addition, n-3 fatty acids have positive effects on the nervous system and brain functions, neurodevelopment and prevention of neurodegenerative diseases [51, 52].

The n-3/n-6 ratio, an indicator of the nutritional value of food, desirable to be high in the human diet for the prevention of cardiovascular and chronic inflammatory diseases and to counter-balance the high intake of n-6 PUFA typical of modern Western diets, was high (2.43–5.01) in all microalgal species except *P. cruentum* (0.37). The PUFA/SFA ratio, a parameter inversely related to cardiovascular risk when considering human diet [53, 54], was favorably high for all microalgal species (1.64–2.19). The n-3/n-6 and PUFA/SFA ratio values found in the microalgae under study are in line with the literature, underlying the healthy properties of microalgal oils [16, 18, 41].

As the main source of LC-n-3 PUFA in the human diet is marine fatty fish, suffering from overfishing and catch

Table 3 Fatty acid profile of the five microalgal species objects of this study (% of total fatty acids)

	P. cruentum	*I. galbana*	*P. tricornutum*	*T. suecica*	*N. gaditana*
C14:0	0.32	16.08	6.65	3.05	5.45
C15:0	0.20	0.22	0.39	0.14	0.36
C16:0	34.11	11.65	14.45	18.29	21.65
C17:0	0.16	–	–	–	0.22
C18:0	0.87	0.63	0.75	1.55	0.37
C22:0	–	0.45	–	–	–
C24:0	–	–	2.38	–	–
C14:1 n-5	–	0.21	–	–	–
C16:1 n-7	2.63	5.56	18.65	6.30	22.25
C17:1 n-7	–	–	–	–	0.38
C18:1 n-9	2.32	16.52	1.42	19.61	4.01
C18:1 n-7	1.16	0.97	1.87	3.42	2.02
C20:1 n-9	–	–	–	1.17	–
C16:2 n-6	–	–	0.70	1.85	0.44
C16:2 n-4	–	–	4.00	–	0.31
C16:3 n-4	–	–	10.35	1.25	–
C16:4 n-1	–	–	0.62	–	–
C16:4 n-3	–	–	–	11.33	–
C18:2 n-6	10.54	10.45	2.80	10.71	2.92
C18:3 n-6	0.63	0.60	0.73	0.66	0.97
C18:3 n-3	–	12.18	0.75	15.70	0.23
C18:4 n-3	–	12.22	0.95	3.88	–
C20:2 n-6	1.43	0.32	–	–	–
C20:3 n-6	1.53	–	–	–	0.65
C20:4 n-6	29.12	–	2.27	1.06	5.84
C20:5 n-3	15.88	1.17	29.31	3.72	36.02
C22:5 n-3	–	1.74	–	–	–
C22:6 n-3	–	9.04	1.56	–	–
Σ SFA	35.66	29.02	24.62	23.02	28.05
Σ MUFA	6.10	23.26	21.94	30.49	28.66
Σ PUFA	59.14	47.72	54.03	50.15	47.38
Σ n-3	15.88	36.36	32.57	34.62	36.25
Σ n-6	43.26	11.36	6.50	14.28	10.82
n-3/n-6	0.37	3.20	5.01	2.43	3.35
PUFA/SFA	1.66	1.64	2.19	2.18	1.69

– not detected

decline, the exploitation of new sustainable sources of these essential nutrients is of great interest.

Dietary considerations

The data here reported suggest some dietary considerations. Microalgal biomass from this study compares favorably to traditional food commodities (eggs, milk, beans, fish, meat, and soybean) for nutrient content [55]. In particular, the concentrations of some mineral elements such as sodium, magnesium, manganese and iron were much higher in microalgae than in most common food items when expressed on a dry matter basis. However, considering that one possible use of microalgal biomass as a food ingredient is in pasta, bakery products or snacks, where the suggested levels of addition generally do not exceed 2–3 g per 100 g of product due to sensory acceptability limits [56–58], the high levels of some minerals here found may not represent a concern.

