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Profiling of carotenoids and antioxidant capacity of microalgae from subtropical coastal and brackish waters

Running title: Carotenoids and antioxidant capacity of subtropical microalgae

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Abstract

Carotenoids are associated with various health benefits, such as prevention of age-related macular degeneration, cataract, certain cancers, rheumatoid arthritis, muscular dystrophy and cardiovascular problems. As microalgae contain considerable amounts of carotenoids, there is a need to find species with high carotenoid content. Out of hundreds of Australian isolates, twelve microalgal species were screened for carotenoid profiles, carotenoid productivity, and

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in vitro antioxidant capacity (total phenolic content (TPC) and ORAC). The top four carotenoid producers at 4.68-6.88 mg/g dry weight (DW) were *Dunaliella salina*, *Tetraselmis suecica*, *Isochrysis galbana*, and *Pavlova salina*. TPC was low, with *D. salina* possessing the highest TPC (1.54 mg Gallic Acid Equivalents/g DW) and ORAC (577 µmol Trolox Equivalents/g DW). Results indicate that *T. suecica, D. salina, P. salina* and *I. galbana* could be further developed for commercial carotenoid production.

Keywords: Carotenoids, microalgae, antioxidant capacity, ORAC, total phenolics

1. Introduction

Carotenoids are found mostly in green leafy and yellow-coloured vegetables and orange-coloured fruits. Carotenoids are lipophilic compounds and have been divided into carotenes and xanthophylls based on their chemical structure. The carotenes are hydrocarbons whereas the xanthophylls have oxygenated functional groups making them more polar than the carotenes (Stahl & Sies, 2012). Carotenoid pigments provide protection to the photosynthetic apparatus in plants by dissipating excess energy. They also have a major role in photosynthesis by harvesting light and by stabilizing protein folding in the photosynthetic apparatus. Carotenoids quench singlet oxygen which mainly arises from sunlight absorption by chromophores and thus protect chlorophylls, lipids, proteins and DNA from oxydative damage. More than 600 different carotenoids are known to be present in nature and their distribution, molecular structure or the presence of specific biosynthesis pathways have been suggested as useful tools for algal classification (Ibañez & Cifuentes, 2013).

Carotenoids have been used as feed for aquaculture and as food colorants for many decades and are increasingly becoming popular as dietary supplements. Carotenoids are known as

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important antioxidants for human health and there is evidence that they are involved in other biological functions, e.g. regulatory effects on intra- and intercellular signaling and gene expression (Stahl & Sies, 2012). Several trials have reported that carotenoids can protect from lung cancer, amyotrophic lateral sclerosis, and several other degenerative diseases (Ibañez & Cifuentes, 2013). Diets rich in carotenoids (in particular, lutein and zeaxanthin) are well known to reduce the prevalence of age-related macular degeneration (Schalch *et al.*, 2007). There are also reports that carotenoids can protect human skin against UV-induced damage. Therefore, it has been suggested that increasing the carotenoid content in food can lead to the improvement of the nutritional quality of the diet, as high conservation of fundamental cellular signaling processes and protective mechanisms are observed in nature (Stahl & Sies, 2012).

Among the various sources of carotenoids, microalgae have recently created a wide interest due to several advantages: they are relatively easy to cultivate, do not need to compete with food production, and can adapt to environmentally changing conditions by producing a great variety of secondary metabolites. The biosynthesis of carotenoids can be triggered by either controling the cultivation conditions or by using genetic engineering approaches. Moreover, microalgae can also be used to purify and take up nutrients from wastewater (Ibañez & Cifuentes, 2013). Two microalgal strains, *Dunaliella salina* and *Haematococcus pluvialis* are well known to accumulate β-carotene (up to 14% dry weight (DW)) and astaxanthin (2–3% DW), respectively, under stress conditions (Ibañez & Cifuentes, 2013). The carotenoids from these two strains are already used as food coloring agents, vitamin supplements for food and animal feed, as well as additives to food and cosmetics (Hu, Lin, Lu, Chou & Yang, 2008). *Muriellopsis* sp. has been exploited for commercial production of lutein due to its high growth rate and high lutein content (up to 35 mg L^{-1}; Eonseon, Lee, Hyund, & Chang, 2003).

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Chlorella vulgaris has also been reported as a high producer of lutein (Plaza, Herrero, Cifuentes, & Ibanez, 2009). *Chlorella ellipsoidea* has been reported to produce violaxanthin, coupled with two other xanthophylls, *viz.* antheraxanthin and zeaxanthin (Plaza *et al.*, 2009). Recently, there has been interest in fucoxanthin, a carotenoid available in brown algae and diatoms, due to claims that it can inhibit cell growth and induce apoptosis in human cancer cells and that it possesses anti-inflammatory, anti-oxidant, anti-diabetic and anti-obesity properties (Maeda, Hosokawa, Sashima, Funayama, & Miyashita, 2005). However, screening studies comparing carotenoid profiles of different algal strains have been studied scarcely so far, with contents reported for 15 strains of chlorophycean (Del Campo, Moreno, Rodriguez, Vargas, Rivas & Guerrero, 2000) and 65 strains of red algae (Schubert and Garcia-Mendoza, 2006).

The oxidative damage caused by reactive oxygen species on lipids, proteins and nucleic acids may trigger various chronic diseases, such as coronary heart disease, atherosclerosis, cancer and ageing. Epidemiological studies have demonstrated an inverse association between intake of fruits and vegetables and mortality from age-related diseases, such as coronary heart disease and cancer, which may be attributed to their phytochemicals and antioxidant capacity (Charles, 2013). Thus, it is important to identify new sources of safe and inexpensive antioxidants of natural origin.

