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ORIGINAL PAPER

Nutritional evaluation and human health-promoting potential of compounds biosynthesized by native microalgae from the Peruvian Amazon

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Abstract

A plausible strategy to mitigate socioeconomic problems in the Peruvian Amazon is through the sustainable exploitation of biodiversity resources, such as native microalgae. Several studies worldwide affirm that these microorganisms are excellent sources of higher value products for human nutrition and possess health-promoting biochemicals, but these attributes are unknown for the native microalgae of Peru. Therefore, the aim of this investigation was to evaluate the nutritional and human health-promoting potential of compounds biosynthesized by native microalgae from the Peruvian Amazon. Ten native microalgae strains of the groups cyanobacteria and chlorophyta were cultured in BG-11 medium and their biomass harvested and dried. Standardized methods were then used to determine proximate composition, fatty acids and amino acids composition, antioxidant activity, and total phenolic content. All ten microalgae strains produce primary nutrients, the entire spectrum of essential amino acids, essential fatty acids, and 3 of the 10 microalgae strains produced eisosapentaenoic acid. Additionally, all microalgae strains exhibited antioxidant activities and contained phenolic compounds. In conclusion, native microalgae strains from the Peruvian Amazon analyzed in this study possess the ability to biosynthesize and accumulate several nutrients and compounds with human health-promoting potential.

Keywords Antioxidant effects · Biochemistry · Essential amino acids · Essential fatty acids · Microalgae · Phenolic compounds

Introduction

Although rates of poverty and malnutrition in Peru have improved greatly over the last decade, both remain common and widespread even in areas of extreme biodiversity. In the Peruvian Amazon, poverty and extreme poverty levels were

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16.5% and 21.1%, respectively, at the end of 2018 (INEI 2019). Perhaps more surprising is the prevalence of anemia (43.6%), which can be explained, in part, by chronic malnutrition rates (22.00–25.99%) of children less than 5 years old (Hernández-Vásquez and Tapia-López 2017; MINSA 2017).

One possible strategy to mitigate Peru's socioeconomic issues is by the biotechnological exploitation of native microalgae from the Peruvian Amazon. There are several advantages of implementing this strategy in Peru. Microalgae are ubiquitous, species rich with approximately 124,000 microalgae species (Andersen 1992; Norton et al. 1996) and endemism is high (Norton et al. 1996). Consequently, as a group, microalgae possess an enormous amount of genomic information that could be used for the production of numerous biological compounds, the vast majority still unknown to science. Finally, microalgae are excellent sources of higher value products for human and animal nutrition and health (Kent et al. 2015; Navarro et al. 2016; Sathasivam et al. 2019; Barkia et al. 2019).

To date, however, little is known of the native microalgae from the Peruvian Amazon. Consequently, it is imperative to conduct bioprospecting studies to establish culture collections of native microalgae in order to explore their multi-use potential (e.g., aquaculture industry, cosmetic industry, food industry, pharmaceutic industry, renewable energy industry, etc.). Our research team has initiated this type of research and established a small collection of native microalgae (~ 100 microalgae strains) that has until recently focused on characterizing these microalgae strains by their potential for biodiesel production (Cobos et al. 2017b). The goal of this investigation is to evaluate the nutritional and human health-promoting potential of compounds biosynthesized by native microalgae from the Peruvian Amazon.

Materials and methods

Microalgae culture and harvest

Ten microalgae strains with the highest specific growth rates were selected (Fig. S1, Table S1) from the Microalgae Culture Collection of the Universidad Científica del Perú. Microalgae cells were inoculated (OD_{680} 0.05) in twenty-five milliliters of BG-11 medium (Stanier et al. 1971) in a 100 mL Erlenmeyer flask and incubated in a controlled culture room under the following conditions: temperature at 25 ± 1 °C, 12/12 h light/dark photoperiod, light intensity at 100 µmol photons m^{-2} s^{-1} supplied by cool-white fluorescent lamps, and continuous agitation at 150 rpm. Based on the microalgae growth, the culture volume was gradually increased until obtaining 5 L (≈ 8 weeks of culture). Cultures were transferred to 50 mL conical-bottom centrifuge tubes and harvested by centrifugation at 2000×*g* for 10 min at 4 °C. Microalgae biomass was rinsed two times with 45 mL of sterilized ultrapure water, centrifuged and the supernatants discarded.

Proximate composition analysis

Fresh microalgae biomass was dried to a constant weight in an oven at 70 °C and dry weight determined gravimetrically using an analytical balance Kern ABJ 220-4NM (Kern & Sohn GmbH, Balingen, Germany). Total lipids from 50 mg of microalgae dry biomass was measured gravimetrically according to the Bligh and Dyer method (1959) using a semi-micro analytical balance (Sartorius, MSU225S-000-DU, Foster City, CA, USA). Carbohydrates (i.e., monosaccharides, oligosaccharides, polysaccharides, and their derivatives) were assayed from 5 mg of microalgae dry biomass following the colorimetric method of DuBois et al. (1956), and protein content was measured from 5 mg of microalgae dry biomass following Hartree (1972). Ash content was measured gravimetrically following the AOAC method (AOAC International 1990) where 100 mg of microalgae dry biomass was incinerated in a Thermolyne™ F6010 muffle furnace (Thermo Fisher Scientific, Walthman, MA, USA) at 550 °C for 16 h. All described composition analyses were carried out in triplicate, and data are expressed as the mean ± SD.

Fatty acid analysis

Fatty acid methyl esters (FAMEs) were first obtained by acid transesterification following a protocol of acid-catalysis methanolysis/methylation (Ichihara and Fukubayashi 2010) and dissolved in 10 µL acetonitrile and resolved by using a gas chromatograph Varian CP-3800 GC (Agilent Technologies, Santa Clara, CA, USA) assembled with an automatic sampler and injector, a flame ionization detector and a 30 m × 0.32 mm × 0.25 µm Stabilwax® capillary column (Restek, Bellefonte, PA, USA). The chromatograph was programmed to operate under the following conditions: injector temperature 250 °C; column temperature gradient 120 °C × 1 min, followed by an increase to 160 °C (rate 30 °C/min), 160 °C for 1 min, followed by an increase to 240 °C (rate 4 °C/min) and 240 °C for 7 min; detector temperature 260 °C. Gas pressures were: synthetic air 60 psi, He 40 psi, and H_2 80 psi. The carrier gas (He) flow rate was constant (1 mL/min). The identification of FAMEs was determined by matching their peak retention times with a known standard mixture (Nu-Check Prep, Elysian, MN, USA). To each sample tricosanoic acid-methyl ester (Sigma-Aldrich, Saint Louis, MO, USA) was added as the internal standard. Chromatograms were analyzed employing the Galaxie™ Chromatography Data System Version 1.9.3.2 (Agilent Technologies, Santa Clara, CA, USA).

Amino acid analysis

For amino acid profiling, 30 mg of microalgae dry biomass was mixed with 2 mL of 6 M hydrochloric acid and incubated in a dry bath at 112 °C for 22 h for acid hydrolysis reaction (Hirs et al. 1954). Further, 50 µL of the hydrolyzed sample was transferred in a test tube where 100 µL of 2.5 mM L-aminobutyric acid (internal standard) and 5 mL of ultrapure water were added. The solution was filtered on a 0.45 µm pore size syringe filter PTFE membrane and 10 µL aliquots were chemically modified using the reactive amine derivatizing reagent 6-aminoquinolyl-N-hydroxysuccinimidyl carbamate (Cohen and Michaud 1993) following instructions of the AccQ-Fluor Reagent Kit (Waters Corporation, Milford, MA, USA). Moreover, 5 µL of the derivatized amino acids solution was resolved using a Hitachi Elite LaChrom HPLC System (Hitachi High Technologies, San Jose, CA, USA) equipped with an L-2200 autosampler, L-2130 HTA pump, L-2350 column oven, L-2455 diode array detector, L-2485 fluorescence detector, and a 150 mm × 4.6 mm × 5 µm Thermo Scientific™ Hypersil GOLD™ C18 Selectivity HPLC Column (Thermo Fisher Scientific, Walthman, MA, USA). The HPLC system was programmed to operate under the following conditions: column temperature: 37 °C, excitation of fluorescent amino acid derivatives: 250 nm, detection of fluorescent emission: 395 nm, flow rate: 1 mL/min. A binary mobile phase consisting of (A) 140 mM sodium acetate pH 5.1 and (B) acetonitrile were filtered using a vacuum filtration system through 0.45 µm membrane filters. The following gradient elution was employed: 0–2 min: 100% A; 2–24 min: 100–83.5% A, 0–16.5% B; 24 –30 min: 83.5–75% A, 16.5–25% B; 30.1–38 min (column equilibration): 100% A. The EZChrom Elite software v 3.2.1 (Agilent Technologies, Santa Clara, CA, USA) was used for data acquisition and analysis.

