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# Chemical composition and nutritional properties of freshwater and marine microalgal biomass cultured in photobioreactors

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**Abstract** Proximate, amino acid and elemental composition, total phenolic content (TPC), and in vitro protein digestibility (IVPD) of microalgal biomass were determined. Microalgae contained low to moderate ash (5–17 %), moderate to high carbohydrate (18–46 %), crude protein (18–46 %), high crude lipid (12–48 %), and energy (19–27 MJ $kg^{-1}$). Characteristic of microalgae, non-essential amino acids (AAs), aspartic and glutamic acids, were predominant (20–30 % of protein; 8–12 % of dry weight). Microalgae had favorable essential AA profiles with high essential amino acid (EAA) indices (0.9–1.2). Expressed as g EAA 100 g $protein^{-1}$, *Porphyridium aerugineum* was rich in leucine (11.9), lysine (8.0), arginine (8.6), and tryptophan (3.3); *Nannochloropsis granulata* (A) in leucine (11.0), lysine (8.5), and tryptophan (2.8); *Tetraselmis chuii* and *Botryococcus braunii* in arginine (9.4 and 20.5, respectively); and *Phaeodactylum tricornutum* in lysine (6.4) and tryptophan (2.6). Mineral compositions (%) were calcium (0.1–3.0), magnesium (0.3–0.7), phosphorous (0.7–1.5), potassium (0.7–2.4), sodium (0.8–2.7), and sulfur (0.4–1.4), and trace element compositions (mg $kg^{-1}$) were copper (18–102), iron (1,395–11,101), manganese (45–454), selenium (0–0.5), and zinc (28–64). Microalgae contained low TPC (6–13 mg gallic acid equivalents (GAE) $g^{-1}$ DW), except *T. chuii* (20 mg GAE $g^{-1}$ DW). IVPD was high (>90 %) for *B. braunii* (A), *P. aerugineum*, and lipid-extracted *N. granulata* (B); mid-range (80–89 %) for *P. tricornutum*, *N. granulata* (A), *B. braunii* (B), *Neochloris oleoabundans*, *T. chuii*, and whole *N. granulata* (B); and lower (<80 %) for *Acutodesmus dimorphus*. Microalgal species *P. tricornutum*, *B. braunii*, *N. granulata*, and *T. chuii* had high protein (40–52 %), IVPD (82–97 %), and digestible protein (35–50 %), comparable to plant proteins used in animal feeds and aquaculture.

**Keywords** Microalgae · Composition · Amino acids · In vitro protein digestibility · Total phenolic content

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## Introduction

As a result of the global non-renewable petroleum (fossil fuels) crisis and "food-for-fuel" and "forest-for-fuel" conflicts associated with first-generation biofuels, there has been a renewed interest in microalgae within the past decade. With increasing advances in microalgal biofuels and expanding industrial-scale algal production for other commercial purposes, it is likely that large quantities of microalgae and algal co-products could become available in the near future. Microalgae are highly efficient at collecting solar energy and transforming it into cellular biomass, much of which is protein, lipid, and carbohydrate (Becker 2013). It has been estimated that under protein-optimized conditions, the protein yield per unit area from mass microalgae culture can be 20–50 times that of soybeans (Chronakis 2000). However, most conceptual algal biorefineries are presently optimized for lipid accumulation for biofuel applications which is understandable considering that lipid yields from microalgae production can be 60–200 times that of terrestrial oilseed crops like soybean and canola (see review of Mata et al. 2010). However, it is now recognized that full utilization of the entire microalgal crop, through a balanced biorefinery approach, is the only feasible strategy to increase the viability of a microalgae industry. Therefore, a more in-depth examination of various algal species, beyond growth rate and lipid production, is necessary. At least in the near to mid-term, the animal feed

and aquaculture sectors appear to be the most promising areas to focus for generating revenues (Pulz and Gross 2004; Brune et al. 2009; Stephens et al. 2010; Subhadra and Grinson 2011; Ahmed et al. 2012). Depending on species, environmental conditions, nutrient supply, and harvesting/processing techniques, whole biomass and residual "cake" after oil extraction may be highly attractive sources of protein, essential amino acids, and other nutrients for terrestrial livestock and aquatic animals. This potential protein source from microalgae and algal co-products for nutrition applications has long been recognized, but commercial success has only been realized to a small extent for a few species (e.g., *Spirulina*, *Chlorella*, and *Dunaliella*) occupying niche markets. In addition to protein, microalgae can contain high-value polyunsaturated fatty acids, sterols, vitamins, polysaccharides, minerals, carotenoids, phycobilins, and other biologically active compounds (see reviews of Borowitzka 2013 and Skjånes et al. 2013). Data on the chemical composition and nutritional value of most algal biomass remains highly variable and only reported for a limited number of commercially established species. With such immense diversity and heterogeneity between algal species, along with large variations in environmental conditions under culture and biomass harvesting/processing, the data varies considerably. In addition, most published literature reports on intact (whole) biomass only, rarely on its fractionated co-products (e.g., de-fatted biomass) and, in many circumstances, have employed analytical methods inadequate for microalgae.

Currently, vast arrays of microalgal species are being screened globally for large-scale cultivation as sources of oil and other co-products. Algae species are known to vary widely in their chemical compositions, physical characteristics, and processing technologies required to extract oil and fractionate their other constituents (Demirbas and Demirbas 2011) and will undoubtedly differ greatly in their nutritional value. Protein quality is generally one of the most important aspects used to define the nutritional value of novel feed ingredients and is largely dependent upon amino acid profile, solubility, extent of chemical and enzymatic hydrolysis in the digestive tract, and physiological utilization after intestinal absorption (McDonald et al. 2010). These can all be affected by their source, processing treatment(s), interactions with other dietary components, presence of anti-nutritional factors, and feeding habits of the target species (NRC 1994, 2011). The use of in vivo biological assays to evaluate algal protein quality using experimental animals are time consuming and costly, while in vitro assays that involve "simulated" digestion of protein suspensions with proteolytic enzymes potentially offer an effective way to screen large numbers of samples (Fernández-Garcia et al. 2009). Although not fully definitive, these methods can complement chemical composition and amino acid profile data as they are relatively inexpensive, do not require live test subjects, results are rapidly obtained using small sample sizes, and they are generally regarded as effective tools for making predictions of potential protein quality for research and industrial use (Fernández-Garcia et al. 2009). The present study evaluated the chemical composition and apparent protein quality of marine and freshwater microalgae cultured in photobioreactors under controlled environmental conditions. Although the microalgae species employed in these studies are not new, novel data has been generated with respect to amino acid profile, elemental composition, in vitro digestibility, and total phenolic content. While several species have been characterized for their growth rate and lipid production potential for biofuels and/or polyunsaturated fatty acids (PUFAs) for use as dietary supplements or live feed for mariculture application, with the exception of *Spirulina* and *Chlorella* most have never been adequately characterized for their proximate and mineral composition, amino acid profile, total phenolic content (TPC), and in vitro protein digestibility (IVPD).

