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Publisher's version / Version de l'éditeur:
https://doi.org/10.1007/s10811-018-1523-1
Journal of Applied Phycology, 2018-05-31

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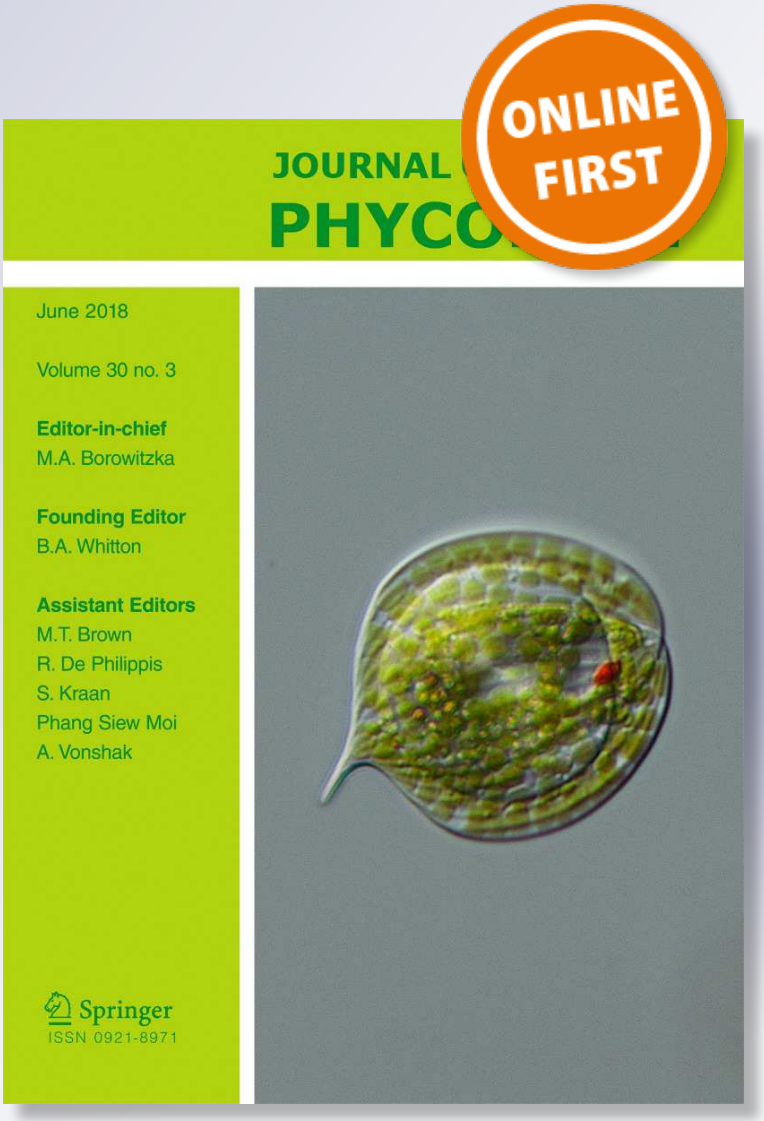
Journal of Applied Phycology

ISSN 0921-8971

J Appl Phycol
DOI 10.1007/s10811-018-1523-1



Springer

Journal of Applied Phycology
https://doi.org/10.1007/s10811-018-1523-1

Antioxidant properties and lipid composition of selected microalgae

Arjun H. Banskota[1] · Sandra Sperker[1] · Roumiana Stefanova[1] · Patrick J. McGinn[1] · Stephen J. B. O'Leary[1]

Received: 3 January 2018 / Revised and accepted: 16 May 2018

Abstract

Marine and freshwater microalgae were evaluated for their antioxidant properties and lipid composition. Both lipophilic (L) and hydrophilic (H) oxygen radical absorbance capacity (ORAC) values were measured. Among the tested microalgae, the marine microalga *Nannochloropsis granulata* showed the highest total ORAC value at 6948 μmol TE (100 g)$^{-1}$, followed by the freshwater species *Neochloris oleoabundans* at 4508 μmol TE (100 g)$^{-1}$ and *Scenedesmus obliquus* at 4406 μmol TE (100 g)$^{-1}$ of algal biomass. Freeze-dried algal biomass was also examined for lipid content by the Folch method. Lipid content ranged from 30.9 to 49.3% and linear regression analysis revealed a statistically significant correlation between the lipid content and the ORAC values. Tested microalgae showed moderate and dose-dependent DPPH radical scavenging activity. The total phenol assay shows that marine microalga *Tetraselmis chui* had the highest phenolic content at 57.5 μmol GAE g^{-1} and *N. oleoabundans* had the lowest at 26.6 μmol GAE g^{-1} of MeOH extract. A statistically significant correlation was detected between DPPH radical scavenging activity and the phenolic content. Fatty acid analysis revealed that polyunsaturated fatty acids constituted > 45% of the total fatty acid content in *N. oleoabundans*, *Phaeodactylum tricornutum*, *Porphyridium aerugineum*, *S. obliquus*, and *Scenedesmus* sp. Total carotenoid concentration ranged from 1.1 to 29.5 mg g^{-1} of dry algal biomass. Lutein, zeaxanthin, and β-carotene are common carotenoids found in most of the algae tested. *P. tricornutum* contains significant amount of fucoxanthin at 24.3 mg g^{-1} algal biomass. These carotenoids also contributed to the overall antioxidative activity of microalgae.

Keywords Microalgae · ORAC · Total phenolic · DPPH radical · Fatty acids · Carotenoids

Introduction

Microalgae are a diverse group of unicellular, primarily aquatic organisms capable of performing photosynthesis. Because some species are capable of producing high level of lipid, these microscopic organisms are considered as a potential source of bio-fuel (Spolaore et al. 2006). Marine species in particular have an added potential advantage if they are developed as an energy crop that can be grown in salt water which covers almost two thirds of the Earth's surface. Microalgae are also known to produce other high-value products including pigments, proteins, polysaccharides, and omega-3 fatty acids (Spolaore et al. 2006; Gantar and Svirčev 2008). *Haematococcus pluvialis* has been commercialized for production of the valuable carotenoid pigment astaxanthin and *Dunaliella salina* for β-carotene (Borowitzka 2013).