Essential amino acid index (EAAI)

EAAI scores predict the biological value of proteins in the sample. This nutrient quality parameter is defined, according to Oser (1959), as the geometric mean of the egg ratios, and was calculated using a FAO/WHO established human reference pattern (FAO/WHO 1991):

$$EAAI = \sqrt[n]{\frac{eaa_1}{eaa_{S1}} \times \frac{eaa_2}{eaa_{S2}} \times \ldots \frac{eaa_n}{eaa_{Sn}}}$$

Where eaa_n expresses the percentage of a type of essential amino acid in the total essential amino acids in the sample; eaa_{Sn} represents the percentage of a type of essential amino acid in the total essential amino acids in the standard (i.e., the standard protein of whole egg). In addition, the biological value of the proteins contained in all ten microalgae biomass was calculated using the equation developed by Oser (1959):

$$Biological\ value = 1.09(EAAI) - 11.7$$

Antioxidant activity and total phenolic content analysis

Two hundred milligrams of microalgae dry biomass were ground using a mortar and pestle containing 2 mL of hydromethanolic solvent (methanol/water 80:20 (v/v) mixture). Homogenized biomass was transferred into a 15 mL capped conical centrifuge tube with 2 mL of hydromethanolic solvent, vortexed (VM-10, Daihan Scientific, DKI Jakarta, Indonesia) for 15 min at 25 °C, centrifuged (5000×*g*, 15 min, 5 °C) in a refrigerated centrifuge LMC-4200R (Biosan, Riga, Latvia), and the supernatant recovered. The extraction was repeated with 2 mL of hydromethanolic solvent. Finally, supernatants were transferred to a 5 mL volumetric flask and brought to a final volume of 5 ml with methanol. Hydromethanolic extracts were stored at − 20 °C and analyzed within 24 h.

Antioxidant activity analysis was determined following a modified method of Sharma and Bhat (2009), which is based on the ability of hydromethanolic extracts to scavenge 2,2-Diphenyl-1-picrylhydrazyl hydrate (DPPH). Briefly, 0.1 mL of hydromethanolic extract was mixed with 3.9 mL of methanolic solution of DPPH radical, incubated in darkness for 30 min at 25 °C, and absorbances measured at 517 nm using an Agilent Cary 60 UV–Vis spectrophotometer (Agilent Technologies, Santa Clara, USA). DPPH radical scavenging ability was quantified using a standard curve (from 0.1 to 10 µM) prepared from 6-hydroxy-2,5,7,8-tetramethyl-3,4-dihydrochromene-2-carboxylic acid (Trolox) (Sigma-Aldrich, Germany). Results of antioxidant activity were expressed as trolox equivalents (µM TE g^{-1} of microalgae biomass dry weight [mbdw]). Antioxidant activity analysis was carried out in triplicate.

Total phenolic content was estimated by the Folin–Ciocalteu method (Velioglu et al. 1998). One hundred microliters of hydromethanolic extract was mixed with 750 µL of 1:10 diluted Folin–Ciocalteu reagent (Sigma-Aldrich, Germany) and incubated for 5 min at 25 °C. Next, 750 µL of saturated sodium carbonate solution (566 mM) was added, incubated for 90 min at 25 °C, and the absorbance measured at 725 nm. Total phenolic content was determined based on the standard curve (from 10 to 100 µM) prepared from 3,4,5-trihydroxybenzoic acid (Gallic acid) (Sigma-Aldrich, Germany). Results of total phenolic content were expressed as gallic acid equivalents (mg GAE g^{-1} of mbdw). Total phenolic content analysis was carried out in triplicate.

Statistical analysis

The homogeneity of variance and normality of the data were assessed both by the Kolmogorov–Smirnov test and Shapiro–Wilk test. Percentage data were arcsine-square root transformed to meet one-way analysis of variance (ANOVA) assumptions. The HSD Tukey post hoc test was utilized to determine if groups were significantly different from each other. Parametric and non-parametric correlation analysis were realized using the Pearson correlation and the Spearman rank correlation measures, respectively. Differences were considered significant at $P < 0.05$. All data presented here are given as the mean ± SD. Statistical analysis were performed using R software v 4.0.0. (Development Core Team R 2020).

Results

Proximate composition

The biochemical composition among the analyzed microalgae strains was significantly different (Fig. 1). Total lipids content ranged from 5.06% to 23.82% ($F > 955$; $P < 0.05$) and *Scenedesmus* sp.2 (> 23%), *Ankistrodesmus* sp. (> 17%), and *Chlorella* sp.1 (> 16%) had the highest percentage values. Carbohydrate content ranged from 9.67% to 34.08% ($F > 1371$; $P < 0.05$) with *Scenedesmus* sp.2 (> 37%), *Chlorella* sp.2 (> 34%), and *Haematococcus* sp.1 (> 24%)

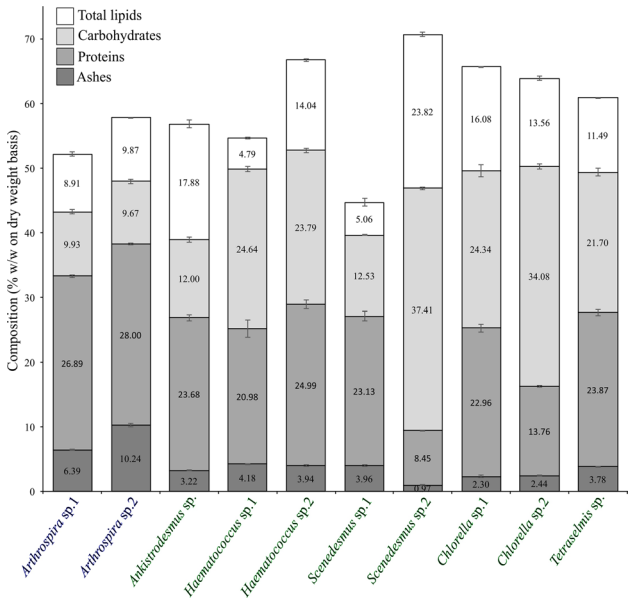

Fig. 1 Proximate composition (total lipids, carbohydrates, proteins, and ashes content) of the dry biomass of ten native microalgae strains of the groups cyanobacteria (blue) and chlorophyta (green) from the Peruvian Amazon

containing the largest values. In terms of proteins content, values ranged from 8.45% to 28.00% ($F > 290$; $P < 0.05$) and *Arthrospira* sp.2 (28%), *Arthrospira* sp.1 (> 26%), and *Haematococcus* sp.2 (~ 25%) contained the highest values. Finally, ash content ranged from 0.97% to 10.24% ($F > 1174$; $P < 0.01$) with *Arthrospira* sp.2 (> 10%), *Arthrospira* sp.1 (> 6%), and *Haematococcus* sp.1 (> 4%) were positioned at the top.

Fatty acid composition

The microalgae strains contained in their biochemical composition the three main classes of fatty acids (i.e., saturated fatty acids [SFA], mono unsaturated fatty acids [MUFAs], and poly unsaturated fatty acids [PUFAs]), but with different fatty acid profiles (Table 1). The three microalgae species with the highest fatty acid content were *Scenedesmus* sp.2 (~ 200 mg g^{-1} of mbdw), *Chlorella* sp.1 (~ 85 mg g^{-1} of mbdw), and *Ankistrodesmus* sp. ~ (81 mg g^{-1} of mbdw). The hydrocarbon chain length of fatty acids ranged from 14 to 20 carbon atoms, in addition, there were differences in the degree, type, and position of unsaturation. Also, the content of identified fatty acids in the microalgae strains were significant different (F value from 153.46 to 29,492.71; $P < 0.05$). For example, linoleic acid (C18:2 n-6) was biosynthesized in large quantities by *Haematococcus* sp.2 (20.19 ± 0.39 mg g^{-1} of mbdw), *Chlorella* sp.1 (18.56 ± 0.14 mg g^{-1} of mbdw), and *Chlorella* sp.2 (16.46 ± 0.28 mg g^{-1} of mbdw). However, α-linoleic acid (C18:3 n3) content was highest in *Scenedesmus* sp.2 (23.13 ± 0.19 mg g^{-1} of mbdw), *Ankistrodesmus* sp. (14.45 ± 0.22 mg g^{-1} of mbdw), and *Scenedesmus* sp.1 (13.02 ± 0.13 mg g^{-1} of mbdw). Eicosapentaenoic acid (EPA, C20:5 n-3) was the only omega-3 very long-chain polyunsaturated fatty acid identified in *Arthrospira* sp.1 (0.26 ± 0.05 mg g^{-1} of mbdw), *Haematococcus* sp.1 (0.21 ± 0.01 mg g^{-1} of mbdw), and *Haematococcus* sp.2 (0.17 ± 0.00 mg g^{-1} of mbdw).