## Materials and methods

### Sample preparation

Microalgae were cultivated in proprietary enclosed "BriteBox" photobioreactors (PBRs) that were developed in our laboratory (Armstrong et al. 2002). These are 1,000 L internally illuminated PBRs enclosed by a fiberglass shell. Continuous light (250 μmol photons $m^{-2}$ $s^{-1}$) was provided by 40, F32T8/TL765 PLUS (32 W) fluorescent bulbs (Alto Technology, Philips Lighting, Canada) arranged in eight horizontal rows of five. The cultures were supplied with titanium heating/cooling loops for temperature control (22 °C), automated on-demand carbon dioxide ($CO_2$) injections for pH control (7.5–7.9), aeration with a turbulent flow of sterilized air introduced into the cultures through perforations in two T-shaped air-lines situated medially at the bottom of the PBR, and either f/2, f/2+Si or Bold's media (Anderson et al. 2005). Microalgal inoculates were obtained from the National Center for Marine Algae and Microbiota, East Boothbay, ME, USA (CCMP), the University of Texas, Austin, TX, USA (UTEX), and the NOAA Milford Microalgal Culture Collection, Milford, CT, USA (PLY). Cell densities were monitored daily, using a particle multi-sizer equipped with a 100-μm aperture tube (model MS3, Beckman-Coulter Inc., USA). Once cultures reached the stationary phase, they were harvested as a paste using a process centrifuge (CEPA Carl Padberg Zentrifugenbau GmbH, model #Z101, Germany) with a 10-L chamber volume and 14,000 rpm (15,760×*g*) and frozen at −20 °C. All samples were lyophilized for 36 h at a low shelf temperature (<5 °C) in a large-capacity freeze-dryer (model 35EL, The Virtis Company, USA). Freeze-dried samples were pulverized (to pass through a 0.8-mm screen) using a

laboratory hammer mill (model 3100, Perten Instruments, Sweden) and stored at −80 °C. In addition, two commercial microalgae products (Earthrise Nutritionals *Spirulina* and Sequel Naturals *Chlorella*) were purchased from a local pharmacy and tested simultaneously (see details in Table 1). Microalgae samples tested included *Phaeodactylum tricornutum*, *Nannochloropsis granulata*, *Botryococcus braunii*, *Neochloris oleoabundans*, *Porphyridium aerugineum*, *Acutodesmus dimorphus*, and *Tetraselmis chuii* and are described in Table 1. Sodium caseinate with 97 % crude protein (N×6.38) supplied by USB Corporation (Cleveland, OH, USA) was used as a reference protein for in vitro protein digestibility studies.

## Analytical techniques

Moisture and ash levels were determined gravimetrically by drying in an oven at 105 °C and after incineration in a muffle furnace at 550 °C, respectively, until constant weight. Total nitrogen (N) was measured by elemental analysis (950 °C furnace) using a Leco N determinator (model FP-528, Leco Corporation, USA) with ultra-high-purity oxygen as the combustion gas and ultra-high-purity helium as the carrier gas. The nitrogen-to-protein conversion factors (e.g., "Jones factors"; Mariotti et al. 2008) used to estimate crude protein content were N×6.38 for sodium caseinate and N×4.78 for microalgae (Lourenço et al. 2004). Crude lipid was extracted with a Soxtec automated system (model 2050, FOSS North America, USA) in 33×80-mm cellulose extraction thimbles (CT33080, Rose Scientific Ltd., Canada) using chloroform/methanol (2:1 *v*/*v*) at 150 °C for 82 min. The final weight of the crude lipid extract was determined gravimetrically after oven-drying (105 °C) for 90 min. Carbohydrate content was estimated by difference (100−[moisture+ash+crude protein+crude lipid]). Caloric content was measured as gross energy (MJ $kg^{-1}$) using an isoperibol oxygen bomb calorimeter (model 6200, Parr Instrument Company, USA) equipped with a Parr 6510 water handling system for closed-loop operation. TPC was extracted and quantified according to the methods of Velioglu et al. (1998) and Singleton et al. (1999). Briefly, 100 mg of sample was incubated for 6 h with 10 mL of 80 % acetone and 1 % hydrochloric acid at 25 °C on an orbital shaker (Gyrotory Shaker, model G-2, New Brunswick Scientific, USA) set at 200 rpm. A 40-μL aliquot was mixed with 360 μL de-ionized water, 200 μL of 2 N Folin-Ciocalteu reagent (F9252, Sigma-Aldrich, USA), and 600 μL of 20 % $Na_2CO_3$. The reaction mixture was thoroughly mixed and allowed to stand at 40 °C for 30 min in darkness. Samples were centrifuged at 10,000 rpm for 2 min at room temperature, and the absorbance was measured at 765 nm using a Spectronic Genesys G5 spectrophotometer. TPC of samples were determined against a standard curve (0–500 μg $mL^{-1}$) of gallic acid (G7384, Sigma-Aldrich) and expressed in terms of gallic acid equivalents (GAE) as "mg GAE g $DW^{-1}$." IVPD was determined by multi-enzyme hydrolysis according to Morris et al. (2008) using commercial enzymes (Sigma-Aldrich) including porcine pancreatic trypsin (Type IX-S, lyophilized powder, 16,700 BAEE units $mg^{-1}$ protein), bovine pancreatic α-chymotrypsin (type II, lyophilized powder, 59.3 units $mg^{-1}$ protein) and porcine intestinal peptidase (115 units $g^{-1}$ solid). Enzyme mixtures were prepared fresh daily in 50 mM Tris, 11.5 mM $CaCl_2$ at pH 8.0 and 37 °C. The pH change after 10 min was used to calculate IVPD and was expressed as a relative percentage of the sodium caseinate (cat. no. 12865, USB Corporation, USA) reference protein normalized to 100 %. Amino acid (AA) profile and elemental composition were determined by a commercial lab for five microalgae containing relatively high crude protein levels

**Table 1** Proximate composition, gross energy, and total phenolic content of microalgae ($n$=3; DW basis)

| Species | Nitrogen (%) | Crude protein (%) | Crude lipid (%) | Carbohydrate (%) | Ash (%) | Gross energy (MJ $kg^{-1}$) | Total phenolic content (mg GAE $g^{-1}$ DW) |
|---|---|---|---|---|---|---|---|
| *Spirulina*[a] | 11.67 | 55.8 | 14.2 | 22.2 | 7.8 | 22.7 | 10.7 |
| *Chlorella*[b] | 11.16 | 53.3 | 15.7 | 25.2 | 5.8 | 24.0 | 7.7 |
| *Phaeodactylum tricornutum* (CCMP-1327) | 8.28 | 39.6 | 18.2 | 25.2 | 17.0 | 20.2 | 9.9 |
| *Nannochloropsis granulata* (A) (CCMP-535) | 7.01 | 33.5 | 23.6 | 36.2 | 6.7 | 24.7 | 8.0 |
| *Nannochloropsis granulata* (B) (CCMP-535) | 3.74 | 17.9 | 47.8 | 27.4 | 6.9 | 26.7 | 6.0 |
| *Botryococcus braunii* (A) (UTEX-572) | 8.35 | 39.9 | 34.4 | 18.5 | 7.2 | 23.6 | 13.2 |
| *Botryococcus braunii* (B) (UTEX-572) | 8.17 | 39.1 | 24.9 | 30.6 | 5.4 | 24.4 | 7.1 |
| *Neochloris oleoabundans* (UTEX-1185) | 6.30 | 30.1 | 15.4 | 37.8 | 16.7 | 19.2 | 9.8 |
| *Porphyridium aerugineum* (UTEX-755) | 6.60 | 31.6 | 13.7 | 45.8 | 8.9 | 20.5 | 6.5 |
| *Acutodesmus dimorphus* (UTEX-1237) | 5.87 | 28.1 | 18.8 | 38.6 | 14.5 | 20.9 | 6.4 |
| *Tetraselmis chuii* (PLY-429) | 9.72 | 46.5 | 12.3 | 25.0 | 16.2 | 19.9 | 20.0 |

[a] Earthrise Nutritionals LLC (Irvine, CA, USA), species *Arthrospira platensis*

[b] Sequel Naturals Ltd. (Port Coquitlam, BC, Canada), species *Chlorella pyrenoidosa* and *Chlorella vulgaris*

(>30 % of DW). Briefly, AA profiles were determined using the Waters Pico-Tag, reverse-phase high-performance liquid chromatography (PT-RP-HPLC) method according to Heinriksen and Meredith (1984) and White et al. (1986). AA concentrations are expressed as mg AA $g^{-1}$ of DW and g AA 100 g $protein^{-1}$, and the protein quality was evaluated on the basis of essential AA indices according to Oser (1951). Elemental compositions were measured by inductively coupled argon plasma optical emission spectrometry (ICP-OES) according to SW-846 Method 6010C (EPA 2007). Briefly, 1 g of sample was digested for 75 min in concentrated nitric acid at 95 °C; after which, dissolution of organic matter was aided with the addition of hydrogen peroxide (30 % *v*/*v*). Samples were then digested for an additional 1 h at 95 °C in concentrated hydrochloric acid, made up to volume with reverse osmosis water and the concentration of mineral and trace elements determined using element-specific wavelengths on an IRIS Intrepid II spectrometer (Thermo Fisher Scientific, USA).