Free radicals including reactive oxygen species (ROS) and reactive nitrogen species (RNS) are generated during normal cellular metabolism. These free radicals are highly reactive species and play a dual role in humans as both beneficial and toxic compounds depending on their concentration. At low or moderate concentration, these reactive species exert beneficial effects on cellular redox signaling and immune function. At high concentration, however, these radical species produce oxidative stress, a harmful process that can lead to cell death through oxidation of protein, lipid, and DNA (Pham-Huy et al. 2008; Sen and Chakraborty 2011). Antioxidants are a crucial defense against free radical-induced damage (Sen and Chakraborty 2011). Several natural compounds such as vitamin C, tocopherol, and numerous plant extracts have been commercialized as natural antioxidants to fight against oxidative stress associated with various chronic diseases including atherosclerosis, diabetes mellitus, neurodegenerative disorders, and certain types of cancer (Pham-Huy et al. 2008; Vadlapudi 2012).

Even though microalgae are a rich source of bioactive molecules, only a limited number of studies have been performed to explore the correlation of antioxidant activity and lipid

✉ Arjun H. Banskota
Arjun.Banskota@nrc-cnrc.gc.ca

[1] Algal Carbon Conversion Flagship Program, Aquatic and Crop Resource Development, National Research Council Canada, 1411 Oxford Street, Halifax, Nova Scotia B3H 3Z1, Canada

Published online: 31 May 2018

composition. Choochote et al. (2014) observed that ethanoic extracts exhibited greater antioxidant activities compared to hot water extracts during their study on three microalgae strains including a *Chlorococcoccum* sp. and two *Chlorella* spp. Similarly, Goiris et al. (2012) screened 32 microalgal biomasses for their antioxidant capacity plus phenolic and carotenoid content. Their results indicated that carotenoid and phenolic compounds contributed to the antioxidant capacity of microalgae even though antioxidative potency varied between species, growth condition, and solvent used for extraction. Murthy et al. (2005) studied the in vivo antioxidant activity of carotenoids from *D. salina*, a green microalga exploited as a commercial source of β-carotene (Borowitzka 2013). Their study found that β-carotene plays a significant role in the reduction of oxidative stress (Murthy et al. 2005). Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish water was done by Ahmed et al. (2014), and an extensive study done by Safafar et al. (2015) tested microalgal species grown in industrial wastewater for their antioxidative properties. Phenolic compounds were found to be primarily responsible for the antioxidant activity, and carotenoids contributed for the DPPH radical scavenging activity, ferrous reduction power (FRAP), and ABTS-radical scavenging capacity activity assays. In our earlier studies, we have also evaluated anti-inflammatory and pancreatic lipase inhibitory activity of both marine and freshwater microalgae and identified several polar lipids as active metabolites (Banskota et al. 2013a–d, 2016). To continue our research on microalgae, we further explored and reported herein the antioxidative properties of several marine and freshwater microalgae species and examined how their chemical composition relates to the antioxidant activity.

Materials and methods

General

HPLC analyses were carried out on an Agilent 1200 Series system (USA) equipped with a diode array detector. Gas chromatography (GC) analyses were carried out on an Agilent Technologies 7890A GC spectrometer with an FID detector using an Omegawax 250 fused silica capillary column (Sigma-Aldrich, USA; 30 m × 0.25 mm × 0.25 μm film thickness). Supelco 37 component FAME mix and PUFA-3 (Supelco, USA) were used as fatty acid methyl ester standards. HPLC-grade solvents were used for the extraction and analyses. Fluorescein disodium, trolox, 2,2-azobis(2-methylpropionamideine) dihydrochloride (AAPH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, and Folin-Ciocalteu (FC) reagent were all obtained from Sigma-Aldrich. Randomly methylated beta cyclodextrin (RMCD) was purchased from Cyclodextrin Technologies Development Inc., Alachua, FL, USA.

Microalgae culture

Production of microalgae (Table 1) was carried out in a proprietary, internally illuminated closed photobioreactor maintaining 500 L of cultivation volume as described previously by Tibbetts et al. (2015). Cultivation conditions at the 500-L scale consisted of 24-h illumination provided by 16 full spectrum T10 (5000 K) fluorescent lamps (Satco Products, USA) that penetrated the volume of the culture, which was maintained at 21 °C. The cultivation medium was composed of either seawater for *Nannochloropsis granulata*, *Phaeodactylum tricornutum*, and *Tetraselmis chui* or fresh water for *Botryococcus braunii*, *Chlorella sorokiniana*, *Neochloris oleoabundans*, *Porphyridium aerugineum*, *Scenedesmus obliquus*, and *Scenedesmus* sp. (0.35 μm filtered, UV treated, and pasteurized for a minimum 8 h at 85 °C) supplemented with f/2 nutrients weekly at a non-limiting rate, and culture pH was maintained at 8.2 by the controlled addition of carbon dioxide. Approximately 400 L of culture was harvested weekly at stationary phase by process centrifugation and the collected material was freeze-dried resulting in a typical weekly yield of 200 g of freeze-dried biomass.

Table 1 List of microalgae tested for antioxidative activity and lipid composition

Microalgae	Collection center	Class	Source
Botryococcus braunii	UTEX 572	Chlorophyceae	Fresh water
Chlorella sorokiniana	UTEX 1230	Chlorophyceae	Fresh water
Nannochloropsis granulata	CCMP 535	Eustigmatophyceae	Marine
Neochloris oleoabundans	UTEX 1185	Chlorophyceae	Fresh water
Phaeodactylum tricornutum	CCMP 1327	Bacillariophyceae	Marine
Porphyridium aerugineum	UTEX LB 755	Porphyridiophyceae	Fresh water
Scenedesmus obliquus	UTEX 2016	Chlorophyceae	Fresh water
Scenedesmus sp.	AMDD - NRC	Chlorophyceae	Fresh water
Tetraselmis chui	PLY429	Prasinophyceae	Marine

Moisture, ash, and lipid content

Moisture content of the freeze-dried algal biomass was determined by oven drying triplicate samples of approximately 100 mg algal biomass at 110 °C for 12 h in a porcelain crucible. The average percentage of moisture content was calculated from the difference in the mass of samples before and after drying. The same samples were used for ash content determination by heating at 550 °C for 12 h in a muffle furnace. The remaining ash was slowly cooled down in a desiccator and its mass used to calculate the ash content percentage. Lipid content was determined by Folch method (Folch et al. 1957); freeze-dried algal biomass (~ 100 mg) was extracted with chloroform:methanol (2:1, 10 mL × 3) in triplicate. The combined lipid extract was dried under reduced pressure and total lipid content was calculated from the mass.