Additionally, the microalgae strains displayed differences in fatty acid parameters, namely ΣSFA, ΣMUFA, ΣPUFA, and Ω-6/Ω-3 ratios. In terms of ΣSFA, *Arthrospira* sp.2 showed the highest value (~ 40%), whereas *Scenedesmus* sp.1 displayed the lowest value (~ 13%). With regard to ΣMUFA, *Arthrospira* sp.2 showed the highest percentage (~ 57%), but *Scenedesmus* sp.1 presented the minimum percentage (~ 14%). With reference to ΣPUFA, *Scenedesmus* sp.1 displayed the greatest percentage (67.01%), contrary to *Arthrospira* sp.2 reported the smallest percentage (1.01%). Additionally, there was a significant difference between pairs of fatty acid parameters (i.e., ΣSFA vs ΣMUFA, ΣSFA vs ΣPUFA), but only the pair ΣSFA vs ΣMUFA showed a statistically significant correlation ($R = 0.99$; $P < 0.01$). Finally, with respect to Ω-6/Ω-3 ratios, the microalgae species can be clustered into three categories: cluster 1 with Ω-6/Ω-3

Table 1 Fatty acids composition of ten native microalgae species from the Peruvian Amazon (mg g^{-1} of mbdw)

Fatty acid	Cyanobacteria		Chlorophyta							
	Arthrospira sp.1	*Arthrospira* sp.2	*Ankistrodesmus* sp.	*Haematococcus* sp.1	*Haematococcus* sp.2	*Scenedesmus* sp.1	*Scenedesmus* sp.2	*Chlorella* sp.1	*Chlorella* sp.2	*Tetraselmis* sp.
Saturated fatty acids (SFA)										
C14:0 (myristic acid)	0.21 ± 0.02	6.75 ± 0.04	0.25 ± 0.02	0.61 ± 0.02	0.25 ± 0.02	0.05 ± 0.00	0.46 ± 0.01	0.24 ± 0.03	0.75 ± 0.01	0.16 ± 0.00
C16:0 (palmitic acid)	9.98 ± 0.20	9.98 ± 0.06	14.04 ± 0.08	5.73 ± 0.06	15.33 ± 0.35	3.67 ± 0.01	47.92 ± 0.74	15.41 ± 0.16	14.92 ± 0.12	9.57 ± 0.20
C18:0 (stearic acid)	0.51 ± 0.02	0.42 ± 0.00	0.59 ± 0.02	0.34 ± 0.01	1.37 ± 0.17	0.15 ± 0.00	5.88 ± 0.02	0.88 ± 0.00	0.96 ± 0.01	1.29 ± 0.02
Mono unsaturated fatty acids (MUFA)										
C16:1 n−7 (palmitoleic acid)	1.71 ± 0.10	20.26 ± 0.01	1.75 ± 0.14	2.99 ± 0.06	0.69 ± 0.02	0.18 ± 0.01	–	0.50 ± 0.01	0.46 ± 0.02	2.34 ± 0.07
C18:1 n−7 (vaccenic acid)	3.44 ± 0.03	3.49 ± 0.00	1.66 ± 0.11	2.00 ± 0.02	2.83 ± 0.11	1.08 ± 0.09	1.55 ± 0.03	1.74 ± 0.01	2.42 ± 0.06	1.54 ± 0.04
C18:1 n−9 (elaidic ú oleic acid)	1.38 ± 0.13	0.39 ± 0.01	15.19 ± 0.40	2.37 ± 0.06	11.19 ± 0.26	3.06 ± 0.08	91.22 ± 1.30	17.48 ± 0.01	16.33 ± 0.25	7.63 ± 0.09
Poly unsaturated fatty acids (PUFA)										
C16:4 n−3 (hexadecatetraenoic Acid)	–	–	4.09 ± 0.11	0.69 ± 0.07	1.20 ± 0.07	3.37 ± 0.07	5.64 ± 0.04	6.41 ± 0.03	4.74 ± 0.09	–
C18:2 n−6 (linoleic acid)	3.92 ± 0.09	0.22 ± 0.00	13.93 ± 0.28	1.74 ± 0.05	20.19 ± 0.39	3.05 ± 0.08	10.32 ± 0.10	18.56 ± 0.14	16.46 ± 0.28	15.82 ± 0.28
C18:3 n-3 (α-linoleic acid)	0.19 ± 0.01	0.21 ± 0.01	14.45 ± 0.22	2.38 ± 0.08	11.92 ± 0.40	13.02 ± 0.13	23.13 ± 0.19	11.23 ± 0.29	8.40 ± 0.20	5.40 ± 0.09
C18:3 n−6 (γ-linoleic acid)	4.29 ± 0.18	–	0.57 ± 0.01	0.16 ± 0.00	0.30 ± 0.02	0.19 ± 0.01	1.37 ± 0.03	1.21 ± 0.03	1.15 ± 0.02	–
C18:4 n−3 (stearidonic acid)	0.10 ± 0.00	–	4.14 ± 0.18	0.42 ± 0.05	0.61 ± 0.02	0.95 ± 0.01	7.61 ± 0.02	2.12 ± 0.03	2.10 ± 0.04	–
C20:4 n−3 (eicosatetraenoic acid)	–	–	–	0.02 ± 0.00	–	–	–	–	–	–

Table 1 (continued)

Fatty acid	Cyanobacteria		Chlorophyta							
	Arthrospira sp.1	*Arthrospira* sp.2	*Ankistrodesmus* sp.	*Haematococcus* sp.1	*Haematococcus* sp.2	*Scenedesmus* sp.1	*Scenedesmus* sp.2	*Chlorella* sp.1	*Chlorella* sp.2	*Tetraselmis* sp.
C20:4 n−6 (arachidonic acid)	0.05 ± 0.01	–	–	0.23 ± 0.01	0.32 ± 0.02	–	–	–	0.24 ± 0.01	–
C20:5 n−3 (eicosapentaenoic acid, EPA)	0.26 ± 0.05	–	–	0.21 ± 0.01	0.17 ± 0.00	–	–	–	–	–
Unknown	1.96 ± 0.01	1.01 ± 0.02	10.48 ± 0.17	1.26 ± 0.01	14.02 ± 0.05	1.93 ± 0.02	3.91 ± 0.01	9.46 ± 0.01	6.77 ± 0.02	7.76 ± 0.03
ΣSFA	10.71 (38.23)	17.14 (40.13)	14.88 (18.34)	6.68 (31.57)	16.95 (21.09)	3.87 (12.61)	54.26 (27.27)	16.53 (19.40)	16.63 (21.97)	11.01 (21.38)
ΣMUFA	6.53 (23.34)	24.14 (56.50)	18.60 (22.92)	7.36 (34.80)	14.71 (18.30)	4.33 (14.09)	92.77 (46.62)	19.71 (23.13)	19.20 (25.37)	11.51 (22.35)
ΣPUFA	8.80 (31.43)	0.43 (1.01)	37.16 (45.81)	5.85 (27.68)	34.70 (43.17)	20.58 (67.01)	48.07 (24.15)	39.53 (46.38)	33.10 (43.73)	21.22 (41.20)
Ω-6/Ω-3	15.11	1.06	0.64	0.57	1.50	0.19	0.32	1.00	1.17	2.93
Total fatty acids	28.00	42.72	81.13	21.15	80.37	30.72	199.01	85.23	75.70	51.51

(–) not detected. The concentration of fatty acids was express in mg g^{-1} of microalgae biomass dry weight (mbdw), with percentage (%) of the total fatty acids in parentheses, and each value represents the mean ± SD of three experiments. ∑SFA, is the sum of the contents of saturated fatty acids, ∑MUFA, is the sum of the contents of mono-unsaturated fatty acids, ∑PUFA, is the sum of the contents of poly unsaturated fatty acids

ratio values < 0.25 includes only *Scenedesmus* sp.1; cluster 2 with Ω-6/Ω-3 ratio values from ≥ 0.25 to ≤ 1.00 includes six microalgae species (*Scenedesmus* sp.2, *Haematococcus* sp.1, *Ankistrodesmus* sp., *Chlorella* sp.1, *Arthrospira* sp.2, and *Chlorella* sp.2); and cluster 3 with Ω-6/Ω-3 ratio values > 1.00 includes *Haematococcus* sp.2, *Tetraselmis* sp., and *Arthrospira* sp.1.

Amino acid composition

Microalgae strains exhibited both essential amino acids (EAA) and non-essential amino acids (NEAA) and their amino acid composition profiles were relatively similar (Table 2), but the quantity of single amino acids were significantly different (F value from 391.34 to 3162.89; $P < 0.01$). The largest amino acids quantities were recorded in *Arthrospira* sp.2 (~ 487 mg g^{-1} of mbdw), *Arthrospira* sp.1 (~ 468 mg g^{-1} of mbdw), and *Scenedesmus* sp.1 (~ 433 mg g^{-1} of mbdw). Also, ΣEAA ranged from 44.73% (*Scenedesmus* sp.2) to 46.77% (*Arthrospira* sp.1), and ΣNEAA from 53.13% (*Haematococcus* sp.2) to 55.27% (*Scenedesmus* sp.2). From the amino acid profiles of the EAA group, the most abundant amino acids were leucine and the aromatic amino acid pair (phenylalanine + tyrosine), whereas the most limiting EAA were methionine + cysteine and histidine. From the amino acid profiles of the NEAA group, the most abundant amino acids were Asx (aspartic acid + asparagine), serine, and alanine, and the scarcest was the imino acid proline. Finally, EAAI scores ranged from 0.81 (*Chlorella* sp.1 and *Chlorella* sp.2) to 0.87 (*Arthrospira* sp.1 and *Haematococcus* sp.2), and the biological value ranged from 76.26 (*Chlorella* sp.1 and *Chlorella* sp.2) to 83.24 (*Arthrospira* sp.1 and *Haematococcus* sp.2).