The amounts of nutrients supplied by 3 g of microalgal biomass are reported in Table 4. In nutritional terms, the most significant contribution of microalgae, when added to food products at a 3% level, is in mineral and PUFA. In fact, regardless of the species, the amounts of protein and lipid supplied by 3 g of dry microalgal biomass correspond to less than 2% the daily protein and lipid requirements of a 60-kg adult on a 2000 kcal diet [59]. On the contrary, the

Table 4 Amounts of selected nutrients supplied by 3 g (dry matter) of microalgal biomass from this study and corresponding percent of recommended dietary allowance (RDA) values

	P. cruentum		*I. galbana*		*P. tricornutum*		*T. suecica*		*N. galbana*	
	Content	% RDA	Content	% RDA	Content	% RDA	Content	% RDA	Content	% RDA
Protein (g)	0.59	1.09	0.87	1.61	0.81	1.50	0.78	1.45	1.00	1.84
Lipid (g)	0.17	0.25	0.93	1.39	0.38	0.57	0.44	0.66	0.84	1.25
EPA + DHA (mg)	24.3	9.72	85.8	34.3	106.2	42.5	14.7	5.88	271.2	108.5
Minerals										
Na (mg)	133.6	8.9	82.1	5.5	129.9	8.7	96.7	6.4	60.7	4.0
Ca (mg)	62.0	6.2	34.4	3.4	87.2	8.7	53.7	5.4	18.9	1.9
K (mg)	33.1	0.8	39.4	1.0	72.3	1.9	40.5	1.0	37.8	1.0
Mg (mg)	14.2	5.9	12.0	5.0	43.4	18.1	18.0	7.5	11.1	4.6
P (mg)	9.5	1.4	18.8	2.7	35.7	5.1	28.9	4.1	26.6	3.8
Fe (mg)	1.1	11.3	0.9	9.3	2.6	26.3	0.9	9.3	1.5	14.7
Zn (mg)	0.3	2.9	0.4	3.4	0.3	2.6	0.2	1.7	0.3	2.5
Mn (mg)	0.1	2.8	0.1	4.0	0.4	15.8	0.1	3.9	0.3	10.3
Cu (μg)	36	4.0	8.0	0.9	165	18.3	33	3.7	87	9.7

% of RDA values have been calculated with reference to a 60-kg adult on a 2000-kcal diet according to current indications [48]

same amount of microalga supplies, depending on the species, 9–26% of the recommended dietary allowance (RDA) for Fe, 5–18% of the RDA for Mg, 2–9% for Ca, 1–18% for Cu. The high Ca:P ratio found in most of the microalgae from this study (see Table 2) is a positive attribute suggesting their possible use as mineral supplements to counteract the Ca:P unbalance that characterizes Western diets and that may negatively affect bone health. As regards PUFA, taking into account the adequate intake of 250 mg per day of EPA + DHA recommended for primary cardiovascular prevention of healthy adults [59–61], 3 g dry biomass of *I. galbana*, *P. tricornutum* or *N. gaditana* supplies about 34%, 42% and 108% of the recommended EPA + DHA amounts, respectively. Therefore, food enrichment with microalgae, along with fish consumption or fish oil supplementation, would help to set the dietary n-3/n-6 PUFA ratio at the desirable levels and to counter-balance the high intake of n-6 PUFA typical of modern Western diets [62].

The dietary pattern changes occurred in the last century in Western industrialized countries have implied not only an increment of the total dietary fat content, but also a qualitative change of fat profile, with a shift towards saturated fat and n-6 PUFA, these latter mainly contributed by vegetable oils rich in linoleic acids, at the expense of n-3 PUFA. The n-6/n-3 PUFA ratio, estimated to be about 1 in the diet of our ancestors, has increased drastically in Western diets, up to a value of 10–20, with several public health implications since a balanced n-6/n-3 ratio is essential for the prevention and treatment of coronary artery disease, hypertension, diabetes, arthritis, osteoporosis, other inflammatory and autoimmune disorders, cancer and mental health [62]. To re-establish a healthy n-6/n-3 ratio, adequate dietary patterns should be promoted, with an increment the consumption of food rich in n-3 PUFA.