Fatty acid analysis

Fatty acid analysis was done according to AOAC official method 991.39 (AOAC 2000) with slight modification in triplicate. In brief, freeze-dried algal biomass (~10 mg) was placed in a dry 5-mL screw-capped reaction vial and MeOH (1.0 mL) containing 0.1 mg methyl tricosanoate as an internal standard (IS) was added. The mixture was sonicated and 1.5 N NaOH solution in MeOH (0.5 mL) was added, blanketed with nitrogen, heated for 5 min at 100 °C, and cooled for 5 min. BF_3 14% solution in MeOH (1.0 mL, Sigma-Aldrich, USA) was added, mixed, blanketed with nitrogen, and heated at 100 °C for 30 min. After cooling, the reaction was quenched by the addition of water (0.5 mL) and the FAME extracted with hexane (2.0 mL). Part of the hexane layer (300–600 μL) was transferred to a GC vial for analysis by GC-FID (described above). Fatty acid content in the freeze-dried algal sample was calculated by the following equation:

$$\text{Fatty acid}\left(\text{mg g}^{-1}\right) = \left(A_X \times W_{\text{IS}} \times \text{CF}_x / A_{\text{IS}} \times W_{\text{S}} \times 1.04\right) \times 1000$$

where A_X is area counts of fatty acid methyl ester; A_{IS} is area counts of internal standard (tricosylic acid methyl ester); CF_X is the theoretical detector correlation factor that is 1 except for EPA or DHA (0.99 for EPA, 0.97 for DHA); W_{IS} is weight of IS added to sample in milligram; W_{S} is sample mass in milligram; and 1.04 is the factor necessary to express result as milligram fatty acid per gram sample.

Carotenoid analysis

For determination of carotenoids, approximately 10 mg of freeze-dried sample was extracted at room temperature homogenizing with $CHCl_3$:MeOH (1:1, 1 mL × 3) using a bead beater (Bead Mill_{24}, Fisher Scientific, USA) in a 2-mL Lysing matrix Y tubes (3 × 1 min cycles) in duplicate. The combined extract was dried under N_2 gas and the residue dissolved in 1.0 mL MeOH for HPLC analysis. Extraction work was performed under dim light to minimize carotenoid decomposition. Carotenoid analysis was performed using an Agilent 1200 series HPLC with a YMC Carotenoid column (5 μm, 2 × 250 mm, 181 YMC Co. Ltd., Japan) eluting with 50 mM NH_4OAc in MeOH/tertiary butyl methyl ether (TBME) linear gradient 5 to 65% B in 30 min at 0.2 mL min^{-1} flow rate for 60 min. Standard curves for astaxanthin, α-carotene, β-carotene, canthaxanthin, fucoxanthin, lutein, lycopene, and zeaxanthin at 450 nm were used for carotenoid quantification. The concentration of unknown carotenoids was calculated using lutein as a standard.

Total phenolic content

Freeze-dried algal biomass (1–10 g) was extracted with MeOH (100 mL × 3). The combined MeOH extract was evaporated under reduced pressure and used to measure total phenolic content. Total phenolic content was determined based on a Folin-Ciocalteu (FC) method adapted to a 96-well microplate format (Zhang et al. 2006) with modifications in triplicate. Twenty microliter of diluted sample or standard was mixed with 40 μL of FC reagent (10%). After 5 min, 160 μL of 700 mM sodium carbonate was added and incubated for 2 h at room temperature. Absorbance was measured at 765 nm using a plate reader SpectraMax Plus (Molecular Devices). Gallic acid was used to generate a calibration curve, and the total phenolic content expressed as gallic acid equivalents (GAE) g^{-1} extract.

Oxygen radical absorbance capacity assay

An ASE 350 Accelerated Solvent Extractor was used for the extraction. Approximately 1.0 g freeze-dried algal biomass was loaded into 5 mL cells fitted with cellulose filters at the bottom and 100 mg of DE (Diatomaceous Earth) at both ends. Algal biomass was extracted in triplicate first with hexane/dichloromethane (50/50 *v*) at 70 °C by 3 cycles of 5-min static extraction (flushed with 60% fresh solvent and purged for 60 s between cycles) followed by acetone/water/acetic acid (70/29.5/0.5 *v*) at 80 °C (same ASE parameters). The hexane/dichloromethane extracts were dried under N_2 at 30 °C and the residue re-dissolved in 10 mL acetone for lipophilic-oxygen radical absorbance capacity (L-ORAC) measurement. The acetone/water/acetic acid extracts were transferred to 25-mL volumetric flasks and the

volume adjusted using the pre-mixed extraction solvent for hydrophilic-ORAC (H-ORAC) measurement. Both L-ORAC and H-ORAC were measured in triplicate.

H-ORAC and L-ORAC assays were performed as described previously by Wu et al. (2004) with modification. Both ORAC assays are based on the same principle, a fluorescent probe is oxidized by the addition of a free radical generator (APPH) which quenches the fluorescent probe over time. Antioxidants present in sample block the generation of free radicals until the antioxidant activity of sample is depleted. Randomly, methylated β-cyclodextrin (RMCD) is used in L-ORAC assay to enhance solubility of sample lipophilic content. For H-ORAC, 25 μL of sample or standard (Trolox) diluted in 75 mM phosphate buffer (pH 7.4) was added to wells followed by the addition of 150 μL of fluorescein (200 nM). Plates were incubated at 37 °C for 10 min and the reaction was initiated by addition of 25 μL of 150 mM AAPH. Fluorescence decay was monitored using a fluorescence plate reader (SpectraMax Gemini XS, Molecular Devices, USA) at an excitation wavelength of 485 nm and emission wavelength of 520 nm at 37 °C. Each extract was tested in triplicate at three concentrations. Trolox, a water-soluble vitamin E analog, was used as the calibration standard and the results were expressed as Trolox equivalents (TE).