Antioxidant activity and total phenolic content

The hydromethanolic extracts of the microalgae strains demonstrated DPPH radical scavenging activity (Fig. 2), with significant differences among microalgae strains ($F = 3357.97$; $P < 0.05$). The highest value of antioxidant activity was recorded in *Arthrospira* sp.2 (> 7 µM TE g^{-1} of mbdw), in contrast, the lowest value was reported in *Scenedesmus* sp.2 (0.34 µM TE g^{-1} of mbdw).

The hydromethanolic extracts of all ten microalgae strains contained phenolic compounds (Fig. 3), but values were variable and significantly different ($F = 528.47$; $P < 0.01$) ($F = 528.47$; $P < 0.01$). The highest total phenolic content was recorded in *Scenedesmus* sp.2 (~ 42 mg GAE g^{-1} of mbdw), *Chlorella* sp.1 (~ 38 mg GAE g^{-1} of mbdw), and *Scenedesmus* sp.1 (~ 30 mg GAE g^{-1} of mbdw). The lowest values were registered in *Arthrospira* sp.1 (~ 15 mg GAE g^{-1} of mbdw), *Haematococcus* sp.1 (~ 13 mg GAE g^{-1} of mbdw), and *Arthrospira* sp.2 (~ 3 mg GAE g^{-1} of mbdw). Additionally, there was no statistically significant correlation between antioxidant activity and total phenolic content of the hydromethanolic extracts (Fig. S2).

Discussion

Proximate composition

The main biochemical components (i.e., total lipids, carbohydrates, proteins, and ash) quantified in all ten microalgae strains revealed interstrain differences and similarities (Fig. 1). These types of findings are very common as evidenced by several reports worldwide (Natrah et al. 2007; Kent et al. 2015; Tibbetts et al. 2015; Molino et al. 2018). Additionally, some studies showed that the proximate composition of microalgae can fluctuate according to the growth phase (i.e., exponential, stationary) (Romero–Romero and Sánchez-Saavedra 2017), culture conditions (Cobos et al. 2017a), nitrogen starvation (Buono et al. 2016), light quality (Romero–Romero and Sánchez-Saavedra 2017), and culture type (i.e., phototrophic, mixotrophic) (Alkhamis and Qin 2016).

Based on the proximate composition these microalgae strains can be used for the extraction of various commodities with biorefinery approaches. For instance, some oleaginous microalgae (containing > 15% total lipids) such as *Scenedesmus* sp.2, *Ankistrodesmus* sp.1, and *Chlorella* sp.1 can be cultivated under conditions to improve lipid biosynthesis and accumulation, specifically triacylglycerols, and then evaluated for biodiesel production using well-established approaches (Arguelles et al. 2018). Simultaneously, essential fatty acids enriched microalgae biomass can be used as food supplements for both humans and animals (e.g., aquaculture, poultry farming, etc.). In addition, *Scenedesmus* sp.2 and *Chlorella* sp.2 were carbohydrate hyperaccumulators (Fig. 1) and thus could be used for bioethanol production, as has been demonstrated by other investigations (Lakatos et al. 2019; Hossain et al. 2019; Abdulla et al. 2020). Finally, in the majority of microalgae (80%) the protein content was > 20% of dry biomass (Fig. 1). This is important, because protein enriched microalgae biomass can also be used in dietary supplements for human and animal nutrition (Benemann 1992; Ginzberg et al. 2000; Görs et al. 2010; Jiang et al. 2019).

Fatty acid composition

All microalgae strains analyzed contained the three fatty acid classes (i.e., SFA, MUFAs, and PUFAs), but the proportion and absolute quantities of each type were highly variable, creating distinct fatty acid profiles (Table 1). These differences were present despite the fact all ten microalgae strains

Table 2 Amino acids composition, essential amino acid index (EAAI), and biological value of ten native microalgae proteins from the Peruvian Amazon (mg g^{-1} of mbdw)

Amino acid	Cyanobacteria		Chlorophyta							
	Arthrospira sp.1	*Arthrospira* sp.2	*Ankistrodesmus* sp.	*Haematococcus* sp.1	*Haematococcus* sp.2	*Scenedesmus* sp.1	*Scenedesmus* sp.2	*Chlorella* sp.1	*Chlorella* sp.2	*Tetraselmis* sp.
Essential amino acids (EAA)										
Valine	35.33 ± 0.69	32.05 ± 0.15	24.49 ± 0.24	23.63 ± 0.36	21.20 ± 0.09	26.50 ± 0.42	8.00 ± 0.04	20.84 ± 0.48	13.07 ± 0.25	23.12 ± 0.59
Threonine	22.97 ± 0.72	24.54 ± 0.01	21.12 ± 0.52	19.95 ± 0.42	17.38 ± 0.18	22.92 ± 0.21	6.95 ± 0.03	17.89 ± 0.20	11.60 ± 0.22	20.29 ± 0.53
Leucine	47.77 ± 0.54	47.39 ± 0.42	39.90 ± 0.29	37.62 ± 0.64	34.19 ± 0.02	40.99 ± 0.60	12.47 ± 0.08	33.34 ± 0.54	20.08 ± 0.41	35.25 ± 0.81
Isoleucine	29.65 ± 0.46	31.06 ± 0.01	18.45 ± 0.28	17.75 ± 0.66	15.53 ± 0.17	19.83 ± 0.62	6.03 ± 0.07	16.00 ± 0.15	9.41 ± 0.17	18.89 ± 0.37
Metionine + cysteine	9.20 ± 0.27	7.36 ± 0.51	7.40 ± 0.08	6.01 ± 0.04	6.01 ± 0.02	7.79 ± 0.21	1.73 ± 0.01	4.41 ± 0.16	1.79 ± 0.06	6.76 ± 0.14
Lysine	20.50 ± 0.70	25.58 ± 0.11	24.53 ± 0.50	17.93 ± 0.50	29.88 ± 0.77	27.54 ± 0.39	9.54 ± 0.05	17.91 ± 0.39	11.87 ± 0.39	32.98 ± 0.74
Histidine	9.46 ± 0.31	8.24 ± 0.01	7.24 ± 0.12	6.34 ± 0.02	6.99 ± 0.20	8.66 ± 0.06	2.64 ± 0.06	6.28 ± 0.35	4.10 ± 0.08	7.05 ± 0.20
Phenilalanine + Tyrosine	44.03 ± 0.65	41.96 ± 0.25	35.20 ± 0.44	33.73 ± 0.21	30.89 ± 0.09	39.85 ± 0.56	10.70 ± 0.09	32.76 ± 0.25	17.61 ± 0.43	34.18 ± 0.49
ΣEAA (%)	218.91 (46.77)	218.18 (44.83)	178.33 (45.69)	162.95 (45.47)	162.08 (46.87)	194.09 (44.84)	58.06 (44.73)	149.43 (45.30)	89.54 (45.62)	178.52 (46.20)
Non-essential amino acids (NEAA)										
Glycine	29.34 ± 0.69	28.41 ± 0.03	27.24 ± 0.51	24.64 ± 0.49	22.34 ± 0.01	33.18 ± 0.36	9.99 ± 0.05	25.34 ± 0.69	15.04 ± 0.43	22.86 ± 0.65
Alanine	42.06 ± 0.55	40.02 ± 0.07	38.25 ± 0.72	33.83 ± 0.28	31.43 ± 0.03	35.07 ± 0.51	10.77 ± 0.07	28.75 ± 0.97	17.54 ± 0.41	32.46 ± 0.67
Serine	49.88 ± 0.64	47.66 ± 0.30	34.55 ± 0.52	34.30 ± 0.38	31.78 ± 0.06	40.17 ± 0.25	13.05 ± 0.20	26.17 ± 0.02	16.84 ± 0.37	37.65 ± 0.45
Proline	18.67 ± 0.41	18.85 ± 0.08	20.97 ± 0.65	18.40 ± 0.07	18.66 ± 0.11	22.79 ± 0.30	7.33 ± 0.07	18.33 ± 0.47	11.18 ± 0.34	18.42 ± 0.58
Arginine	30.66 ± 0.82	38.46 ± 0.41	26.85 ± 0.33	21.99 ± 0.38	23.33 ± 0.09	25.43 ± 0.27	6.56 ± 0.14	19.97 ± 0.07	11.92 ± 0.40	27.48 ± 0.63
Aspartic acid (Asx)	46.24 ± 0.72	61.63 ± 0.13	38.21 ± 0.74	36.86 ± 0.43	34.16 ± 0.18	53.23 ± 0.32	15.20 ± 0.01	33.07 ± 0.61	20.94 ± 0.52	43.05 ± 0.73
Glutamic acid (Glx)	32.35 ± 0.53	33.47 ± 0.09	25.94 ± 0.59	25.42 ± 0.57	22.02 ± 0.17	28.90 ± 0.31	8.84 ± 0.02	28.82 ± 0.59	13.29 ± 0.37	26.02 ± 0.51
ΣNEAA (%)	249.20 (53.23)	268.51 (55.17)	212.01 (54.31)	195.43 (54.53)	183.73 (53.13)	238.76 (55.16)	71.74 (55.27)	180.44 (54.70)	106.74 (54.38)	207.93 (53.80)
Total AA	468.11	486.69	390.34	358.38	345.80	432.84	129.80	329.87	196.28	386.46
EAAI	0.87	0.83	0.86	0.83	0.87	0.84	0.83	0.81	0.81	0.86
Biological value	83.24	78.55	81.71	78.55	83.24	79.97	78.99	76.26	76.26	82.04