Reducing the total fat intake within the recommended range (20–35% of dietary energy) and substituting saturated and *trans*-fatty acids with mono- and polyunsaturated fatty acids, both n-6 and n-3, result in a favorable modulation of lipid and lipoprotein blood profile with consequent advantages on health and chronic disease risk reduction [63]. In practical terms, the desirable dietary lipid profile change may be actuated through the reduction of red meat, full-fat dairy products and industrially processed product consumption, while incrementing the consumption of lean and poultry meat, seafood products, especially fatty fish, nuts and seeds, rich in omega-3 fatty acids, and olive oil as a source of oleic acid [64]. Within this context, the integration of the diet with marine microalgal oil, rich in LC n-3 PUFA, or with functional products containing microalgae as an ingredient, may represent a valid alternative strategy to help re-equilibrate the dietary lipid profile and provide an additional source of the healthy n-3 fatty acids. Clinical trials of microalgal oil supplementation report beneficial changes of cardio-metabolic risk markers, comparable to those reported with fish oil consumption but with the advantage of lower gastrointestinal complaints at high-dose therapies and no adverse effects [51].

Furthermore, based on the results of our previous study [20], food enrichment with microalgae would provide it with significant amounts of carotenoids, mainly xanthophylls, thus incrementing the antioxidant potential and healthy value of the original food.

Nutrient bioaccessibility and bioavailability, as well as sensory properties and shelf life of food enriched with

microalgal biomass, represent relevant issues to be considered in the development of food products enriched with microalgae and deserving specific evaluations for each product and microalgal strain. Other bioactive compounds not covered by this study (vitamins, sterols and phenolic compounds) deserving an in-depth study would be also contributed by microalgae addition to food, thus positively affecting their healthiness and antioxidant potential.

Conclusions

The microalgal biomass of *P. cruentum, I. galbana*, *P. tricornutum*, *T. suecica* and *N. gaditana* studied was characterized by a good nutritional value and confirmed to be a potentially valuable ingredient for nutritional or nutraceutical purposes.

All species proved to be a good source of PUFA and minerals. *P. tricornutum*, *N. gaditana* and *I. galbana* were the species highest in nutritionally important n-3 LC-PUFA, with positive implications when included in a balanced diet.

The high nutrient density, healthy lipid profile and environmentally sustainable origin make these microalgae a potential food ingredient to be considered in substitution to or at integration of traditional ones and as a viable alternative to fish oil, with the advantage of reducing the dependency on traditional terrestrial and marine finite resources.