For L-ORAC, samples and standards were diluted in 7% RMCD solution (acetone:water, 1:1 *v*/*v*) and the same procedure was followed as in the H-ORAC assay.

DPPH radical scavenging activity

DPPH radical scavenging activity was done according to the procedure described by Hatano et al. (1989) with minor modification. MeOH extract was used for this assay. In brief, 50 μL of extract at various concentrations was mixed with equal volume of 60 μM DPPH solution in MeOH; the resulting solution was thoroughly mixed and absorbance measured at 520 nm after 30 min using a SpectraMax Plus Spectrophotometer plate reader (Molecular Devices, USA). The scavenging activity was determined by comparing the absorbance with that of controls containing only DPPH and MeOH (100%). Vitamin C, a known antioxidant, was used as a positive control having IC_{50} value of 4.75 μg mL^{-1}. Measurements were carried out in triplicate.

Statistical analyses of correlation

Statistical analysis was performed using Microsoft Excel 2013 software. Linear regression analysis was conducted to test for correlations between microalgae lipid contents and oxygen radical absorbance capacities and between DPPH radical scavenging capacity and total phenolic contents. *p* values less than 0.05 were considered significant and positive *R*-squared values indicate the positive correlation between lipid contents and ORAC values as well as phenolic content and DPPH scavenging capacity.

Results

Lipids, moisture, and ash content

Lipids were extracted from freeze-dried algal biomass with $CHCl_3$:MeOH (2:1, Folch method). Total lipid content ranged from 30.9 to 49.3% of the freeze- dried algal biomass (Table 2). The highest lipid content was observed for *N. granulata* with 49.3%, followed by *P. tricornutum* 44.8% and *B. braunii* 41.1%. *Neochloris oleoabundans* had the lowest lipid content among the microalgae tested with lipid comprising only 31.8% of algal biomass. The moisture and ash content were also measured. The marine microalgae were found to have higher ash content than the freshwater strains. The highest levels of moisture and ash content were measured in *T. chui* at 5.4 and 14.6%, respectively. *Porphyridium aerugineum* had the lowest moisture content (0.9%) and *B. braunii* the lowest ash content at 2.9% of algal biomass (Table 2).

Fatty acid analysis

The resulting FAME profiles are reported in Table 3. The highest fatty acid content was observed for *B. brauni* at 153.3 mg g^{-1} algal biomass followed by *N. granulata* at 137.0 mg g^{-1}. The lowest fatty acid content was observed for *P. aerugineum* with 36.0 mg g^{-1}. Palmitic acid was the most common fatty acid found in all microalgae species. Fatty acids with various degrees of saturation were detected, including saturated (SFA) and mono- and polyunsaturated fatty acids (MUFA, PUFA). *Neochloris oleoabundans* contained the highest amount of PUFA at 48.4% of total fatty acid. *B.*

Table 2 Total lipid, moisture, and ash content

Microalgae	Moisture content (%)	Ash content (%)	Lipids (%)
B. braunii	2.3 ± 0.2	2.9 ± 0.0	41.1 ± 5.5
C. sorokiniana	4.8 ± 0.2	3.8 ± 0.2	32.3 ± 2.4
N. granulata	4.4 ± 0.3	6.8 ± 0.1	49.3 ± 4.0
N. oleoabundans	3.5 ± 0.1	11.6 ± 0.1	31.8 ± 6.7
P. tricornutum	2.9 ± 0.0	14.4 ± 0.1	44.8 ± 3.6
P. aerugineum	0.9 ± 0.1	5.3 ± 0.1	30.9 ± 6.1
S. obliquus	3.2 ± 0.3	3.4 ± 0.1	40.5 ± 7.8
Scenedesmus sp.	3.7 ± 0.1	3.6 ± 0.0	36.3 ± 12.5
T. chui	5.4 ± 0.0	14.6 ± 0.4	32.1 ± 5.5

Each value represents the mean ± SD of three experiments

Table 3 Composition of fatty acids in microalgae species used in this study (mg g^{-1} dry weight)