### Statistical methods

Data is reported as the mean±standard deviation. Statistical analyses were performed using one-way analysis of variance (ANOVA) (SigmaStat v.3.5) with a 5 % level of probability ($P<0.05$) selected in advance to sufficiently demonstrate a statistically significant difference. Where significant differences were observed, treatment means were differentiated using pairwise multiple comparison procedures (Tukey multiple range test). Correlations made between variables were calculated in SigmaStat v.3.5 by simple Pearson correlation matrix. All raw data were confirmed to have a normal distribution using the Kolmogorov-Smirnov test (SigmaStat v.3.5).

## Results

### Chemical composition

Proximate composition, gross energy, and total phenolic contents of microalgae are shown in Table 1. Most species studied contained similar levels of nitrogen (N, 5.87–8.35 %), representing 28.1–39.9 % crude protein. Alternatively, *N. granulata* (B) was lower in N (3.74 %) and crude protein (17.9 %) while *T. chuii* contained the highest levels of N (9.72 %) and crude protein (46.5 %). *T. chuii*, *P. aerugineum*, and *N. oleoabundans* contained the lowest crude lipid levels (12.3–15.4 %); *B. braunii* (A) and *N. granulata* (B) the highest (34.4–47.8 %); and the others at intermediate levels (18.2–24.9 %). *B. braunii* (A) contained the lowest carbohydrate level (18.5 %), *P. aerugineum* the highest (45.8 %), and the others at intermediate levels (25.0–38.6 %). Ash levels were generally low (5.4–8.9 %) for most species, except *P. tricornutum*, *N. oleoabundans*, *A. dimorphus*, and *T. chuii* (14.5–17.0 %). A similar gross energy range (19.2–20.9 MJ $kg^{-1}$) was observed for *P. tricornutum*, *N. oleoabundans*, *P. aerugineum*, *A. dimorphus*, and *T. chuii*, while the values were higher (23.6–26.7 MJ $kg^{-1}$) for *N. granulata* (A and B) and *B. braunii* (A and B). TPC was low (6.0–9.9 mg GAE $g^{-1}$ DW) for most species studied while moderate levels were observed for *B. braunii* (A) and *T. chuii* (13.2–20.0 mg GAE $g^{-1}$ DW).

### Amino acid profile and elemental composition

Amino acid profiles and elemental composition of selected microalgae are reported in Tables 2 and 3. *P. aerugineum* contained significantly higher levels (g100 g $protein^{-1}$) of most essential amino acids (EAA) and non-essential amino acids (NEAA) than other species at threonine (5.8), valine (7.3), methionine (3.7), isoleucine (7.1), leucine (11.9), phenylalanine (6.3), tryptophan (3.3), aspartic acid (15.0), serine (7.0), alanine (8.4), and tyrosine (5.8). *N. granulata* (A) was richest in histidine (2.3), lysine (8.5), proline (11.2), and glycine (7.5). *P. tricornutum* contained the highest level of glutamic acid (18.8); *B. braunii* (A) had by far the highest level of arginine (20.5); and *T. chuii* was highest in cysteine (2.8). EAA indices followed accordingly: *P. aerugineum* (1.25)>*N. granulata* (A) (1.18)>*B. braunii* (A) (1.03)>*P. tricornutum* and *T. chuii* (0.89–0.91). *T. chuii* contained significantly higher levels of most minerals and trace elements than other species at calcium (2.99 %), phosphorous (1.46 %), sulfur (1.38 %), copper (102.2 mg $kg^{-1}$), and zinc (63.7 mg $kg^{-1}$). *P. tricornutum* was richest in magnesium (0.71 %), potassium (2.39 %), sodium (2.66 %), and sulfur (1.38 %). *B. braunii* (A) contained the highest levels of phosphorous (1.45 %) and manganese (453.7 mg $kg^{-1}$), and *P. aerugineum* was highest in iron (11,100.7 mg $kg^{-1}$).

### In vitro protein digestibility

IVPD of whole and lipid-extracted microalgae is reported in Table 4. With regard to the whole microalgae, *A. dimorphus* had significantly lower IVPD (77.8 %) than all other species studied, *P. aerugineum* had the highest (93.7 %), and other species were intermediate and in a range of 82.5–90.0 %. A similar trend was observed for lipid-extracted microalgae where *A. dimorphus* also had significantly lower IVPD (78.1 %) than all other species studied, *P. aerugineum* and *B. braunii* (A) had highest (92.6–96.6 %), and other species were intermediate and in a range of 85.1–91.1 %. Within

**Table 2** Amino acid profile of selected microalgae (g100 g protein$^{-1}$; $n$=2; DW basis)

| | *Phaeodactylum tricornutum* | *Nannochloropsis granulata* (A) | *Botryococcus braunii* (A) | *Porphyridium aerugineum* | *Tetraselmis chuii* | Egg albumin[a] | *Chlorella*[b] |
|---|---|---|---|---|---|---|---|
| Essential amino acids (EAA) | | | | | | | |
| Threonine | 4.8±0.00 b | 5.4±0.02 c | 3.7±0.01 a | 5.8±0.07 d | 4.0±0.16 a | 5.0 | 4.0 |
| Valine | 5.1±0.02 a | 7.1±0.02 b | 4.4±0.02 c | 7.3±0.12 d | 4.8±0.05 e | 7.2 | 5.3 |
| Methionine | 2.7±0.02 a | 3.5±0.01 b | 2.5±0.03 c | 3.7±0.03 d | 2.4±0.04 e | 3.2 | 2.2 |
| Isoleucine | 4.6±0.02 b | 5.6±0.02 c | 3.4±0.01 a | 7.1±0.10 d | 3.4±0.08 a | 6.6 | 3.8 |
| Leucine | 7.0±0.02 a | 11.0±0.04 b | 7.1±0.03 a | 11.9±0.13 c | 7.3±0.11 a | 8.8 | 7.8 |
| Phenylalanine | 4.8±0.01 b | 6.2±0.01 a | 4.4±0.02 c | 6.3±0.05 a | 4.7±0.10 b | 5.8 | 4.7 |
| Histidine | 1.5±0.01 a | 2.3±0.00 b | 1.5±0.01 a | 1.9±0.03 c | 1.6±0.01 a | 2.4 | 1.8 |
| Lysine | 6.4±0.01 a | 8.5±0.06 b | 4.7±0.01 c | 8.0±0.20 d | 5.6±0.00 e | 5.3 | 5.2 |
| Arginine | 5.7±0.00 a | 7.4±0.04 b | 20.5±0.01 c | 8.6±0.07 d | 9.4±0.19 e | 6.2 | 5.5 |
| Tryptophan | 2.6±0.09 ab | 2.8±0.07 b | 2.2±0.01 a | 3.3±0.15 c | 2.3±0.11 ab | 1.7 | 0.8 |
| EAAI[c] | 0.91±0.01 a | 1.18±0.01 c | 1.03±0.00 b | 1.25±0.00 d | 0.89±0.00 a | 1.0 | 0.76 |
| Non-essential amino acids (NEAA) | | | | | | | |
| Aspartic acid | 11.6±0.04 a | 11.4±0.15 a | 8.7±0.02 b | 15.0±0.31 c | 14.1±0.08 d | 11.0 | 7.8 |
| Serine | 4.8±0.02 a | 5.6±0.01 b | 3.5±0.01 c | 7.0±0.04 d | 4.2±0.03 e | 6.9 | 3.3 |
| Glutamic acid | 18.8±0.00 a | 14.1±0.09 b | 12.7±0.02 c | 15.6±0.26 d | 12.0±0.19 e | 12.6 | 9.7 |
| Proline | 7.1±0.01 a | 11.2±0.03 b | 4.6±0.02 c | 5.0±0.02 d | 3.6±0.10 e | 4.2 | 4.2 |
| Glycine | 5.5±0.01 a | 7.5±0.00 b | 4.9±0.03 c | 7.0±0.06 d | 6.5±0.09 e | 4.2 | 5.2 |
| Alanine | 7.3±0.00 a | 7.1±0.08 a | 6.4±0.06 b | 8.4±0.08 c | 6.0±0.13 d | – | 7.2 |
| Cysteine | 1.5±0.02 a | 1.6±0.01 a | 1.4±0.00 b | 2.2±0.03 c | 2.8±0.03 d | 2.3 | – |
| Tyrosine | 3.4±0.00 b | 4.2±0.01 c | 2.8±0.01 a | 5.8±0.06 d | 3.0±0.12 a | 4.2 | – |