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References

1. Singh UB, Ahluwalia AS (2013) Microalgae: a promising tool for carbon sequestration. Mitig Adapt Strategy Glob Change 18:3–95
2. Draaisma RB, Wijffels RH, Slegers PM, Brentner LB, Roy A, Barbosa MJ (2013) Food commodities from microalgae. Curr Opin Biotechnol 24:169–177
3. Chew KW, Yap JY, Show PL, Suan NH, Juan JC, Ling TC, Lee DJ, Chang JS (2017) Microalgae biorefinery: high value products perspectives. Biores Technol 229:53–62
4. Chacón-Lee TL, González-Mariño GE (2010) Microalgae for "healthy" foods—possibilities and challenges. Comp Rev Food Sci Food Saf 9:655–675
5. Vaz BDS, Moreira JB, Morais MGD, Costa JAV (2016) Microalgae as a new source of bioactive compounds in food supplements. Curr Opin Food Sci 7:73–77
6. Villarruel-López A, Ascencio F, Nunõ K (2017) Microalgae, a potential natural functional food source—a review. Pol J Food Nutr Sci 67:251–263
7. Guedes AC, Malcata FX (2012) Nutritional value and uses of microalgae in aquaculture. In: Muchlisin ZA (ed) Aquaculture. IntechOpen. https://doi.org/10.5772/30576. Available from: https://www.intechopen.com/books/aquaculture/nutritional-value-and-uses-of-microalgae-in-aquaculture
8. Conceição LEC, Yúfera M, Makridis P, Morais S, Dinis MT (2010) Live feeds for early stages of fish rearing. Aquac Res 41:613–640
9. Camacho-Rodríguez J, González-Céspedes AM, Cerón-García MC, Fernández-Sevilla JM, Acién-Fernández FG, Molina-Grima EA (2014) A quantitative study of eicosapentaenoic acid (EPA) production by *Nannochloropsis gaditana* for aquaculture as a function of dilution rate, temperature and average irradiance. Appl Microbiol Biotechnol 98:2429–2440
10. Chauton MS, Reitan KI, Norsker NH, Tveterås R, Kleivdal HTA (2015) A techno-economic analysis of industrial production of marine microalgae as a source of EPA and DHA-rich raw material for aquafeed: research challenges and possibilities. Aquaculture 436:95–103
11. Ma XN, Chen TP, Yang B, Liu J, Chen F (2016) Lipid production from *Nannochloropsis*. Mar Drugs 14:61
12. Reyimu Z, Özçimen D (2017) Batch cultivation of marine microalgae *Nannochloropsis oculata* and *Tetraselmis suecica* in treated municipal wastewater toward bioethanol production. J Clean Prod 150:40–46
13. Sun Z, Wei H, Zhou ZG, Ashokkumar M, Liu J (2018) Screening of Isochrysis strains and utilization of a two-stage outdoor cultivation strategy for algal biomass and lipid production. Appl Biochem Biotechnol 185:1100–1117
14. Arad S, Levy-Ontman O (2010) Red microalgal cell-wall polysaccharides: biotechnological aspects. Curr Opin Biotechnol 21:358–364
15. Guihéneuf F, Stengel DB (2015) Towards the biorefinery concept: interaction of light, temperature and nitrogen for optimizing the co-production of high-value compounds in *Porphyridium purpureum*. Algal Res 10:152–163
16. Batista AP, Gouveia L, Bandarra NM, Franco JM, Raymundo A (2013) Comparison of microalgal biomass profiles as novel functional ingredient for food products. Algal Res 2:164–173
17. De Jesus Raposo MF, De Morais RMSC, De Morais AMMB (2013) Bioactivity and applications of sulphated polysaccharides from marine microalgae. Mar Drugs 11:233–252
18. Matos ÂP, Feller R, Moecke EHS, de Oliveira JV, Junior AF, Derner RB, Sant'Anna ES (2016) Chemical characterization of six microalgae with potential utility for food application. J AOCS 93:963–972
19. Bernaerts TMM, Gheysen L, Kyomugasho C, Jamsazzadeh Kermani Z, Vandionant S, Foubert I, Hendrickx ME, Van Loey AM (2018) Comparison of microalgal biomasses as functional food ingredients: focus on the composition of cell wall related polysaccharides. Algal Res 32:150–161
20. Di Lena G, Casini I, Lucarini M, Lombardi-Boccia G (2018) Carotenoid profiling of five microalgae species from large-scale production. Food Res Int. https://doi.org/10.1016/j.foodres.2018.11.043
21. Guillard RRL (1975) Culture of phytoplankton for feeding marine invertebrates. In: Smith WL, Chanley MH (eds) Culture of marine invertebrate animals. Plenum Press, New York, pp 26–60
22. AOAC (2012) Official methods of analysis, 19th edn. Association of Official Analytical Chemists, Arlington
23. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

24. Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917
25. Ma Y, Wang Z, Zhu M, Yu C, Cao Y, Zhang D, Zhou G (2013) Increased lipid productivity and TAG content in Nannochloropsis by heavy-ion irradiation mutagenesis. Biores Technol 136:360–367
26. Di Lena G, Casini I, Caproni R, Fusari A, Orban E (2017) Total mercury levels in commercial fish species from Italian fishery and aquaculture. Food Add Contam Part B Surveill 10:118–127
27. Metcalfe LD, Schmitz AA (1961) The rapid preparation of fatty acid esters for gas chromatographic analysis. Anal Chem 33:363–364
28. Exler J, Kinsella JE, Watt BK (1975) Lipids and fatty acids of important finfish. New data for nutrient tables. J AOCS 52:154–159
29. Barka A, Blecker C (2016) Microalgae as a potential source of single-cell proteins. A review. Biotechnol Agron Soc Environ 20:427–436
30. Lourenço SO, Barbarino E, Lavín PL, Lanfer Marquez UM, Aidar E (2004) Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. Eur J Phycol 39:17–32
31. Lourenço SO, Barbarino E, Lanfer Marquez UM, Aidar E (1998) Distribution of intracellular nitrogen in marine microalgae: basis for the calculation of specific nitrogen-to-protein conversion factors. J Phycol 34:798–811
32. Rebolloso Fuentes MM, Acién Fernández GG, Sánchez Pérez JA, Guil Guerrero JL (2000) Biomass nutrient profiles of the microalga *Porphyridium cruentum*. Food Chem 70:345–353
33. Ryckebosch E, Bruneel C, Termote-Verhalle R, Goiris K, Muylaert K, Foubert I (2014) Nutritional evaluation of microalgae oils rich in omega-3 long chain polyunsaturated fatty acids as an alternative for fish oil. Food Chem 160:393–400
34. Assunção MFG, Varejão JMTB, Santos LMA (2017) Nutritional characterization of the microalga *Ruttnera lamellosa* compared to *Porphyridium purpureum*. Algal Res 26:8–14
35. Tibbetts SM, Milley JE, Lall SP (2015) Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors. J Appl Phycol 27:1109–1119
36. Kermanshahi-pour A, Sommer TJ, Anastas PT, Zimmerman JB (2014) Enzymatic and acid hydrolysis of *Tetraselmis suecica* for polysaccharide characterization. Biores Technol 173:415–421
37. De Jesus Raposo MFJ, De Morais AMMB, De Morais RMSC (2015) Carotenoids from marine microalgae: a valuable natural source for the prevention of chronic diseases. Mar Drugs 13:5128–5155
38. Bernaerts TMM, Panozzo A, Doumen V, Foubert I, Gheysen L, Goiris K, Moldenaers P, Hendrickx ME, Van Loey AM (2017) Microalgal biomass as a (multi)functional ingredient in food products: rheological properties of microalgal suspensions as affected by mechanical and thermal processing. Algal Res 25:452–463
39. Scholz MJ, Weiss TL, Jinkerson RE, Jing J, Roth R, Goodenough U, Posewitz MC, Gerken HG (2014) Ultrastructure and composition of the *Nannochloropsis gaditana* cell wall. Eukar Cell 13:1450–1464
40. Le Costaouëc T, Unamunzaga C, Mantecon L, Helbert W (2017) New structural insights into the cell-wall polysaccharide of the diatom *Phaeodactylum tricornutum*. Algal Res 26:172–179
41. Bonfanti C, Cardoso C, Afonso C, Matos J, Garcia T, Tanni S, Bandarra NM (2018) Potential of microalga *Isochrysis galbana*: bioactivity and bioaccessibility. Algal Res 29:242–248
42. Hulatt CJ, Wijffels RH, Bolla S, Kiron V (2017) Production of fatty acids and protein by Nannochloropsis in flat-plate photobioreactors. PLoS One 12:e0170440
43. Adarme-Vega TC, Lim DKY, Timmins M, Vernen F, Li Y, Schenk PM (2012) Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb Cell Fact 11:96
44. [EU] European Union Commission 2008. Commission Regulation (EC) No. 629/2008 of 2 July 2008 amending Regulation (EC) No 1881/2006. Official Journal of the European Communities, 3/7/2008 L 173/6. Brussels, Belgium