Fatty acid	*B. braunii*	*C. sorokiniana*	*N. granulata*	*N. oleoabundans*	*P. tricornutum*	*P. aerugineum*	*S. obliquus*	*Scenedesmus* sp.	*T. chui*
C8:0	–	–	–	–	–	–	–	–	–
C10:0	–	–	–	–	–	–	–	–	–
C11:0	1.4 ± 0.1	–	–	–	–	–	–	–	–
C12:0 (lauric acid)	–	–	1.4 ± 0.1	–	–	–	–	–	–
C13:0 (tridecanoic acid)	–	–	–	1.1 ± 0.1	–	–	1.3 ± 0.1	–	–
C14:0 (myristic acid)	–	–	11.3 ± 0.1	–	7.1 ± 0.4	–	–	–	–
C14:1 (myristoleic acid)	–	–	–	0.3 ± 0.5	–	–	0.8 ± 0.6	0.4 ± 0.1	–
C15:0 (pentadecanoic acid)	–	–	–	–	–	–	–	–	–
C15:1 (cis-10-pentadecenoic acid)	–	–	–	–	–	–	–	–	–
C16:0 (palmitic acid)	13.5 ± 0.7	18.4 ± 1.2	27.7 ± 0.1	16.9 ± 0.3	11.7 ± 0.4	17.9 ± 0.5	16.6 ± 0.2	14.6 ± 0.7	13.9 ± 1.7
C16:1 n-7 (palmitoleic acid)	–	–	41.5 ± 0.1	–	21.2 ± 0.7	–	–	–	–
C16:2 n-4	–	–	–	–	–	–	1.4 ± 0.0	–	1.8 ± 0.3
C17:0 (heptadecanoic acid)	–	–	–	–	–	–	–	–	–
C16:3	–	–	–	–	9.6 ± 0.4	–	–	–	–
C17:1 (cis-10-heptadecenoic acid)	3.2 ± 0.1	3.1 ± 0.5	–	11.6 ± 0.2	–	–	1.1 ± 0.2	2.6 ± 0.2	–
C16:3 n-1	–	–	–	–	–	–	–	–	–
C18:0 (stearic acid)	–	–	–	–	–	–	–	–	–
C18:1 n-9 (elaidic acid)	88.2 ± 2.7	11.1 ± 0.3	11.0 ± 3.9	1.5 ± 0.1	–	–	16.4 ± 0.2	14.1 ± 0.7	6.0 ± 0.7
C18:1 n-7	2.2 ± 0.1	–	–	–	–	–	–	–	–
C18:2 n-6 (linoleic acid)	12.3 ± 0.5	21.3 ± 2.7	7.7 ± 0.1	18.1 ± 0.5	–	4.3 ± 0.1	23.4 ± 0.1	11.0 ± 0.6	6.2 ± 0.8
C18:2 n-6t	–	–	–	–	–	–	–	–	–
C18:3 n-4	–	–	–	–	–	–	–	–	–
C18:3 n-6 (γ-linolenic acid)/C19:0	–	–	–	–	–	–	–	–	–
C18:4 n-3	–	–	–	–	–	–	–	–	–
C18:3 n-3 (α-linolenic acid)	26.4 ± 1.4	3.7 ± 0.6	–	23.1 ± 0.4	–	–	12.5 ± 0.1	28.2 ± 1.3	10.8 ± 0.5
C20:0 (arachidic acid)	–	–	–	–	–	–	–	–	–
C20:1 n-9 (cis-11-eicosenoic acid)	–	–	–	–	–	–	–	–	1.2 ± 0.1
C20:2 (cis-11,14-eicosadienoic acid)	–	–	–	–	–	–	–	–	–
C20:3 n-6 (cis-8,11,14-eicosatrienoic acid)	–	–	–	–	–	7.6 ± 0.1	–	–	–
C21:0 (henicosanoic acid)	–	–	–	–	–	–	–	–	–
C20:3 n-3 (cis-8,11,14-eicosatrienoic acid)	–	–	4.8 ± 0.1	–	–	–	–	–	–
C20:4 n-6 (arachidonic acid)	–	–	–	–	–	–	–	–	–
C20:4 n-3	–	–	–	–	–	–	–	–	–
C20:5 n-3 (eicosapentaenoic acid)	6.2 ± 0.1	–	31.7 ± 0.1	–	28.5 ± 1.2	6.2 ± 0.1	–	–	3.8 ± 0.6

Table 3 (continued)

Fatty acid	*B. braunii*	*C. sorokiniana*	*N. granulata*	*N. oleoabundans*	*P. tricornutum*	*P. aerugineum*	*S. obliquus*	*Scenedesmus* sp.	*T. chui*
C22:0 (behenic acid)	–	–	–	–	–	–	–	–	–
C22:1 n-9 (erucic acid)	–	–	–	–	–	–	–	–	–
C22:2 n-3 (docosadienoic acid)	–	–	–	–	–	–	–	–	–
C24:0 (lignoceric acid)	–	–	–	–	1.4 ± 0.1	–	–	–	–
C22:6 n-3 (docosahexaenoic acid)/C24:1 n-9	–	–	–	–	–	–	–	–	–
Unknown	–	13.1 (18.5)	–	12.6 (14.8)	–	–	10.0 (11.9)	14.4 (16.9)	27.9 (39.1)
∑SFA	14.9 (9.7)	18.4 (26.0)	40.4 (29.5)	17.9 (21.1)	20.2 (25.4)	17.9 (49.8)	17.9 (21.4)	14.6 (17.1)	13.9 (19.5)
∑MUFA	93.5 (61.0)	14.3 (20.1)	52.4 (38.3)	13.4 (15.7)	21.2 (26.7)	0	18.3 (21.9)	17.2 (20.1)	7.1 (10.0)
∑PUFA	44.9 (29.3)	25.0 (35.3)	44.2 (32.3)	41.3 (48.4)	38.1 (47.9)	18.1 (50.2)	37.4 (44.8)	39.2 (45.9)	22.5 (31.5)
Total fatty acid	153.3	70.8	137.0	85.2	79.5	36.0	83.5	85.4	71.5

(–) not detected. The concentration of fatty acids was express in mg g^{-1} dry weight of microalgae with percentage (%) of the total fatty acids in parentheses, and each value represents the mean ± SD of three experiments

braunii had the highest amount of MUFA at 61% of total fatty acid and *P. aerugineum* has the highest amount of SFA at 49.8% of total fatty acid. Eicosapentaenoic acid (EPA) was detected in all marine microalgae species and in the freshwater *P. aerugineum*. Another omega-3 fatty acid, α-linolenic acid (ALA), was present in *B. braunii*, *C. sorokiniana*, *N. oleoabundans*, *S. obliquus*, *Scenedesmus* sp., and *T. chui*. Lignoceric acid (C24:0) a long-chain fatty acid was detected only in *P. tricornutum*.

Carotenoid content

Fucoxanthin, astaxanthin, lutein, zeaxanthin, canthaxanthin, α-carotene, β-carotene, and lycopene were quantified individually by HPLC and total carotenoid contents was also determined (Table 4). Total carotenoids range from 1.1 to 29.5 mg g^{-1} freeze-dried algal biomass. Beta-carotene was found in all microalgae tested. Lutein and zeaxanthin were produced by almost all of species. *Phaeodactylum tricornutum* produced the highest amount of carotenoids at 29.5 mg g^{-1} of dry biomass, which was comprised predominantly of fucoxanthin (24.3 mg g^{-1}). Significant amounts of lutein, more than 2 mg g^{-1}, were detected in *N. oleoabundanas*, *P. tricornutum*, and *Scenedesmus* sp. Small amount of zeaxanthin was detected in almost all the tested microalgae and the highest amount was accumulated in *S. obliquus* at 1.6 mg g^{-1}. Lycopene and astaxanthin were detected only in *T. chui.*

Phenolic contents

The highest phenolic content was found in *T. chui* at 57.5 ± 8.7 μmol GAE g^{-1} methanolic extract. Similarly, *B. braunii* had 52.9 ± 2.0 μmol GAE g^{-1} phenolic content. The lowest phenolic content was measured in *N. oleoabundans* at 26.6 ± 2.5 μmol GAE g^{-1} (Table 5).