Values within the same row having different letters are significantly different ($P$<0.05)

[a] Reported by Becker (2007)

[b] Product specification sheet

[c] Essential amino acid indices calculated according to Oser (1951)

**Table 3** Elemental composition of selected microalgae ($n$=2; DW basis)

| | *Phaeodactylum tricornutum* | *Nannochloropsis granulata* (A) | *Botryococcus braunii* (A) | *Porphyridium aerugineum* | *Tetraselmnis chuii* | Literature values[a] |
|---|---|---|---|---|---|---|
| Minerals (%) | | | | | | |
| Calcium | 0.26±0.00 c | 0.09±0.00 a | 0.10±0.00 b | 0.64±0.01 d | 2.99±0.00 e | 0.3–2.1 |
| Magnesium | 0.71±0.00 e | 0.26±0.00 a | 0.36±0.01 b | 0.55±0.01 d | 0.43±0.00 c | 0.1–1.1 |
| Phosphorous | 1.17±0.00 b | 0.73±0.00 a | 1.45±0.02 d | 1.39±0.01 c | 1.46±0.00 d | 1.7–3.0 |
| Potassium | 2.39±0.01 e | 1.50±0.01 c | 0.75±0.01 b | 0.67±0.01 a | 1.86±0.01 d | 0.6–1.2 |
| Sodium | 2.66±0.02 d | 1.03±0.00 c | 0.94±0.02 b | 0.81±0.02 a | 0.89±0.00 b | 0.7–1.1 |
| Sulfur | 1.38±0.01 d | 0.58±0.01 b | 0.41±0.01 a | 0.64±0.01 c | 1.38±0.00 d | 1.4 |
| Trace elements (mg kg$^{-1}$) | | | | | | |
| Copper | 54.8±0.4 d | 17.8±1.2 a | 35.2±1.4 b | 45.3±1.1 c | 102.2±0.7 e | 12–650 |
| Iron | 4,772.7±22.4 b | 1,394.8±0.0 a | 6,203.1±97.2 c | 11,100.7±221.1 d | 1,773.7±14.7 a | 1,000–7,000 |
| Manganese | 45.1±0.2 a | 150.8±0.0 b | 453.7±7.5 e | 258.5±4.4 d | 191.4±0.7 c | 37–592 |
| Selenium | 0.5±0.0 ns | 0.5±0.1 | <DL | <DL | 0.5±0.1 | ND |
| Zinc | 50.0±0.4 c | 32.0±2.3 a | 27.8±0.5 a | 41.0±0.6 b | 63.7±0.5 d | 239–3,700 |

Values within the same row with different letters are significantly different ($P$<0.05).

<*DL* below detection limit, *ND* no data

[a] From Campanella et al. (1998) and Volkman and Brown (2006)

**Table 4** Crude protein, in vitro protein digestibility (IVPD) and digestible protein content of whole and lipid-extracted microalgae ($n$=3; DW basis)

| Species | Whole microalgae | | | Lipid-extracted microalgae | | | $P$ value[a] |
|---|---|---|---|---|---|---|---|
| | Crude protein (%) | IVPD (%) | Digestible protein (%) | Crude protein | IVPD (%) | Digestible protein (%) | |
| *Spirulina* | 55.8 | 87.8±0.7 fg | 49.0 | 59.9 | 88.6±0.4 cd | 53.1 | 0.194 |
| *Chlorella* | 53.3 | 87.2±1.3 def | 46.5 | 56.5 | 85.9±0.1 bc | 48.5 | 0.148 |
| *Phaeodactylum tricornutum* | 39.6 | 88.7±0.4 fg | 35.1 | 42.9 | 89.5±0.9 de | 38.4 | 0.204 |
| *Nannochloropsis granulata* (A) | 33.5 | 84.5±1.3 bcd | 28.3 | 41.3 | 87.8±1.2 bcd | 36.3 | 0.033** |
| *Nannochloropsis granulata* (B) | 17.9 | 84.9±0.4 cde | 15.2 | 33.9 | 91.1±0.1 de | 30.9 | <0.001** |
| *Botryococcus braunii* (A) | 39.9 | 90.0±0.9 g | 35.9 | 52.1 | 96.6±0.3 f | 50.3 | 0.003** |
| *Botryococcus braunii* (B) | 39.1 | 87.4±0.7 efg | 34.2 | 49.9 | 88.6±2.3 bcd | 44.2 | 0.561 |
| *Neochloris oleoabundans* | 30.1 | 88.8±0.2 fg | 26.7 | 33.3 | 89.8±1.1 de | 29.9 | 0.205 |
| *Porphyridium aerugineum* | 31.6 | 93.7±0.1 h | 29.6 | 32.3 | 92.6±0.7 ef | 29.9 | 0.177 |
| *Acutodesmus dimorphus* | 28.1 | 77.8±0.9 a | 21.9 | 33.4 | 78.1±1.9 a | 26.1 | 0.813 |
| *Tetraselmis chuii* | 46.5 | 82.5±0.4 b | 38.4 | 50.5 | 85.1±0.3 b | 43.0 | <0.001** |

In vitro multi-enzyme protein digestibility using sodium caseinate as the control and the in vitro multi-enzyme protein digestibility of test microalgae expressed as a relative percentage of that of sodium caseinate normalized at 100 %. Values within the same column with different letters represent a significant difference between species ($P$<0.05)

[a] Indicates horizontal comparison significance levels within the same species (e.g., whole IVPD vs lipid-extracted IVPD)

species, there were no significant differences in IVPD observed between whole and lipid-extracted microalgae for *P. tricornutum* (88.7 and 89.5 %, $P$=0.204), *B. braunii* (B) (87.4 and 88.6 %, $P$=0.561), *N. oleoabundans* (88.8 and 89.8 %, $P$=0.205), *P. aerugineum* (93.7 and 92.6, $P$=0.177), and *A. dimorphus* (77.8 and 78.1 %, $P$=0.913). In contrast, IVPD of lipid-extracted microalgae was significantly higher than whole microalgae for *N. granulata* (A) (84.5 and 87.8, $P$=0.033), *N. granulata* (B) (84.9 and 91.1 %, $P$<0.001), *B. braunii* (A) (90.0 and 96.6 %, $P$=0.003), and *T. chuii* (82.5 and 85.1 %, $P$<0.001).

## Discussion

Since algal biomass characterization is still in its infancy, analytical methods established for other conventional ingredients are far from standardized and often inadequate for microalgae (see review of Laurens et al. 2012). Most samples were low-ash (<10 %) while others (e.g., *P. tricornutum*, *N. oleoabundans*, *A. dimorphus*, and *T. chuii*) contained moderate ash levels (14–17 %). The observed range (5–17 %) is consistent for freshwater and marine microalgae (4–20 %) with exception of silica-rich diatoms that may contain up to 43 % ash (Rebolloso-Fuentes et al. 2001; Volkman and Brown 2006). Crude lipid content was relatively high for most microalgae (12–48 %). Crude lipid describes the sum of all substances soluble in lipophilic organic solvents and is highly dependent upon the specificity and polarity of the solvents used and extraction protocols employed. Microalgae pose a particular challenge for the determination of reliable lipid content data since the organic solvents used in the extraction co-extract chlorophyll and other pigments to varying degrees (Palmquist and Jenkins 2003). Lipid content of various freshwater and marine microalgae is highly variable with reports typically in the 1–38 % range (see review of Becker 2013). Microalgae contained 18–46 % carbohydrate which is in the vast range typically reported for microalgae at 4–64 % (Volkman and Brown 2006; Demirbas and Demirbas 2011; Becker 2013). Carbohydrate content of algae is not only highly variable depending upon species, growth phase, and culture conditions but also due to differing analytical methods and the terms in which carbohydrate content is expressed. The differences in carbohydrate quantification and reporting methods used in the literature make it difficult to compare studies between and within species and under the same culture conditions. Although carbohydrate values calculated based on the difference in the sum of other biomass components (e.g., moisture, ash, protein (N×6.25), and fat) is a routine practice in animal nutrition (McDonald et al. 2010), the values reported here may be overestimated to some degree. This method does not "directly" take into account the content of nucleic acids, which can reach up to 6 % of microalgae (Becker 2013). Since crude protein values in this study were calculated using a nitrogen-to-protein conversion factor (N×4.78) that effectively excludes microalgae-specific nucleic acids, any contribution, although likely minimal, will be accounted for in the estimation of carbohydrate content. Crude protein (CP) content of whole and lipid-extracted microalgae (Table 4) ranged