The concentration of amino acids was express in mg g^{-1} of microalgae biomass dry weight (mbdw), with percentage (%) of the amino acids in parentheses, and each value represents the mean ± SD of three experiments. ΣEAA, is the sum of the essential amino acids, ΣNEAA, is the sum of the non-essential amino acids, EAAI, is the essential amino acid index

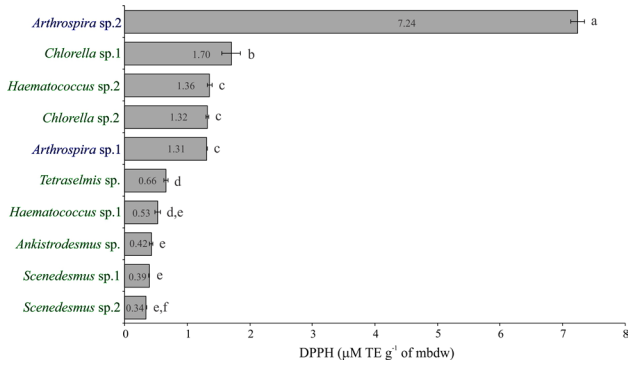

Fig. 2 DPPH radical scavenging activity of the dry biomass of ten native microalgae strains of the groups cyanobacteria (blue) and chlorophyta (green) from the Peruvian Amazon

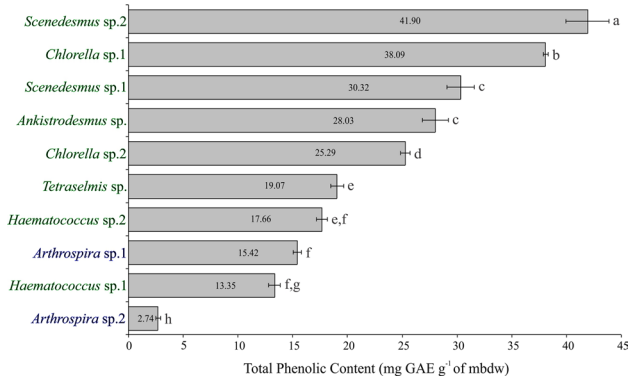

Fig. 3 Total phenolic content of the dry biomass of ten native microalgae strains of the groups cyanobacteria (blue) and chlorophyta (green) from the Peruvian Amazon

were cultivated under the same conditions. Interesting, to complicate the problem further, a recent study found that the same microalgae strain produced different fatty acid profiles when cultivated under variable culture conditions (Santhakumaran et al. 2020). Frequently, the great diversities in fatty acid profiles is a common phenomenon of several microalgae studied to date, both from marine and freshwater origin (Natrah et al. 2007; Maadane et al. 2015; Banskota et al. 2019; Soares et al. 2019).

In terms of health-promoting short-chain PUFAs, all ten microalgae species in this study were able to biosynthesize both essential fatty acids (i.e., linoleic acid, α-linoleic acid), but differences between strains were pronounced (Table 1). No all microalgae are able to produce these essential PUFAs. For example, *Nannochloropsis granulata*, *Porphyridium aerugineum, and Phaeodactylum tricornutum* do not produce α-linoleic acid (Banskota et al. 2019). In terms of very long-chain PUFAs (i.e., eisosapentaenoic acid [EPA], docosahexaenoic acid [DHA]) only three microalgae strains produced EPA (*Arthrospir*a sp.1, *Haematococcus* sp.1, and *Haematococcus* sp.2), but all ten microalgae species were unable to produce DHA. Similar results were reported by Banskota et al. (2019), who found that only two of six freshwater microalgae species produced EPA, however, all three marine microalgae species synthesized this compound. On the other hand, identical to our findings, these researchers also demonstrated that all nine microalgae species analyzed were incapable of biosynthesizing DHA. Analogous results were also published by other research groups, who reported the existence of microalgae species (from marine and freshwater origin) that are able or unable to biosynthesize one or both very long-chain PUFAs (Maadane et al. 2015; Banskota et al. 2019; Soares et al. 2019). For example, some representative microalgae genus of both groups (chlorophyta and cyanobacteria) with capabilities to biosynthesize EPA and/or DHA are: *Arthrospira*, *Chlorella*, *Gloeothece*, *Haematococcus*, *Isocrysis, Nannochloropsis*, *Nostoc*, *Phaeodactylum*, *Pavlova, Pinguiococcus*, *Scenedesmus*, and *Thraustochytrium* (Tokuşoglu and üUnal 2003; Guedes et al. 2011; Lei et al. 2012; Adarme-Vega et al. 2012; Los and Mironov 2015). Undoubtedly, the biosynthetic capabilities of certain microalgae species are coded by genes which were recently identified through genomic and transcriptomic approaches (Chi et al. 2008; Rismani-Yazdi et al. 2011; Radakovits et al. 2012; Wang et al. 2014; Ma et al. 2018; Liang et al. 2019; Gao et al. 2020), and several of these genes have been cloned and functionally characterized, specifically elongases and the membrane-bound enzymes called front-end desaturases (Qi et al. 2002; Pereira et al. 2004; Khozin-Goldberg et al. 2011, 2016; Mühlroth et al. 2013; Ben Amor et al. 2018). From an evolutionary viewpoint, the gene encoding the prototype of prokaryotic delta-6 desaturase could be the common ancestor of all the front-end desaturases identified to date in eukaryotes (Sperling et al. 2003; Meesapyodsuk and Qiu 2012).

Finally, with respect to Ω-6/Ω-3 ratios, 50% of the microalgae species (Table 1) had values within the range (from 0.25 to 1) considered most beneficial for human and animal health (Simopoulos 2008; DiNicolantonio and O'Keefe 2018; DiNicolantonio and OKeefe 2019; Kaliannan et al. 2019; Xiao et al. 2019). Over 10 years ago, evidence was presented that ingestion of enormous quantities of omega-6 PUFAs and a very high Ω-6/Ω-3 ratio, which is common in western diets, boost the pathogenic effects of several human chronic diseases, including autoimmune diseases, cancer, cardiovascular diseases, and inflammatory diseases, while increased levels of omega-3 PUFAs (i.e., a reduced Ω-6/Ω-3 ratio) carry out an important role in the suppressive effects (Simopoulos 2008).

Amino acid composition

All ten microalgae species exhibited both classes of dietary amino acids (i.e., EAA, NEAA), but amino acid profiles were variable (Table 2). Our results are in agreement with several studies that determined both marine and freshwater microalgae can produce the 20 amino acids commonly found in proteins (Zhang et al. 2009; Kent et al. 2015; Tibbetts et al. 2015, 2019; Pereira et al. 2019). Moreover, the molecular machinery necessary to produce these amino acids was corroborated by an abundance of genomic resources from several microalgae species from classical biochemical investigations and studies at the genomics, transcriptomics, proteomics, and metabolomics levels (Baba et al. 2012; Gao et al. 2014; Gudmundsson et al. 2017; Brown et al. 2019).

Based on the EAAI to test the nutritional quality of proteins contained in microalgae biomass, our results showed that these microalgae proteins can be ranked, according to Zhang et al. (2009), as useful proteins (EAAI value from 0.75 to 0.86) and good quality proteins (EAAI value from 0.86 to 0.95). These results are similar to marine and freshwater microalgae that had EAAI values from 0.76 to 1.25 (Kent et al. 2015; Tibbetts et al. 2015), and thus ranked as useful proteins, good quality proteins, and high-quality proteins (EAAI value > 0.95) (Zhang et al. 2009). In *Dunaliella salina* EAA productivity and yield is impacted by both light/dark conditions and growth phases of the microalgae culture. Thus, the greatest EAA quantities were recorded in the stationary phase and the EAAI scores revealed an accumulating tendency during the growth phases (Sui et al. 2019).

The biological values of the microalgae proteins are characterized as good quality and exhibited a strong correlation with EAAI values, which is consistent with the results reported by Oser (1959). Finally, the equation for calculating EAAI, which integrates the totality of human essential amino acids, indicates that microalgae proteins are the richest source of essential amino acids relevant for human and animal nutrition.

Antioxidant activity and total phenolic content

All ten microalgae strains demonstrated capabilities to neutralize the oxidative effect of DPPH radicals (Fig. 2). This antioxidant activity was also demonstrated in both marine and freshwater microalgae species researched worldwide (Li et al. 2007; Ahmed et al. 2014; Maadane et al. 2015; Safafar et al. 2015; Assunção et al. 2017; Banskota et al. 2019, but see Natrah et al. 2007).