ORAC values

Freeze-dried algal biomass was extracted using an accelerated solvent extractor with hexanes/dichloromethane for L-ORAC and acetone/water/acetic acid for H-ORAC as described by Wu et al. 2004. *Nannochloropsis granulata* showed the highest total ORAC values at 6948 μmol TE $(100\ g)^{-1}$ dry biomass, followed by *N. oleoabundans* (4508 μmol TE $(100\ g)^{-1}$) and *S. obliquus* (4406 μmol TE $(100\ g)^{-1}$). The lowest ORAC value was measured in *T. chui* at 2121 μmol TE $(100\ g)^{-1}$ dry biomass (Table 5). Total ORAC values for *B. braunii*, *N. oleoabundans*, and *S. obliquus* were all > 4000 μmol TE $(100\ g)^{-1}$ dry biomass. The remaining microalgae species examined had ORAC values of 3500 μmol TE $(100\ g)^{-1}$ or less. The L-ORAC

Table 4 Carotenoid content; results are express in mg g^{-1} dry weight

Microalgae	Fucoxanthin	Astaxanthin	Lutein	Zeaxanthin	Cantaxanthin	α-Carotene	β-Carotene	Lycopene	Other	Total
B. braunii	–	–	0.7 ± 0.01	0.1 ± 0.01	–	0.1 ± 0.02	0.2 ± 0.03	–	0.6 ± 0.00	1.7 ± 0.08
C. sorokiniana	–	–	0.8 ± 0.01	–	–	–	0.1 ± 0.04	–	0.2 ± 0.01	1.1 ± 0.02
N. granulata	–	–	–	0.1 ± 0.00	0.4 ± 0.03	–	1.0 ± 0.03	–	3.6 ± 0.06	5.1 ± 0.07
N. oleoabundans	–	–	3.4 ± 0.04	0.1 ± 0.01	0.5 ± 0.01	0.6 ± 0.01	0.6 ± 0.00	–	2.5 ± 0.08	7.8 ± 0.15
P. tricornutum	24.3 ± 0.67	–	2.1 ± 0.11	0.1 ± 0.00	0.6 ± 0.04	0.1 ± 0.01	1.6 ± 0.18	–	2.2 ± 0.24	29.5 ± 1.26
P. aerugineum	–	–	–	0.4 ± 0.01	–	–	0.4 ± 0.07	–	3.3 ± 0.18	4.0 ± 0.10
S. obliquus	–	–	1.6 ± 0.09	1.6 ± 0.09	0.3 ± 0.03	0.2 ± 0.02	0.4 ± 0.02	–	1.4 ± 0.08	5.4 ± 0.34
Scenedesmus sp.	–	–	2.6 ± 0.03	0.2 ± 0.00	0.2 ± 0.14	0.1 ± 0.02	0.7 ± 0.02	–	0.6 ± 0.04	4.5 ± 0.21
T. chui	–	0.1 ± 0.00	0.6 ± 0.05	0.1 ± 0.01	0.4 ± 0.02	1.7 ± 0.05	1.0 ± 0.03	1.5 ± 0.21	3.5 ± 0.11	8.5 ± 0.16

Each value represents the mean ± SD of two experiments

and H-ORAC values vary depending upon the algal species (Table 5).

DPPH radical scavenging activity

Methanolic extracts of microalgal biomass were tested for their DPPH radical scavenging activity at 50, 100, and 200 μg mL^{-1} concentrations. All the tested extracts showed moderate and dose-dependent DPPH radical scavenging activity as compared to vitamin C, which was used as a positive control (Fig. 1). The *T. chui* extract showed the highest DPPH radical scavenging potency; at 200 μg mL^{-1} concentration, it scavenged 45.0% DPPH of radicals. The *N. oleoabundans* extract had the lowest DPPH radical scavenging potency.

Discussion

Microalgae are well known for producing various biologically active compounds including pigments, fatty acids, proteins, and polysaccharides (Borowitzka 2013). They are rich in lipids including long-chain polyunsaturated fatty acids (PUFA) and carotenoids. PUFA, especially omega-3 fatty acids including eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), are reported to have beneficial effects for heart health (Holub and Holub 2004) and carotenoids are considered a strong natural antioxidant (Krinsky 2001).

In the current study, we measured the ORAC (L-ORAC and H-ORAC) values of six freshwater and three marine microalgal species (Table 1). The total ORAC values ranged from 2121 to 6948 μmol TE $(100\ g)^{-1}$ dry algal biomass. Close comparison of these ORAC values, especially L-ORAC (Table 5) and lipid content (Table 2), revealed that biomass containing higher lipid content also had higher total ORAC and L-ORAC values. Moreover, linear regression analysis revealed a statistically significant correlation ($p < 0.05$) between the assayed ORAC values and the total lipid content as shown in Fig. 2a, b. *Nannochloropsis granulata* had the highest measured lipid content (49.3%) and possessed the highest ORAC value (total ORAC 6948 and L-ORAC 5298 μmol TE $(100\ g)^{-1}$). Conversely, biomass samples of *P. aerugineum*, *N. oleoabundans*, and *T. chui* were found to have low lipid content (32% or less) and correspondingly low total ORAC values in general, particularly the L-ORAC values. Our results suggest that the higher the unsaturation level (especially PUFA content), the better the L-ORAC values, except for *N. granulata* which had moderate PUFA content (32.3% of total fatty acid content) and the highest measured L-ORAC value. *Tetraselmis chui* had the lowest L-ORAC value (669 μmol of TE $(100\ g)^{-1}$) and also low MUFA and PUFA content. In our earlier studies, we have observed that nitric oxide (NO) inhibitory activities of *T. chui*, *N. granulata*, and *P. aerugineum* were due to the presence of polar lipids, i.e., galactolipids and betaine lipids (Banskota et al. 2013a, b, c, d). Their potency towards NO inhibition was enhanced by increasing degree of unsaturation on the acyl side of the fatty acids. Together with the findings of the current study, these results provide strong evidence to support the hypothesis that lipids with higher degrees of unsaturation enhance the antioxidant potency of microalgal extracts.