between 18–60 % with most species containing relatively high levels (>30 %). Elemental analysis of total N and the use of appropriate nitrogen-to-protein (N-to-P) conversion factor (N×4.78) were used to evaluate the CP content of microalgae in this study. This approach eliminates the need for prior protein extraction (e.g., cell wall disruption) and interferences caused by carotenoids and chlorophyll that are difficult to reproduce and may lead to conflicting results. Comparison of CP data with literature values is difficult because the N-to-P conversion factor routinely used for plant and animal feed proteins and for labeling (N×6.25) has been indiscriminately and erroneously applied to microalgae by most authors (Chronakis 2000; Becker 2007). This N-to-P factor overlooks the assumption that the protein source contains 16 % N (Mariotti et al. 2008) and does not adequately take into account the content of non-protein nitrogen (NPN) such as structural proteins, bioactive peptides, free amino acids, nucleic acids, and ammonia found in microalgae. The NPN content of microalgae has been reported to be 4 to 40 % depending upon species, season, and growth phase (Lourenço et al. 2004; González López et al. 2010; Becker 2013). The most comprehensive and commonly cited summary of CP values for microalgae (Chacón-Lee and González-Marino 2010) range between 6 and 71 % and likely overestimate the CP content. The commercial *Spirulina* and *Chlorella* products used in this study both report containing 67 % CP, as-is basis (70 % DW basis). Had the N-to-P factor of N×4.78 been used, the CP content of these products would be 53 % DW basis, which more closely resembles values reported here (53–56 % of DW). Of the microalgae studied, *P. tricornutum*, *B. braunii* (A), and *T. chuii* had the highest CP levels (>40 %). *P. tricornutum* and *B. braunii* (A) had virtually the same crude protein content (average 39.7 %) which is in agreement with the value (39.6 %) reported by Sydney et al. (2010) for *B. braunii*. The marked difference in CP and crude lipid contents between *N. granulata* (A) (34 and 24 %, respectively) and *N. granulata* (B) (18 and 48 %, respectively) is consistent with reports showing that accumulation of non-protein energy, such as lipid, can be greatly enhanced under nitrogen-limited conditions (Cohen et al. 1995). The energy density of microalgal biomass has not been widely reported. Since microalgae were generally high in crude lipid and crude protein, energy content was relatively high as well (19–27 MJ $kg^{-1}$). This is consistent (19–29 MJ $kg^{-1}$) with other species cultured for biofuel applications (McGinn et al. 2011) and higher than levels (10–20 MJ $kg^{-1}$) reported for species cultured for mariculture (Whyte 1987). This is not surprising as the species in this study and the aforementioned study had similar protein levels (18–46 and 20–42 %, respectively) but quite different lipid levels (12–48 and 6–23 %, respectively) which likely reflect very different optimization strategies.

Phenolic compounds are a diverse class of secondary metabolites (e.g., flavonoids, phenolic acids, phenolic alcohols, stilbenes, tannins, and lignans) that contain a polyphenol structure consisting of two or more six-carbon aromatic rings. TPC has been reported recently for various microalgae species in relation to their antioxidant potential (Duval et al. 2000; Li et al. 2007; Goh et al. 2010; Hajimahmoodi et al. 2010; Custódio et al. 2012; Goiris et al. 2012). However, since plant-based phenolic compounds also play both beneficial and detrimental roles in animal nutrition (in particular protein bioavailability), their measurement is important and has not yet been reported for most of the species studied here. Specific phenolic compounds such as terrestrial plant-derived tannins have been shown to provide beneficial effects by inhibiting harmful bacterial colonization of the intestine, as demonstrated in terrestrial livestock such as poultry (Jamroz et al. 2003), swine (Van Parys et al. 2010), and rabbits (Króliczewska et al. 2011). However, this has yet to be demonstrated in farmed animals and fish fed algae-derived phenolic sources. As mentioned, the determination of TPC in the present study was important because of their potential negative effects on protein quality. Plant-based phenolic compounds have the ability to precipitate dietary protein, causing reduced protein digestibility and amino acid availability (Julkunen-Tiitto 1985). The mechanism by which these compounds may reduce protein digestibility is through hydrogen-binding and/or oxidation of proteins and amino acids rendering them unavailable to proteolytic enzyme hydrolysis and subsequent intestinal absorption. Several recent studies have reported the TPC of algal-derived ingredients including seaweeds (Wong and Cheung 2001; Rupérez et al. 2002; Heffernan et al. 2014) and microalgae (Duval et al. 2000; Li et al. 2007; Goh et al. 2010; Hajimahmoodi et al. 2010; Custódio et al. 2012; Goiris et al. 2012). However, comparison of TPC between feed ingredients is difficult because of the high level of heterogeneity of the broad classification of phenolic compounds; thus, it is only possible to get relative equivalents with the standards used (Julkunen-Tiitto 1985). In addition, various studies have reported TPC for very different algal species and employing different extraction methods (e.g., hot water, alcohols, and organic solvents). For this particular assay, Singleton et al. (1999) used ethanol, Velioglu et al. (1998) used methanol, and Wong and Cheung (2001) used acetone. Although Singleton et al. (1999) used ethanol in those studies, these authors recommended that diluted aqueous solutions of other solvents un-reactive in the assay may also be acceptable (acetone, methanol, dimethylformamide), provided that testing and preparation of standards were performed in the same solution. Although our pilot studies using different extraction solvents (acetone, ethanol, methanol) showed acetone to be the preferred solvent for this assay, which agrees with Wong and Cheung (2001) also studying algae, further studies are warranted to optimize the extraction of phenolic compounds

from microalgal biomass. The generally low to moderate TPC of microalgae species in this study (6–20 mg GAE $g^{-1}$ DW) is in good agreement with other reports for other microalgae (0–20 mg GAE $g^{-1}$ DW). As such, it seems unlikely that TPC in microalgal biomass will contribute to any significant detrimental effects on protein bioavailability.

The microalgae species studied had EAA and NEAA levels similar to and often exceeding that of the commercially purchased *Chlorella* product. Characteristic of microalgae, the NEAAs aspartic acid and glutamic acid were predominant at 20–30 % of the protein (8–12 % of DW). With respect to protein quality, ingredients for human and animal consumption are said to be of high nutritional value if they have an EAA index of >0.90, moderate quality when 0.70–0.89, and low quality when <0.70 (Brown and Jeffrey 1992). The EAA indices of *P. tricornutum* (0.9) and *T. chuii* (0.9) exceeded that of *Chlorella* (0.8) while that of *N. granulata* (A) (1.2), *B. braunii* (A) (1.0), and *P. aerugineum* (1.2) were equal or superior to that of an ideally balanced protein (egg albumin protein, 1.0). EAA index values for microalgal biomass are rarely found in published literature and have not been reported for these particular algal species. Therefore, this is novel data for these species and is in general agreement with Brown and Jeffrey (1992) who also observed good protein quality of other microalgae species based on moderate to high EAA indices (0.80–0.93). In comparison with the EAA content (expressed as g EAA 100 g $protein^{-1}$) of egg albumin protein (considered to be "ideal"), *P. aerugineum* was particularly rich in leucine (11.9), lysine (8.0), arginine (8.6), and tryptophan (3.3); *N. granulata* (A) rich in leucine (11.0), lysine (8.5), and tryptophan (2.8); *B. braunii* and *T. chuii* rich in arginine (9.4–20.5); and *P. tricornutum* rich in lysine (6.4) and tryptophan (2.6). Although limited AA data for certain microalgal species has been published previously (see review of Brown et al. 1997), cultures have typically been grown as live feed for mariculture and generally harvested during exponential growth (log phase). To our knowledge, this is one of the first reports of AA profile data for these particular algal species cultured in enclosed 1,000-L photobioreactors and harvested in the stationary phase with a focus on lipid accumulation for biofuel applications. The results provide additional consensus that the balance of amino acids of microalgal biomass compare very well and often exceed those of other conventional plant-based protein sources. For instance, the EAAs lysine, methionine, and tryptophan are typically limiting and often deficient in plant-based protein meals commonly used in animal nutrition (e.g., soybean, canola, sunflower, and flax). Reported levels (% of DW) for these products are 1.2–2.2 % (lysine), 0.6–1.5 % (methionine), and 0.4–0.7 % (tryptophan) (NRC 1994, 2011) while the algal biomass studied here generally contained higher levels: 1.9–2.8 % (lysine), 1.0–1.2 % (methionine), and 0.9–1.1 % (tryptophan). Algal biomass could be desirable as a supplement in animal feeds and aquaculture to provide EAAs and reduce the costs associated with crystalline amino acid supplementation, provided that adequate bioavailability can be demonstrated.