Without exception, the hydromethanolic extracts of evaluated microalgae contained phenolic compounds (Fig. 3). These compounds have beneficial effects on human health (Barkia et al. 2019) and are commonly found in marine and freshwater microalgae (Li et al. 2007; Zhang et al. 2007; Hajimahmoodi et al. 2010; Goiris et al. 2012; Ahmed et al. 2014; Safafar et al. 2015; Banskota et al. 2019; Bulut et al. 2019), indicating that these microorganism possess metabolic pathways to biosynthesize phenolic compounds (Tohge et al. 2013; Goiris et al. 2014; Yonekura-Sakakibara et al. 2019). Several human health-promoting effects of phenolic compounds are associated with their direct scavenging actions against free radicals (i.e., reactive oxygen species [ROS], reactive nitrogen species [RNS]). These abilities, to efficiently remove ROS and RNS, are related with their structural properties and are dose-dependently modulated (Onofrejová et al. 2010; Fernando et al. 2016). Curiously, antioxidant activity on total phenolic were not correlated (Fig. S2.), but similar results were also reported in a study of several microalgae species (Li et al. 2007). However, discrepant results were published by another research group from Canada, who display a statistically significant Pearson correlation ($r^2 = 0.66$, $P < 0.005$) between antioxidant activity and total phenolic content (Banskota et al. 2019).

The lack of correlation between antioxidant activity and total phenolic content found in our study could be partially explained by the influence of several factors. First, the antioxidant action of the hydromethanolic extracts is a consequence of individual and synergistic action of various biochemical compounds such as phenolics (e.g., phenolic acids, flavonoids, etc.), carotenoids (e.g., astaxanthin, β-carotene, lutein, zeaxanthin, etc.), chlorophylls, peptides, polysaccharides, and vitamins (Ko et al. 2012; Trabelsi et al. 2016; Fimbres-Olivarria et al. 2018; Soares et al. 2019; Sathasivam et al. 2019; Di Lena et al. 2019). Second, particularly *Arthospira* sp.2 displayed the highest value (7.24 µM TE g-1 of mbdw) of DPPH radical scavenging activity (Fig. 2), but surprisingly, presented the lowest value (2.74 mg GAE g^{-1} of mbdw) in total phenolic content (Fig. 3). This cyanobacteria species, in addition, showed a high protein content (28%, see Fig. 1), therefore, the incongruity mentioned, could be interpreted considering that in the protein fraction of this cyanobacteria exist a great proportion of phycobiliproteins. These proteins covalently bound to open tetrapyrroles constitute an abundant (i.e., *Limnothrix* sp. produces 18% of C-phycocyanin of total dry biomass) and very common component of cyanobacteria that displays a potent scavenging capacity against DPPH free radicals (Gantar et al. 2012; Mysliwa-Kurdziel and Solymosi 2017; Park et al. 2018; Li et al. 2019; Pagels et al. 2019).

Conclusion

All ten native microalgae strains from the Peruvian Amazon analyzed in this study had the ability to biosynthesize and accumulate several nutrients and compounds that have the potential to promote human health, but their absolute

and relative quantities were highly variable both within and between genera. Total lipids and carbohydrates were more abundant in *Scenedesmus* sp.2, however, proteins and ashes were more concentrated in the *Arthrospira* genus. Essential fatty acids such as linoleic acid and α-linoleic acid were mainly produced and accumulated by microalgae of the chlorophyta group, but γ-linoleic acid was highest in the cyanobacteria *Arthrospira* sp.1. EPA was biosynthesized by the cyanobacteria *Arthrospira* sp.1 and both strains of the genus *Haematococcus*. Essential amino acids were produced by cyanobacteria and chlorophyta groups, however the cyanobacteria group was the principal producer of these nutrients.

In the case of compounds with human health-promoting potential, all microalgae were able to neutralize the oxidative effect of DPPH radicals, but the cyanobacteria *Arthrospira* sp.2 was by far the most efficient. Also, phenolic compounds were commonly more concentrated in the chlorophyta group, in contrast the cyanobacteria group produced low levels of these compounds. These phenolic compounds should be identified and functionally characterized to demonstrate their biosafety and beneficial effects on human health.

Now that our bioprospecting has established that select microalgae strains of the Peruvian Amazon have attributes that are likely to be of social and economic value, future studies should focus on their potential use and feasibility of industrial cultivation using biorefinery approaches. Additional bioprospecting studies should also be encouraged, especially in the Amazon, as only a minuscule proportion of extant species has been characterized.

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References

Abdulla R, King TK, Jambo SA, Faik AA (2020) *Microalgae chlorella* as a sustainable feedstock for bioethanol production. In: Yaser AZ (ed) Green engineering for campus sustainability. Springer, Singapore, pp 81–103

Adarme-Vega TC, Lim DKY, Timmins M et al (2012) Microalgal biofactories: a promising approach towards sustainable omega-3 fatty acid production. Microb Cell Factories 11:96. https://doi.org/10.1186/1475-2859-11-96

Ahmed F, Fanning K, Netzel M et al (2014) Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish waters. Food Chem 165:300–306. https://doi.org/10.1016/j.foodchem.2014.05.107

Alkhamis Y, Qin JG (2016) Comparison of pigment and proximate compositions of *Tisochrysis lutea* in phototrophic and mixotrophic cultures. J Appl Phycol 28:35–42. https://doi.org/10.1007/s10811-015-0599-0

Andersen RA (1992) Diversity of eukaryotic algae. Biodivers Conserv 1:267–292. https://doi.org/10.1007/BF00693765

AOAC International (1990) AOAC Official Methods of Analysis of the Association of Official Analytical Chemists (AOAC), 15th edn. AOAC International, Washington

Arguelles EDLR, Laurena AC, Monsalud RG, Martinez-Goss MR (2018) Fatty acid profile and fuel-derived physico-chemical properties of biodiesel obtained from an indigenous green microalga, *Desmodesmus* sp. (I-AU1), as potential source of renewable lipid and high quality biodiesel. J Appl Phycol 30:411–419. https://doi.org/10.1007/s10811-017-1264-6

Assunção MFG, Amaral R, Martins CB et al (2017) Screening microalgae as potential sources of antioxidants. J Appl Phycol 29:865–877. https://doi.org/10.1007/s10811-016-0980-7

Baba M, Ioki M, Nakajima N et al (2012) Transcriptome analysis of an oil-rich race A strain of *Botryococcus braunii* (BOT-88-2) by de novo assembly of pyrosequencing cDNA reads. Bioresour Technol 109:282–286. https://doi.org/10.1016/j.biortech.2011.10.033

Banskota AH, Sperker S, Stefanova R et al (2019) Antioxidant properties and lipid composition of selected microalgae. J Appl Phycol 31:309–318. https://doi.org/10.1007/s10811-018-1523-1

Barkia I, Saari N, Manning SR (2019) Microalgae for high-value products towards human health and nutrition. Mar Drugs. https://doi.org/10.3390/md17050304

Ben Amor F, Ben Hlima H, Abdelkafi S, Fendri I (2018) Insight into *Arthrospira platensis* Δ9 desaturase: a key enzyme in poly-unsaturated fatty acid synthesis. Mol Biol Rep 45:1873–1879. https://doi.org/10.1007/s11033-018-4333-2

Benemann JR (1992) Microalgae aquaculture feeds. J Appl Phycol 4:233–245. https://doi.org/10.1007/BF02161209

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37:911–917. https://doi.org/10.1139/o59-099

Brown RB, Wass TJ, Thomas-Hall SR, Schenk PM (2019) Chromosome-scale genome assembly of two Australian *Nannochloropsis oceanica* isolates exhibiting superior lipid characteristics. Microbiol Resour Announc. https://doi.org/10.1128/MRA.01288-19

Bulut O, Akın D, Sönmez Ç et al (2019) Phenolic compounds, carotenoids, and antioxidant capacities of a thermo-tolerant *Scenedesmus* sp. (Chlorophyta) extracted with different solvents. J Appl Phycol 31:1675–1683. https://doi.org/10.1007/s10811-018-1726-5

Buono S, Colucci A, Angelini A et al (2016) Productivity and biochemical composition of *Tetradesmus obliquus* and *Phaeodactylum tricornutum*: effects of different cultivation approaches. J Appl Phycol 28:3179–3192. https://doi.org/10.1007/s10811-016-0876-6

Chi X, Yang Q, Zhao F et al (2008) Comparative analysis of fatty acid desaturases in cyanobacterial genomes. Comp Funct Genomics. https://doi.org/10.1155/2008/284508

Cobos M, Lopez LC, Paredes JD et al (2017a) Media composition affects the fatty acids profiles of three oleaginous microalgae from the Peruvian Amazon. Asian J Microbiol Biotechnol 1:14–24

Cobos M, Paredes JD, Maddox JD et al (2017b) Isolation and characterization of native microalgae from the peruvian amazon with potential for biodiesel production. Energies 10:224. https://doi.org/10.3390/en10020224