45. [EFSA] European Food Safety Authority (2012) Scientific opinion on the risk for public health related to the presence of mercury and methylmercury in food. EFSA J 10:1–241
46. Rodolfi L, Biondi N, Guccione A, Bassi N, D'Ottavio M, Arganaraz G, Tredici MR (2017) Oil and eicosapentaenoic acid production by the diatom *Phaeodactylum tricornutum* cultivated outdoors in Green Wall Panel (GWP®) reactors. Biotechnol Bioeng 114:2204–2210
47. Hamilton ML, Haslam RP, Napier JA, Sayanova O (2014) Metabolic engineering of *Phaeodactylum tricornutum* for the enhanced accumulation of omega-3 long chain polyunsaturated fatty acids. Metab Eng 22:3–9
48. Martins DA, Custódio L, Barreira L, Pereira H, Ben-Hamadou R, Varela J, Abu-Salah KM (2013) Alternative sources of n-3 long-chain polyunsaturated fatty acids in marine microalgae. Mar Drugs 11:2259–2281
49. Hu H, Gao K (2006) Response of growth and fatty acid compositions of Nannochloropsis sp. to environmental factors under elevated CO_2 concentration. Biotechnol Lett 28:987–992
50. Pal D, Khozin-Goldberg I, Cohen Z, Boussiba S (2011) The effect of light, salinity, and nitrogen availability on lipid production by Nannochloropsis sp. Appl Microbiol Biotechnol 90:1429–1441
51. Tur JA, Bibiloni MM, Sureda A, Pons A (2012) Dietary sources of omega 3 fatty acids: public health risks and benefits. Br J Nutr 107(SUPPL. 2):S23–S52
52. Calder PC (2014) Very long chain omega-3 (n-3) fatty acids and human health. Eur J Lipid Sci Technol 116:1280–1300
53. Erkkila A, de Mello VD, Risérus U, Laaksonen DE (2008) Dietary fatty acids and cardiovascular disease: an epidemiological approach. Prog Lipid Res 47:172–187
54. Vannice G, Rasmussen H (2014) Position of the academy of nutrition and dietetics: dietary fatty acids for healthy adults. J Acad Nutr Diet 114:136–153
55. CREA. Italian food composition tables. Research Centre for Food and Nutrition, Rome, Italy. http://nut.entecra.it/646/tabelle_di_composizione_degli_alimenti.html
56. Gouveia L, Coutinho C, Mendonça E, Batista AP, Sousa I, Bandarra NM, Raymundo A (2008) Functional biscuits with PUFA-ω3 from Isochrysis galbana. J Sci Food Agric 88:891–896
57. Fradique M, Batista AP, Nunes MC, Gouveia L, Bandarra NM, Raymundo A (2013) *Isochrysis galbana* and *Diacronema vlkianum* biomass incorporation in pasta products as PUFA's source. LWT Food Sci Technol 50:312–319
58. Babuskin S, Krishnan KR, Babu PAS, Sivarajan M, Sukumar M (2014) Functional foods enriched with marine microalga *Nannochloropsis oculata* as a source of ω-3 fatty acids. Food Technol Biotechnol 52:292–299
59. SINU, Società Italiana di Nutrizione Umana. LARN, Livelli di assunzione di riferimento di nutrienti ed energia per la popolazione italiana. IV Revisione. Milano: SICS, 2016
60. Food and Agriculture Organization of the United Nations. Fats and fatty acids in human nutrition. Report of an expert consultation. FAO Food and Nutrition Paper 91. (Final report). 2010. Rome: FAO
61. European Food Safety Authority (2010) EFSA panel on dietetic products, nutrition, and allergies (NDA); scientific opinion on dietary reference values for fats, including saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, trans fatty acids, and cholesterol. EFSA J 8:107

62. Simopoulos AP (2003) Importance of the ratio omega-6/omega-3 essential fatty acids: evolutionary aspects. In: Simopoulos AP, Cleland LG (eds) Omega-6/omega-3 essential fatty acid ratio: the scientific evidence. World Review of Nutrition and Dietetics, vol 92. Karger, Basel, pp 1–22
63. Wang DD (2018) Dietary n-6 polyunsaturated fatty acids and cardiovascular disease: epidemiologic evidence. Prostaglandins Leukot Essent Fatty Acids 135:5–9
64. US Department of Health and Human Services and US Department of Agriculture (2015–2020) Dietary guidelines for Americans, 8th edn. http://health.gov/dietaryguidelines/2015/guidelines/