Mineral compositions (%) of microalgae were calcium (0.1–3.0), magnesium (0.3–0.7), phosphorous (0.7–1.5), potassium (0.7–2.4), sodium (0.8–2.7), and sulfur (0.4–1.4), and trace element compositions (mg $kg^{-1}$) were copper (18–102), iron (1,395–11,101), manganese (45–454), selenium (0–0.5), and zinc (28–64). There is limited data available on the elemental composition of microalgal biomass, which is in contrast to macroalgae (seaweeds) biomass where numerous species have been well characterized (see reviews of MacArtain et al. 2007; Venugopal 2009). This lack of information may be related to the fact that major efforts toward commercialization of microalgae have generally focused on its organic constituents (e.g., essential PUFA, high-value pigments, and energy feedstock) and less on their inorganic elemental content. This is not entirely unexpected because, although microalgae may contain certain elements of interest for nutrition, the inorganic elemental composition (ash) of microalgae (excluding diatoms) is generally much lower (4–20 %) than macroalgae (22–64 %) (McDermid and Stuercke 2003; Volkman and Brown 2006). We could only find two reports that provide data on the elemental content in algal biomass (Campanella et al. 1998; Volkman and Brown 2006). Although useful for comparison, the data did not include certain elements and was limited to species which were not part of this study. Mineral compositions (%) reported in these studies were calcium (0.3–2.1), magnesium (0.1–1.1), phosphorous (1.7–3.0), potassium (0.6–1.2), sodium (0.7–1.1), and sulfur (1.4), and trace element compositions (mg $kg^{-1}$) were copper (12–650), iron (1,000–7,000), manganese (37–592), and zinc (239–3,700).

The total protein content of feed ingredients has limited application for making nutritional claims because it does not provide information regarding the extent of hydrolysis during digestion prior to absorption of amino acids and other hydrolytic products (e.g., peptides). Generally, this is accomplished through in vivo and/or in vitro protein digestibility bioassays. In vivo protein digestibility data of microalgae fed to farmed animals and fish is scarce and inconclusive with reported values typically determined with rats which are highly variable (11–87 %) in the literature (Mišurcova et al. 2010). A limited number of reports using varying in vitro methods have shown a wide range of IVPD (27–97 %) for a small selection of microalgae species. Reported IVPD for *Chlorella* are 55–66 % (Tamiya 1962), 27–93 % (Hedenskog et al. 1969), 44–57 % (Janczyk et al. 2005), 70–97 % (Morris et al. 2008), and 61–79 % (Mišurcova et al. 2010). IVPD for *Spirulina* has been reported to be 84 % (Lipinsky and Litchfield 1974) and 70–85 % (Devi et al. 1981), and work with other species

(*Nostoc*, *Scenedesmus*, and *Microcystis*) have shown IVPD values of 29–93 % (Hedenskog et al. 1969; de la Fuente et al. 1977; Hori et al. 1990). This large variability may be related to species differences but more likely due to differences in methods used such as differences in enzyme mixtures, sample processing, assay conditions, and the extent of algal cell wall disruption. The assay used in the present study involved proteolytic enzymatic hydrolysis of the dried algae protein substrate exposed to a standardized mixture of commercial purified enzymes (trypsin, chymotrypsin, and peptidase) at 37 °C and an initial pH of 8.0. The method is referred to as an indirect "pH-drop" assay since the enzymatic cleavage of peptide bonds releases hydrogen ions, thus decreasing the pH. This particular IVPD assay has been used previously with microalgal biomass (Morris et al. 2008), but data was only generated for one freshwater species (*Chlorella vulgaris*). The pH of the solution after 10 min has been highly correlated with in vivo protein digestibility measured in humans and rats (Hsu et al. 1977). In this study, the two commercial microalgae products (*Spirulina* and *Chlorella*) both report >80 % in vitro protein digestibility (using pepsin) which generally agrees well with Morris et al. (2008) who reported an average of 81 % (range 70–97 %) and also our IVPD values of 86–89 %. IVPD was high (>90 %) for whole and lipid-extracted *B. braunii* (A) and *P. aerugineum*, and lipid-extracted *N. granulata* (B); mid-range (80–89 %) for whole and lipid-extracted *P. tricornutum*, *N. granulata* (A), *B. braunii* (B), *N. oleoabundans*, *T. chuii*, and whole *N. granulata* (B); and lower (<80 %) for whole and lipid-extracted *A. dimorphus*. The lower IVPD observed for *A. dimorphus* is consistent with Subbulakshmi et al. (1976) who also found lower IVPD for a related strain (*Scenedesmus acutus*=*Scenedesmus obliquus*) and may be related to its rigid cell wall (Miranda et al. 2012). Reduced protein digestibility of algae can also be attributed to relatively high levels of soluble polysaccharide fiber that can entrap proteins in the cellular matrix, rendering them less bioavailable to enzymatic hydrolysis (Marrion et al. 2005) and also to inhibitory effects of TPC (Mabeau and Fleurence 1993; Bobin-Dubigeon et al. 1997). For the majority of microalgae studied here, IVPD was mid-range to high (>82 %) and TPC was generally low (<20 mg GAE $g^{-1}$ DW; Table 1); however, no significant correlation between TPC and IVPD was found ($r=-0.12$, $R^2=0.0145$, $P=0.497$). This is in contrast to macroalgae (seaweeds) that typically contain high TPC and which have been positively correlated to reductions in protein digestibility of seaweed biomass (Bobin-Dubigeon et al. 1997; Chronakis 2000; Wong and Cheung 2001).

Lipid extraction of the microalgae concentrated the non-lipid components such that crude protein contents were 2–21 % (average 9.3 %) higher than that of the whole microalgae. For the most part, lipid extraction had no significant effect ($P>0.05$) or only a marginal (<2 %) effect on IVPD with values for whole microalgae of 78–94 % (average 86.7 %) and lipid-extracted microalgae of 78–97 % (average 88.5 %). The exceptions were a few microalgae with relatively high crude lipid levels (*N. granulata* (A and B), *B. braunii* (A), and *T. chuii*) where small, but significant ($P<0.05$) increases in IVPD (5 %) were observed for whole microalgae of 82–90 % (average 85.5 %) and lipid-extracted microalgae of 85–97 % (average 90.1 %). These small increases are consistent with Hsu et al. (1977) who observed a similar trend toward increased IVPD after the samples had been lipid-extracted. These authors reported that since the "trend" was generally insignificant, extraction of fat prior to multi-enzyme protein hydrolysis is unnecessary, and our results generally support this conclusion. However, since a small but significant increase in IVPD was observed for a few microalgae samples with high crude lipid levels in this study, it may be that this recommendation has limitations. In the study reported by Hsu et al. (1977), the crude lipid levels were low (<11 %), whereas in this study, crude lipid levels ranged from 12 to 48 %. The significant increase in IVPD in lipid-extracted microalgae generally occurred with samples having the highest crude lipid contents (>24 %). In fact, the trend was significant ($P<0.001$) with increased IVPD in lipid-extracted microalgae positively correlated ($r=0.76$, $R^2=0.73$) with original crude lipid content, over the range of 15–48 % crude lipid. Further evidence to support a lipid extraction treatment prior to utilizing this particular in vitro assay is provided by Morris et al. (2008) who reported a modest (5.5 %) but significantly higher IVPD in lipid-extracted *C. vulgaris* biomass relative to its non-extracted counterpart. The IVPD results for freshwater and marine microalgal biomass observed in this study (78–97 %) are comparable to those reported for other commonly consumed ingredients such as vegetables (68–80 %), tubers (71–88 %), grains (69–84 %), legumes (72–99 %), oilseeds (75–99 %), fruits (72–92 %), seaweeds (78–95 %), and *C. vulgaris* biomass (70–97 %). To our knowledge, this is the first study to utilize this in vitro method to evaluate the IVPD of whole and lipid-extracted microalgal biomass (with the exception of whole *C. vulgaris*) derived from a wide range of freshwater and marine microalgae.