Cohen SA, Michaud DP (1993) Synthesis of a fluorescent derivatizing reagent, 6-Aminoquinolyl-N-Hydroxysuccinimidyl carbamate, and its application for the analysis of hydrolysate amino acids via high-performance liquid chromatography. Anal Biochem 211:279–287. https://doi.org/10.1006/abio.1993.1270

Development Core Team R (2020) R: The R Project for Statistical Computing. https://www.r-project.org/. Accessed 24 May 2020

Di Lena G, Casini I, Lucarini M, Lombardi-Boccia G (2019) Carotenoid profiling of five microalgae species from large-scale production. Food Res Int 120:810–818. https://doi.org/10.1016/j.foodres.2018.11.043

DiNicolantonio JJ, O'Keefe JH (2018) Importance of maintaining a low omega-6/omega-3 ratio for reducing inflammation. Open Heart 5:e000946. https://doi.org/10.1136/openhrt-2018-000946

DiNicolantonio JJ, OKeefe J (2019) Importance of maintaining a low omega-6/omega-3 ratio for reducing platelet aggregation, coagulation and thrombosis. Open Heart 6:e001011. https://doi.org/10.1136/openhrt-2019-001011

FAO, WHO (1991) Protein quality evaluation: report of joint FAO/WHO expert consultation. Food and Agriculture Organization of the United Nations, Rome

Fernando IPS, Kim M, Son K-T et al (2016) Antioxidant activity of marine algal polyphenolic compounds: a mechanistic approach. J Med Food 19:615–628. https://doi.org/10.1089/jmf.2016.3706

Fimbres-Olivarria D, Carvajal-Millan E, Lopez-Elias JA et al (2018) Chemical characterization and antioxidant activity of sulfated polysaccharides from *Navicula* sp. Food Hydrocoll 75:229–236. https://doi.org/10.1016/j.foodhyd.2017.08.002

Gantar M, Simović D, Djilas S et al (2012) Isolation, characterization and antioxidative activity of C-phycocyanin from *Limnothrix* sp. strain 37-2-1. J Biotechnol 159:21–26. https://doi.org/10.1016/j.jbiotec.2012.02.004

Gao C, Wang Y, Shen Y et al (2014) Oil accumulation mechanisms of the oleaginous microalga *Chlorella protothecoides* revealed through its genome, transcriptomes, and proteomes. BMC Genomics 15:582. https://doi.org/10.1186/1471-2164-15-582

Gao B, Huang L, Lei X et al (2020) Evaluation and transcriptome analysis of the novel oleaginous microalga *Lobosphaera bisecta* (Trebouxiophyceae, Chlorophyta) for arachidonic acid production. Mar Drugs 18:229. https://doi.org/10.3390/md18050229

Ginzberg A, Cohen M, Sod-Moriah UA et al (2000) Chickens fed with biomass of the red microalga *Porphyridium* sp. have reduced blood cholesterol level and modified fatty acid composition in egg yolk. J Appl Phycol 12:325–330. https://doi.org/10.1023/A:1008102622276

Goiris K, Muylaert K, Fraeye I et al (2012) Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. J Appl Phycol 24:1477–1486. https://doi.org/10.1007/s10811-012-9804-6

Goiris K, Muylaert K, Voorspoels S et al (2014) Detection of flavonoids in microalgae from different evolutionary lineages. J Phycol 50:483–492. https://doi.org/10.1111/jpy.12180

Görs M, Schumann R, Hepperle D, Karsten U (2010) Quality analysis of commercial Chlorella products used as dietary supplement in human nutrition. J Appl Phycol 22:265–276. https://doi.org/10.1007/s10811-009-9455-4

Gudmundsson S, Agudo L, Nogales J (2017) 4—applications of genome-scale metabolic models of microalgae and cyanobacteria in biotechnology. In: Gonzalez-Fernandez C, Muñoz R (eds) Microalgae-based biofuels and bioproducts. Woodhead Publishing, Cambrigde, pp 93–111

Guedes AC, Amaro HM, Barbosa CR et al (2011) Fatty acid composition of several wild microalgae and cyanobacteria, with a focus on eicosapentaenoic, docosahexaenoic and α-linolenic acids for eventual dietary uses. Food Res Int 44:2721–2729. https://doi.org/10.1016/j.foodres.2011.05.020

Hajimahmoodi M, Faramarzi MA, Mohammadi N et al (2010) Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. J Appl Phycol 22:43–50. https://doi.org/10.1007/s10811-009-9424-y

Hartree EF (1972) Determination of protein: a modification of the Lowry method that gives a linear photometric response. Anal Biochem 48:422–427. https://doi.org/10.1016/0003-2697(72)90094-2

Hernández-Vásquez A, Tapia-López E (2017) Chronic malnutrition among children under five in Peru: spátial analysis of nutrition data (2010–2016). Rev Esp Salud Publica 91:e1–e10

Hirs CH, Stein WH, Moore S (1954) The amino acid composition of ribonuclease. J Biol Chem 211:941–950

Hossain N, Zaini J, Indra Mahlia TM (2019) Life cycle assessment, energy balance and sensitivity analysis of bioethanol production from microalgae in a tropical country. Renew Sustain Energy Rev 115:109371. https://doi.org/10.1016/j.rser.2019.109371

Ichihara K, Fukubayashi Y (2010) Preparation of fatty acid methyl esters for gas-liquid chromatography. J Lipid Res 51:635–640. https://doi.org/10.1194/jlr.D001065

INEI (2019) Evolución de la Pobreza Monetaria (2007–2018). Instituto Nacional de Estadística e Informática, Lima

Jiang M, Zhao HH, Zai SW et al (2019) A defatted microalgae meal (*Haematococcus pluvialis*) as a partial protein source to replace fishmeal for feeding juvenile yellow perch Perca flavescens. J Appl Phycol 31:1197–1205. https://doi.org/10.1007/s10811-018-1610-3

Kaliannan K, Li X-Y, Wang B et al (2019) Multi-omic analysis in transgenic mice implicates omega-6/omega-3 fatty acid imbalance as a risk factor for chronic disease. Commun Biol 2:276. https://doi.org/10.1038/s42003-019-0521-4

Kent M, Welladsen HM, Mangott A, Li Y (2015) Nutritional evaluation of australian microalgae as potential human health supplements. PLoS ONE. https://doi.org/10.1371/journal.pone.0118985

Khozin-Goldberg I, Iskandarov U, Cohen Z (2011) LC-PUFA from photosynthetic microalgae: occurrence, biosynthesis, and prospects in biotechnology. Appl Microbiol Biotechnol 91:905–915. https://doi.org/10.1007/s00253-011-3441-x

Khozin-Goldberg I, Leu S, Boussiba S (2016) Microalgae as a source for VLC-PUFA production. Subcell Biochem 86:471–510. https://doi.org/10.1007/978-3-319-25979-6_19

Ko S-C, Kim D, Jeon Y-J (2012) Protective effect of a novel antioxidative peptide purified from a marine *Chlorella ellipsoidea* protein against free radical-induced oxidative stress. Food Chem Toxicol Int J Publ Br Ind Biol Res Assoc 50:2294–2302. https://doi.org/10.1016/j.fct.2012.04.022

Lakatos GE, Ranglová K, Manoel JC et al (2019) Bioethanol production from microalgae polysaccharides. Folia Microbiol (Praha) 64:627–644. https://doi.org/10.1007/s12223-019-00732-0

Lei A, Chen H, Shen G et al (2012) Expression of fatty acid synthesis genes and fatty acid accumulation in *Haematococcus pluvialis* under different stressors. Biotechnol Biofuels 5:18. https://doi.org/10.1186/1754-6834-5-18

Li H-B, Cheng K-W, Wong C-C et al (2007) Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem 102:771–776. https://doi.org/10.1016/j.foodchem.2006.06.022

Li W, Su H-N, Pu Y et al (2019) Phycobiliproteins: molecular structure, production, applications, and prospects. Biotechnol Adv 37:340–353. https://doi.org/10.1016/j.biotechadv.2019.01.008

Liang J, Wen F, Liu J (2019) Transcriptomic and lipidomic analysis of an EPA-containing *Nannochloropsis* sp. PJ12 in response to nitrogen deprivation. Sci Rep 9:1–18. https://doi.org/10.1038/s41598-019-41169-2

Los DA, Mironov KS (2015) Modes of fatty acid desaturation in cyanobacteria: an update. Life 5:554–567. https://doi.org/10.3390/life5010554

Ma R, Thomas-Hall SR, Chua ET et al (2018) Gene expression profiling of astaxanthin and fatty acid pathways in *Haematococcus pluvialis* in response to different LED lighting conditions. Bioresour Technol 250:591–602. https://doi.org/10.1016/j.biortech.2017.11.094