Carotenoids and phenolics are two major groups of natural compounds having strong antioxidant activity (Krinsky 2001; Stahl and Sies 2003). We measured the total phenolic content of MeOH extracts, which ranged from 26.6 to 57.5 μmol GAE g^{-1} by Folin-Ciocalteu method. Total carotenoid content varied from 1.1 to 29.5 mg g^{-1} algal biomass. Even though no direct correlation was observed in the present study between antioxidant value and the carotenoid content, these pigments certainly contributed to the overall antioxidant potency of the tested microalgae because of their strong antioxidant properties (Krinsky 2001). Interestingly, linear regression analysis revealed that DPPH radical scavenging activities (Fig. 1) correlated significantly with the total phenolic content of the

Table 5 Total ORAC values of microalgae μmol TE (100 g)$^{-1}$ dry algal biomass and total phenolic (TP) content μmol GAE g^{-1} of MeOH extract

Microalgae		Min	Max	Mean	SD
B. braunii	H-ORAC	1462	1808	1635	245
	L-ORAC	2506	2743	2625	167
	Total ORAC	*3968*	*4550*	*4259*	*412*
	TP			52.9	2.0
C. sorokiniana	H-ORAC	1352	1519	1436	118
	L-ORAC	1347	2460	1904	787
	Total ORAC	*2699*	*3979*	*3339*	*905*
	TP			37.2	3.6
N. granulata	H-ORAC	1587	1713	1650	89
	L-ORAC	4278	6318	5298	1442
	Total ORAC	*5865*	*8031*	*6948*	*1532*
	TP			43.6	1.2
N. oleoabundans	H-ORAC	2987	3208	3097	156
	L-ORAC	1141	1681	1411	382
	Total ORAC	*4128*	*4889*	*4508*	*538*
	TP			26.6	2.5
P. tricornutum	H-ORAC	1534	1835	1685	213
	L-ORAC	1204	1720	1462	365
	Total ORAC	*2738*	*3556*	*3147*	*578*
	TP			44.7	2.4
P. aerugineum	H-ORAC	1513	1830	1671	225
	L-ORAC	990	1043	1016	38
	Total ORAC	*2502*	*2873*	*2688*	*262*
	TP			36.4	30.5
S. obliquus	H-ORAC	2686	3164	2925	338
	L-ORAC	1194	1769	1481	407
	Total ORAC	*3880*	*4933*	*4406*	*745*
	TP			29.3	1.7
Scenedesmus sp.	H-ORAC	2403	2495	2449	65
	L-ORAC	941	1120	1031	126
	Total ORAC	*3344*	*3615*	*3480*	*191*
	TP			39.8	5.6
T. chui	H-ORAC	1047	1857	1452	573
	L-ORAC	284	1053	669	544
	Total ORAC	*1331*	*2911*	*2121*	*1117*
	TP			57.5	8.7

Min, minimum; *Max*, maximum. ORAC value represents mean ± SD of three experiments

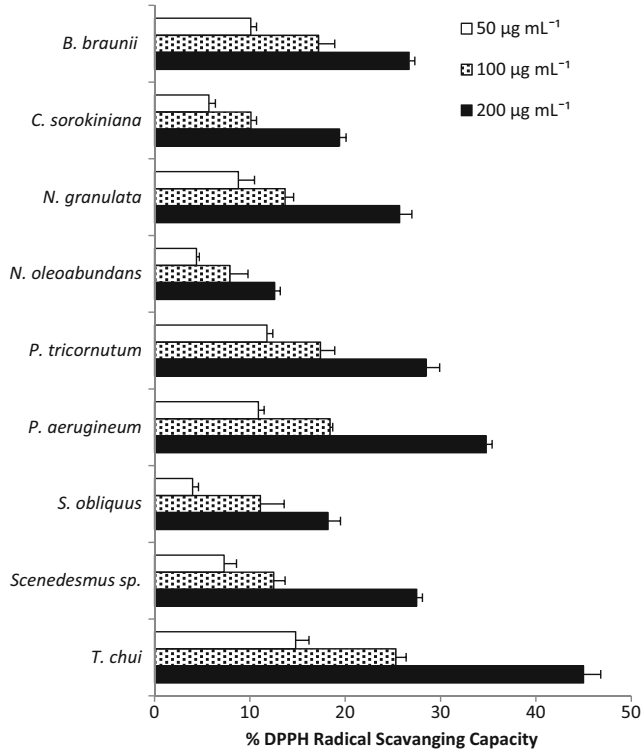


Fig. 1 DPPH radical scavenging activity of the MeOH extracts of tested microalgae. Each value represents the mean ± SD of three replicates

microalgae ($p < 0.005$; Fig. 2c). An exception to this is *B. braunii* which had the second highest phenolic content yet relatively weak DPPH radical scavenging compared to *P. tricornutum* and *P. aerugineum*. *Tetraselmis chui* had the strongest DPPH radical scavenging activity among the tested algae (45.0% scavenging capacity) and the highest phenolic content (57.5 μmol GAE g^{-1} extract). *Neochloris oleaoabundans* possessed the lowest DPPH radical scavenging potency and the lowest phenolic content (26.6 μmol GAE g^{-1} extract) among the tested species. These results are in accordance with the previous finding by Ahmed et al. (2014) and Safafar et al. (2015) that phenolic compounds are responsible for the antioxidant properties of microalgae.