The microalgae studied here were highly heterogeneous in their chemical composition even when cultivated under similar environmental conditions, reflecting their wide genetic and phenotypic diversity. We provide further evidence that microalgal biomass can contain not only high protein levels but also that the balance of amino acids compare well and in some cases (e.g., *N. granulata* (A), *B. braunii* (A), and *P. aerugineum*), exceed those of other conventional plant-based protein sources used in animal and fish nutrition. EAAs often limiting in conventional feed ingredients (e.g., lysine, methionine, and tryptophan), essential minerals (e.g.,

calcium, magnesium, phosphorous, potassium, and sodium), and certain trace elements (e.g., copper and manganese) were generally found at higher levels in microalgal biomass. Of the microalgae species studied here, *P. aerugineum* and *N. granulata* (A) were the richest source of most EAAs, *T. chuii* and *P. tricornutum* were the richest in most essential minerals and *T. chuii*, and *P. aerugineum* in key trace elements, especially copper and iron. These characteristics could make these species attractive as feed supplements if high bioavailability can be demonstrated in further studies. Total phenolic content of microalgae was relatively low (<20 mg GAE $g^{-1}$ DW) and seems unlikely to contribute to any significant detrimental effects on protein bioavailability and, indeed, preliminary assessment of protein quality (in vitro multi-enzyme digestibility) was generally mid-range to high for whole (78–94 %) and lipid-extracted microalgae (78–97 %), indicating good potential for use in animal feeds and aquaculture. More definitive investigations involving "species-specific" enzymatic in vitro digestibility and in vivo biological performance of target animals fed diets supplemented with these products are required.

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**Conflict of Interest** We certify that there is no conflict of interest with any financial organization regarding the material discussed in this manuscript.

## References

Ahmed F, Li Y, Schenk PM (2012) Algal biorefinery: sustainable production of biofuels and aquaculture feed? In: Gordon R, Seckbach J (eds) The science of algal fuels—phycology, geology, biophotonics, genomics and nanotechnology. Springer, NY, pp 23–41

Anderson RA, Berges JA, Harrison PJ, Watanabe MM (2005) Recipes for freshwater and seawater media. In: Anderson RA (ed) Algal culturing techniques. Elsevier, Burlington, p 507

Armstrong SM, Staples LS, Bauder AG, Craigie JS (2002) Photobioreactor, Canadian Patent CA2394518

Becker EW (2007) Micro-algae as a source of protein. Biotechnol Adv 25:207–210

Becker EW (2013) Microalgae for human and animal nutrition. In: Richmond A, Hu Q (eds) Handbook of microalgal culture: applied phycology and biotechnology, 2nd edn. Wiley-Blackwell, Oxford, pp 461–503

Bobin-Dubigeon C, Hoebler C, Lognone V, Dagorn-Scaviner C, Mabeau S, Barry JL, Lahaye M (1997) Chemical composition, physicochemical properties, enzymatic inhibition and fermentative characteristics of dietary fibres from edible seaweeds. Sci Aliments 17:619–639

Borowitzka MA (2013) High-value products from microalgae-their development and commercialization. J Appl Phycol 25:743–756

Brown MR, Jeffrey SW (1992) Biochemical composition of microalgae from the green algal classes *Chlorophyceae* and *Prasinophyceae*. 1. Amino acids, sugars and pigments. J Exp Mar Biol Ecol 161:91–113

Brown MR, Jeffrey SW, Volkman JK, Dunstan GA (1997) Nutritional properties of microalgae for mariculture. Aquaculture 151:315–331

Brune D, Lundquist T, Benemann J (2009) Microalgal biomass for greenhouse gas reductions: potential for replacement of fossil fuels and animal feeds. J Env Engineer 135:1136–1144

Campanella L, Crescentini G, Avino P, Moauro A (1998) Determination of macrominerals and trace elements in the alga *Spirulina platensis*. Analusis 26:210–214

Chacón-Lee TL, González-Marino GE (2010) Microalgae for "Healthy" foods—possibilities and challenges. Comp Rev Food Sci Food Safety 9:655–675

Chronakis IS (2000) Biosolar proteins from aquatic algae. Dev Food Sci 41:39–75

Cohen Z, Norman HA, Heimer YM (1995) Microalgae as a source of omega-3 fatty acids. World Rev Nutr Diet 77:1–31

Custódio L, Justo T, Silvestre L, Barradas A, Duarte CV, Pereira H, Barreira L, Rauter AP, Alberício F, Varela J (2012) Microalgae of different phyla display antioxidant, metal chelating and acetylcholinesterase inhibitory activities. Food Chem 131:134–140

de la Fuentes G, Flores A, Molina MR, Almengor L, Bressani R (1977) Some nutritional characteristics of a naturally occurring alga (*Microcystis* sp.) in a Guatemalan Lake. Appl Environ Microbiol 33:6–9

Demirbas A, Demirbas MF (2011) Importance of algae oil as a source of biodiesel. Energy Conver Manag 52:163–170

Devi MA, Subbulakshmi G, Devi KM, Venkataraman LV (1981) Studies on the proteins of mass-cultivated, blue-green alga (*Spirulina platensis*). J Agric Food Chem 29:522–525

Duval B, Shetty K, Thomas WH (2000) Phenolic compounds and antioxidant properties in the snow alga *Chlamydomonas nivalis* after exposure to UV light. J Appl Phycol 11:559–566

Environmental Protection Agency (2007) Test methods for evaluating solid waste physical/chemical methods, Methods EPA 6010C and 7471B

Fernández-Garcia E, Lérida-Carvajal I, Pérez-Gálvez A (2009) *In vitro* bioaccessibility assessment as a prediction tool of nutritional efficiency. Nutr Res 29:751–760

Goh SH, Yusoff FM, Loh SP (2010) A comparison of the antioxidant properties and total phenolic content in a diatom, *Chaetoceros* sp. and a green microalga, *Nannochloropsis* sp. J Agric Sci 2:123–130

Goiris K, Muylaert K, Fraeye I, Foubert I, Brabanter J, Cooman L (2012) Antioxidant potential of microalgae in relation to their phenolic and carotenoid content. J Appl Phycol 24:1477–1486

González López CV, Cerón García MC, Acién Fernández FC, Bustos CS, Chisti Y, Fernández Sevilla JM (2010) Protein measurements of microalgal and cyanobacterial biomass. Bioresource Technol 101: 7587–7591

Hajimahmoodi M, Faramarzi M, Mohammadi N, Soltani N, Oveisi M, Nafissi-Varcheh N (2010) Evaluation of antioxidant properties and total phenolic contents of some strains of microalgae. J Appl Phycol 22:43–50

Hedenskog G, Enebo L, Vendlová J, Prokeš B (1969) Investigation of some methods for increasing the digestibility *in vitro* of microalgae. Biotechnol Bioeng 6:37–51

Heffernan N, Smyth TJ, Soler-Villa A, Fitzgerald RJ, Brunton NP (2014) Phenolic content and antioxidant activity of fractions obtained from selected Irish macroalgae species (*Laminaria digitata*, *Fucus serratus*,