Maadane A, Merghoub N, Ainane T et al (2015) Antioxidant activity of some Moroccan marine microalgae: pufa profiles, carotenoids

and phenolic content. J Biotechnol 215:13–19. https://doi.org/10.1016/j.jbiotec.2015.06.400
Meesapyodsuk D, Qiu X (2012) The front-end desaturase: structure, function, evolution and biotechnological use. Lipids 47:227–237. https://doi.org/10.1007/s11745-011-3617-2
Michel D, Gilles KA, Hamilton JK et al (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356. https://doi.org/10.1021/ac60111a017
MINSA (2017) Plan Nacional Para la Reducción y Control de la Anemia Materno Infantil y la Desnutrición Crónica Infantil en el Perú (2017-2021), 1ra. Edición, Ministerio de Salud
Molino A, Iovine A, Casella P et al (2018) Microalgae characterization for consolidated and new application in human food, animal feed and nutraceuticals. Int J Environ Res Public Health. https://doi.org/10.3390/ijerph15112436
Mühlroth A, Li K, Røkke G et al (2013) Pathways of lipid metabolism in marine algae, co-expression network, bottlenecks and candidate genes for enhanced production of EPA and DHA in species of Chromista. Mar Drugs 11:4662–4697. https://doi.org/10.3390/md11114662
Mysliwa-Kurdziel B, Solymosi K (2017) Phycobilins and phycobiliproteins used in food industry and medicine. Mini Rev Med Chem 17:1173–1193. https://doi.org/10.2174/1389557516666160912180155
Natrah FMI, Yusoff FM, Shariff M et al (2007) Screening of Malaysian indigenous microalgae for antioxidant properties and nutritional value. J Appl Phycol 19:711–718. https://doi.org/10.1007/s10811-007-9192-5
Navarro F, Forján E, Vázquez M et al (2016) Microalgae as a safe food source for animals: nutritional characteristics of the acidophilic microalga *Coccomyxa onubensis*. Food Nutr Res. https://doi.org/10.3402/fnr.v60.30472
Norton TA, Melkonian M, Andersen RA (1996) Algal biodiversity. Phycologia 35:308–326. https://doi.org/10.2216/i0031-8884-35-4-308.1
Onofrejová L, Vašíčková J, Klejdus B et al (2010) Bioactive phenols in algae: the application of pressurized-liquid and solid-phase extraction techniques. J Pharm Biomed Anal 51:464–470. https://doi.org/10.1016/j.jpba.2009.03.027
Oser BL (1959) An integrated essential amino acid index for predicting the biological value of proteins. In: Albanese AA (ed) Protein and Amino Acid Nutrition. Academic Press, Amsterdam, pp 281–295
Pagels F, Guedes AC, Amaro HM et al (2019) Phycobiliproteins from cyanobacteria: chemistry and biotechnological applications. Biotechnol Adv 37:422–443. https://doi.org/10.1016/j.biotechadv.2019.02.010
Park WS, Kim H-J, Li M et al (2018) Two classes of pigments, carotenoids and C-phycocyanin, in spirulina powder and their antioxidant activities. Mol J Synth Chem Nat Prod Chem. https://doi.org/10.3390/molecules23082065
Pereira SL, Leonard AE, Huang Y-S et al (2004) Identification of two novel microalgal enzymes involved in the conversion of the ω3-fatty acid, eicosapentaenoic acid, into docosahexaenoic acid. Biochem J 384:357. https://doi.org/10.1042/BJ20040970
Pereira H, Silva J, Santos T et al (2019) Nutritional potential and toxicological evaluation of *Tetraselmis* sp. CTP4 microalgal biomass produced in industrial photobioreactors. Molecules. https://doi.org/10.3390/molecules24173192
Qi B, Beaudoin F, Fraser T et al (2002) Identification of a cDNA encoding a novel C18-Δ9 polyunsaturated fatty acid-specific elongating activity from the docosahexaenoic acid (DHA)-producing microalga, *Isochrysis galbana* 1. FEBS Lett 510:159–165. https://doi.org/10.1016/S0014-5793(01)03247-1
Radakovits R, Jinkerson RE, Fuerstenberg SI et al (2012) Draft genome sequence and genetic transformation of the oleaginous alga *Nannochloropsis gaditana*. Nat Commun 3:686. https://doi.org/10.1038/ncomms1688
Rismani-Yazdi H, Haznedaroglu BZ, Bibby K, Peccia J (2011) Transcriptome sequencing and annotation of the microalgae *Dunaliella tertiolecta*: pathway description and gene discovery for production of next-generation biofuels. BMC Genomics 12:148. https://doi.org/10.1186/1471-2164-12-148
Romero-Romero CC, del Sánchez-Saavedra MP (2017) Effect of light quality on the growth and proximal composition of *Amphora* sp. J Appl Phycol 29:1203–1211. https://doi.org/10.1007/s10811-016-1029-7
Safafar H, van Wagenen J, Møller P, Jacobsen C (2015) Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. Mar Drugs 13:7339–7356. https://doi.org/10.3390/md13127069
Santhakumaran P, Kookal SK, Mathew L, Ray JG (2020) Experimental evaluation of the culture parameters for optimum yield of lipids and other nutraceutically valuable compounds in *Chloroidium saccharophillum* (Kruger) comb. Nov. Renew Energy 147:1082–1097. https://doi.org/10.1016/j.renene.2019.09.071
Sathasivam R, Radhakrishnan R, Hashem A, Abd_Allah EF (2019) Microalgae metabolites: a rich source for food and medicine. Saudi J Biol Sci 26:709–722. https://doi.org/10.1016/j.sjbs.2017.11.003
Sharma OP, Bhat TK (2009) DPPH antioxidant assay revisited. Food Chem 113:1202–1205. https://doi.org/10.1016/j.foodchem.2008.08.008
Simopoulos AP (2008) The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med Maywood NJ 233:674–688. https://doi.org/10.3181/0711-MR-311
Soares AT, da Costa DC, Vieira AAH, Antoniosi Filho NR (2019) Analysis of major carotenoids and fatty acid composition of freshwater microalgae. Heliyon 5:e01529. https://doi.org/10.1016/j.heliyon.2019.e01529
Sperling P, Ternes P, Zank TK, Heinz E (2003) The evolution of desaturases. Prostaglandins Leukot Essent Fatty Acids 68:73–95. https://doi.org/10.1016/S0952-3278(02)00258-2
Stanier RY, Kunisawa R, Mandel M, Cohen-Bazire G (1971) Purification and properties of unicellular blue-green algae (order Chroococcales). Bacteriol Rev 35:171–205
Sui Y, Muys M, Vermeir P et al (2019) Light regime and growth phase affect the microalgal production of protein quantity and quality with *Dunaliella salina*. Bioresour Technol 275:145–152. https://doi.org/10.1016/j.biortech.2018.12.046
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. https://doi.org/10.1007/s10811-014-0428-x
Tibbetts SM, Patelakis SJJ, Whitney-Lalonde CG et al (2019) Nutrient composition and protein quality of microalgae meals produced from the marine prymnesiophyte *Pavlova* sp. 459 mass-cultivated in enclosed photobioreactors for potential use in salmonid aquafeeds. J Appl Phycol. https://doi.org/10.1007/s10811-019-01942-2
Tohge T, Watanabe M, Hoefgen R, Fernie AR (2013) The evolution of phenylpropanoid metabolism in the green lineage. Crit Rev Biochem Mol Biol 48:123–152. https://doi.org/10.3109/10409238.2012.758083
Tokuşoglu Ö, Üunal MK (2003) Biomass nutrient profiles of three microalgae: *spirulina platensis*, *Chlorella vulgaris*, and *Isochrisis galbana*. J Food Sci 68:1144–1148. https://doi.org/10.1111/j.1365-2621.2003.tb09615.x
Trabelsi L, Chaieb O, Mnari A et al (2016) Partial characterization and antioxidant and antiproliferative activities of the aqueous extracellular polysaccharides from the thermophilic microalgae

Graesiella sp. BMC Complement Altern Med 16:210. https://doi.org/10.1186/s12906-016-1198-6

Velioglu YS, Mazza G, Gao L, Oomah BD (1998) Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. J Agric Food Chem 46:4113–4117. https://doi.org/10.1021/jf9801973

Wang D, Ning K, Li J et al (2014) Nannochloropsis genomes reveal evolution of microalgal oleaginous traits. PLoS Genet 10:e1004094. https://doi.org/10.1371/journal.pgen.1004094

Xiao Y, Li X, Zeng X et al (2019) A low ω-6/ω-3 ratio high-fat diet improves rat metabolism via purine and tryptophan metabolism in the intestinal tract, while reversed by inulin. J Agric Food Chem 67:7315–7324. https://doi.org/10.1021/acs.jafc.9b02110

Yonekura-Sakakibara K, Higashi Y, Nakabayashi R (2019) The origin and evolution of plant flavonoid metabolism. Front Plant Sci. https://doi.org/10.3389/fpls.2019.00943

Zhang W-W, Duan X-J, Huang H-L et al (2007) Evaluation of 28 marine algae from the Qingdao coast for antioxidative capacity and determination of antioxidant efficiency and total phenolic content of fractions and subfractions derived from *Symphyocladia latiuscula* (Rhodomelaceae). J Appl Phycol 19:97–108. https://doi.org/10.1007/s10811-006-9115-x

Zhang T, Chi Z, Sheng J (2009) A highly thermosensitive and permeable mutant of the marine yeast *Cryptococcus aureus* G7a potentially useful for single-cell protein production and its nutritive components. Mar Biotechnol N Y N 11:280–286. https://doi.org/10.1007/s10126-008-9144-3