In addition to lipids and phenolics, cyanobacteria contain C-phycocyanin, a protein-bound pigment with antioxidant activity (Romay et al. 1998). Three polysaccharide fractions have been isolated from the marine microalga *Isochrysis galbana* with moderate scavenging activities against superoxide and hydroxyl radicals (Yingying et al. 2014). Moreover, Tannin-Spitz et al. (2005) evaluated the antioxidant properties of the water-soluble polysaccharide of *Porphyridium* sp. by determining the ability of a polysaccharide solution to inhibit auto-oxidation of linoleic acid and oxidative damage to 3T3 cells. The polysaccharide inhibited oxidative damage in a dose-dependent manner. *Arthrospira* (*Spirulina*) *maxima* has been reported to provide some antioxidant protection both in vitro and in vivo because of the presence of α-tocopherol, β-carotene, and phenolic acids (Miranda et al. 1998). Taken together, these findings suggest that in addition to lipid fractions, proteins, polysaccharides, and possibly other compounds may also contribute to the antioxidative activity of microalgal biomass. It is possible that these microalgae produce antioxidative compounds to protect their lipids against oxidation.

To the best of our knowledge, we are reporting for the first time the direct correlation between lipid content and ORAC values of microalgal biomass. The ORAC values may also be

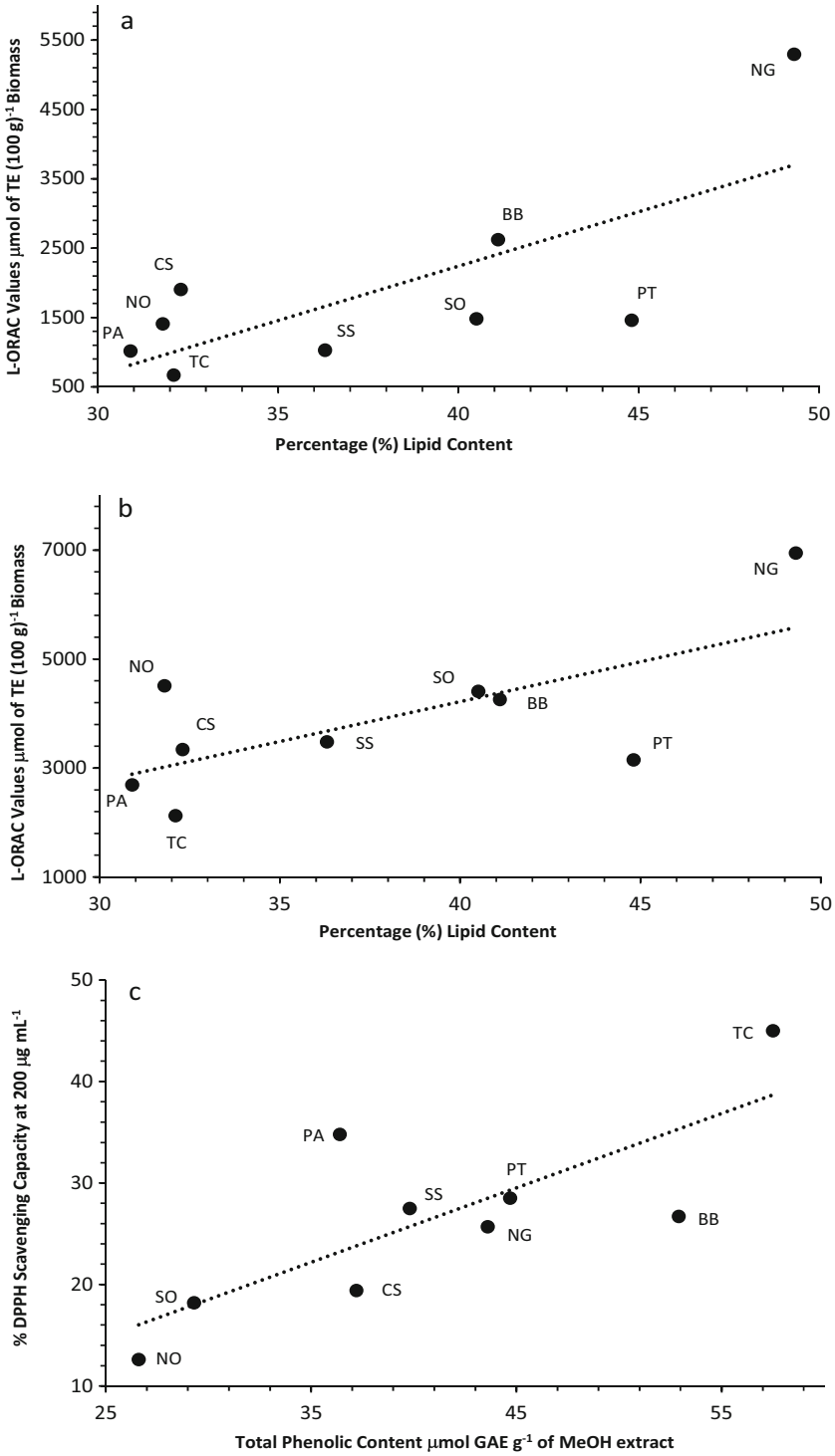


Fig. 2 Linear regression of **a** L-ORAC value vs percentage (%) lipid content dry microalgae biomass ($r^2 = 0.54$ and $p < 0.05$), **b** total ORAC value vs percentage (%) lipid content dry microalgae biomass ($r^2 = 0.47$ and $p < 0.05$) and **c** phenolic content vs percentage (%) DPPH radical scavenging capacity of MeOH extract at 200 µg mL^{-1} concentration ($r^2 = 0.66$ and $p < 0.005$). BB, *B. braunii*; CS, *C. sorokiniana*; NG, *N. granulata*; NO, *N. oleoabundans*; PT, *P. tricornutum*; PA, *P. aerugineum*; SO, *S. obliquus*; SS, *Scenedesmus* sp.; TC, *T. chui*

enhanced by increasing the degree of unsaturation in fatty acid chains. We also found that the degree of DPPH radical scavenging potency correlated well with total phenolic content of the algal biomass.

Acknowledgements The authors are thankful to all NRC colleagues involved in algal biomass production which was used for this study. This is NRC publication no. 56282.

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