*Gracilaria gracilis* and *Codium fragile*). J Appl Phycol. doi:10.1007/s10811-014-0291-9

Heinriksen RL, Meredith SC (1984) Amino acid analysis by reverse-phase high-performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. Anal Biochem 136:65–74

Hori K, Ueno-Mohri T, Okita T, Ishibashi G (1990) Chemical composition, *in vitro* digestibility and *in vitro* available iron of blue green alga, *Nostoc commune*. Plant Foods Hum Nutr 40:223–229

Hsu HW, Vavak DL, Satterlee LD, Miller GA (1977) A multienzyme technique for estimating protein digestibility. J Food Sci 42:1269–1273

Jamroz D, Orda J, Kamel C, Wiliczkiewicz A, Wertelecki T, Skorupinska J (2003) The influence of phytogenic extracts on performance, nutrient digestibility, carcass characteristics, and gut microbial status in broiler chickens. J Anim Feed Sci 12:583–596

Janczyk P, Wolf C, Souffrant WB (2005) Evaluation of nutritional value and safety of the green microalgae *Chlorella vulgaris* treated with novel processing methods. Arch Zootech 8:132–147

Julkunen-Tiitto R (1985) Phenolic constituents in the leaves of Northern willows: methods for the analysis of certain phenolics. J Agric Food Chem 33:213–217

Króliczewska B, Miśta D, Zawadzki W, Wypchlo A, Króliczewski J (2011) Effects of a skullcap root supplement on haematology, serum parameters and antioxidant enzymes in rabbits on a high-cholesterol diet. J Anim Physiol Anim Nutr 95:114–124

Laurens LM, Dempster TA, Jones HDT, Wolfrum EJ, Van Wychen S, McAllister JSP, Rencenberger M, Parchert KJ, Gloe LM (2012) Algal biomass constituent analysis: method uncertainties and investigation of the underlying measuring chemistries. Anal Chem 84:1879–1887

Li HB, Cheng KW, Wong CC, Fan KW, Chen F, Jiang Y (2007) Evaluation of antioxidant capacity and total phenolic content of different fractions of selected microalgae. Food Chem 102:771–776

Lipinsky ES, Litchfield JH (1974) Single-cell protein in perspective. Food Technol 28:16–24

Lourenço SO, Barbarino E, Lavín PL, Marquez UML, Aidar E (2004) Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. Eur J Phycol 39:17–32

Mabeau S, Fleurence J (1993) Seaweeds in food products: biochemical and nutritional aspects. Trends Food Sci Technol 4:103–107

MacArtain P, Gill CIR, Brooks M, Campbell R, Rowland IR (2007) Nutritional value of edible seaweeds. Nutr Rev 65:535–543

Mariotti F, Tome D, Mirand PP (2008) Converting nitrogen into protein—beyond 6.25 and Jones' factors. Crit Rev Food Sci Nutr 48:177–184

Marrion O, Fleurence J, Schwertz A, Guéant JL, Mamelouk L, Ksouri J, Villaume C (2005) Evaluation of protein *in vitro* digestibility of *Palmaria palmata* and *Gracilaria verrucosa*. J Appl Phycol 17:99–102

Mata TM, Martins AA, Caetano NS (2010) Microalgae for biodiesel production and other applications: a review. Renew Sustain Energy Rev 14:217–232

McDermid KJ, Stuercke B (2003) Nutritional composition of edible Hawaiian seaweeds. J Appl Phycol 15:513–524

McDonald P, Edwards RA, Greenhalgh JFD, Morgan CA, Sinclair LA (2010) Animal Nutrition, 7th edn. Prentice Hall, New York, 692 p

McGinn PJ, Dickinson KE, Bhatti S, Frigon J-C, Guiot SR, O'Leary SJB (2011) Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: opportunities and limitations. Photosynth Res 109:231–247

Miranda JR, Passarinho PC, Gouveia L (2012) Pre-treatment optimization of *Scenedesmus obliquus* microalga for bioethanol production. Bioresource Technol 104:342–348

Mišurcova L, Kračmar S, Klejdus B, Vacek J (2010) Nitrogen content, dietary fiber, and digestibility in algal food products. Czech J Food Sci 28:27–35

Morris HJ, Almerales A, Carrillo O, Bermúdez RC (2008) Utilisation of *Chlorella vulgaris* cell biomass for the production of enzymatic protein hydrolysates. Bioresource Technol 99:7723–7729

National Research Council (2011) Nutrient requirements of fish and shrimp. National Academy Press, Washington, DC, 376 p

National Research Council (1994) Nutrient requirements of poultry. National Academy Press, Washington, DC, 155 p

Oser BL (1951) Method for integrating essential amino acid content in the nutritional evaluation of protein. J Am Diet Assoc 27:396–402

Palmquist DL, Jenkins TC (2003) Challenges with fats and fatty acid methods. J Anim Sci 81:3250–3254

Pulz O, Gross W (2004) Valuable products from biotechnology of microalgae. Appl Microbiol Biotechnol 65:635–648

Rebolloso-Fuentes MM, Navarro-Pérez A, García-Camacho F, Ramos-Miras JJ, Guil-Guerrero JL (2001) Biomass nutrient profiles of the microalga *Nannochloropsis*. J Agric Food Chem 49:2966–2972

Rupérez P, Ahrazem O, Leal JA (2002) Potential antioxidant capacity of sulfated polysaccharides from the edible marine brown seaweed *Fucus vesiculosis*. J Agric Food Chem 50:840–845

Singleton VL, Orthofer R, Lamuela-Raventós RM (1999) Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In: Abelson JN, Simon MI (eds) Methods in enzymology. Academic, New York, pp 152–178

Skjånes K, Rebours C, Lindblad P (2013) Potential for green microalgae to produce hydrogen, pharmaceuticals and other high value products in a combined process. Crit Rev Biotechnol 33:172–215

Stephens E, Ross II, King Z, Mussgnug JH, Kruse O, Posten C, Borowitzka MA, Hankamer B (2010) An economic and technical evaluation of microalgal biofuels. Nature Biotechnol 28:126–128

Subbulakshmi G, Becker WE, Venkataraman LV (1976) Effect of processing on the nutrient content of the green alga *Scenedesmus acutus*. Nutr Rep Int 14:581–591

Subhadra B, Grinson G (2011) Algal biorefinery-based industry: an approach to address fuel and food insecurity for a carbon-smart world. J Sci Food Agric 91:2–13

Sydney EB, Sturm W, Cesar de Carvalho J, Thomaz-Soccol V, Larroche C, Pandey A, Soccol CR (2010) Potential carbon dioxide fixation by industrially important microalgae. Bioresource Technol 101:5892–5896

Tamiya H (1962) Chemical composition and applicability as food and feed of mass cultured unicellular algae. Final Report no. 1 on Contract NODA 92-557-FEC-33129, U.S. Army Research and Development Group (9852)

Van Parys A, Boyen F, Dewulf J, Haesebrouck F, Pasmans F (2010) The use of tannins to control *Salmonella typhimurium* infection in pigs. Zoonoses Public Health 57:423–428

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

Venugopal V (2009) Seaweed: nutritional value, bioactive properties, and uses. In: Venugopal V (ed) Marine products for healthcare: functional and bioactive nutraceutical compounds from the ocean. CRC Press, Boca Raton, pp 261–295

Volkman JK, Brown MR (2006) Nutritional value of microalgae and applications. In: Subba Rao DV (ed) Algal cultures, analogues of blooms and applications. CABI, pp 407–457

White JA, Hart RJ, Fry JC (1986) An evaluation of the Waters Pico-Tag system for the amino-acid analysis of food materials. J Clin Lab Auto 8:170–177

Whyte JNC (1987) Biochemical composition and energy content of six species of phytoplankton used in mariculture of bivalves. Aquaculture 60:231–241

Wong KH, Cheung PCK (2001) Nutritional evaluation of some subtropical red and green seaweeds. Part II - *In vitro* protein digestibility and amino acid profiles of protein concentrates. Food Chem 72:11–17