Phenolic compounds constitute one of the most numerous and ubiquitous groups of phytochemicals which possess a high spectrum of biological activities, including antioxidant, anti-inflammatory, and antimicrobial functions. A large body of preclinical research and epidemiological data suggest that plant phenolics can slow the progression of certain cancers and reduce the risks of cardiovascular disease, neurodegenerative diseases, and diabetes

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101 (Kim, Shin, & Jang, 2009). Due to these beneficial characteristics of phenolic compounds for
102 human health, they have been the focus of intensive research (Martins, Mussatto, &
103 Martinez-Avila, 2011), and there is considerable interest in the application of phenolic
104 compounds from plants in the nutraceutical and pharmaceutical industries. Due to the
105 advantages discussed earlier, microalgae rather than vascular plants can potentially be a more
106 useful source of these bioactive compounds.
107
108 Although macroalgae have received much attention as a potential source of natural
109 antioxidants (Duan, Zhang, Li, & Wang, 2006), there has been very limited information on
110 antioxidant capacity of microalgae. There are a number of reports on the evaluation of
111 antioxidant capacity of some species belonging to the genera *Botryococcus* (Rao, Sarada,
112 Baskaran, & Ravishankar, 2006), *Chlorella* (Wu, Ho, Shieh, & Lu, 2005), *Dunaliella*
113 (Herrero, Jaime, Martín-Álvarez, Cifuentes, & Ibáñez, 2006), *Nostoc* (Li, Cheng, Wong, Fan,
114 Chen, & Jiang, 2007), *Phaeodactylum* (Guzmán, Gato, & Calleja, 2001), *Polysiphonia* (Duan
115 *et al.*, 2006), *Scytosiphon* (Kuda *et al.*, 2005), *Arthrospira* (Herrero, Martín-Álvarez,
116 Senorans, Cifuentes, & Ibáñez, 2005; Jaime, Mendiola, Herrero, Soler-Rivas, Santoyo,
117 Senorans, Cifuentes, & Ibáñez, 2005) and *Synechocystis* (Plaza, Santoyo, Jaime, Reina,
118 Herrero, Senorans, & Ibáñez, 2010).
119
120 In this study, we present carotenoid profiles of twelve microalgal strains collected from
121 subtropical marine and brackish waters of commercial interest especially in the aquaculture
122 industry. We also present for the first time antioxidant capacity data of these microalgae to
123 identify local strains with the highest potential for large-scale cultivation. Such information
124 will help to identify the usefulness of these microalgae strains for human health as food
125 additives or as dietary supplements.

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2. Materials and methods

2.1. Chemicals

Chemicals were purchased from Merck (Kilsyth, VIC, Australia) or Sigma-Aldrich (Castle Hill, NSW, Australia) and were of analytical or HPLC grade. Lutein, zeaxanthin and *β*-cryptoxanthin, astaxanthin and *β*-carotene were purchased from Extrasynthese (Genay, France). Milli-Q water was used throughout unless otherwise stated.

2.2. Preparation of microalgal samples

Out of several hundred microalgal isolates that were collected in coastal or brackish water and maintained at the Algae Biotechnology Laboratory at the University of Queensland, Australia, twelve strains were selected, mainly based on their rapid growth and ease of handling compared to many other cultures. The microalgal strains *Dunaliella salina, Isochrysis galbana, Nannochloropsis* sp. BR2, *Pavlova lutheri, P. salina, Chaetoceros muelleri, C. calcitrans, Tetraselmis chui, T. suecica, Tetraselmis* sp. M8 have been described previously (Lim, Garg, Timmins, Zhang, Thomas-Hall, Schuhmann, *et al.* 2012). *Phaeodactylum tricornutum,* and *Dunaliella tertiolecta* were supplied by CSIRO's collection of living microalgae (Strain IDs: CS 29/8 and CS 175/8; Genbank accessions EF140622.1 and EF537907.1; respectively). Pure cultures were obtained and 18S rDNA sequencing was carried out as described previously (Lim *et al.* 2012; respectively). Microalgal cultures were inoculated from master cultures in 250 mL flasks with F/2 media (AlgaBoost^{TM} F/2 2000x). When they reached the late exponential growth phase (doubling times were twice as long as during highest exponential growth), the cultures (100 mL) were centrifuged, the supernatant

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was decanted and the remaining biomass was washed in MilliQ water before freeze-drying. The freeze-dried samples were kept at -20°C until extraction.

2.3. Analysis of carotenoids

The carotenoid extraction was based on a previously published method (Fanning *et al*., 2010) with slight modifications. The samples were crushed using a mortar and pestle and then weighed (10-60 mg) into 50 mL Falcon tubes. The samples were then kept on ice throughout the extraction. A total of 10 mL acetone was added to the samples followed by vortexing. Then, 10 mL hexane and 5 mL 10% NaCl were added. The mixture was vortexed and then centrifuged at 3000 x g at 4°C for 3 min. The top layer of the supernatant was then transferred to another Falcon tube and another 10 mL hexane was added followed by vortexing. The process was repeated until the supernatant became colourless. The combined hexane fractions were then dried in a centrifugal evaporator prior to being reconstituted in 2.5 mL methanol/dichloromethane (50/50, v/v) for HPLC analysis.

The HPLC-PDA analysis was undertaken as previously described (Fanning *et al.*, 2010) with only minor modifications to the gradient. The following 54 min gradient was used: 0 min, 80% phase A; 48 min, 20% phase A; 49 min, 80% phase A; 54 min, 80% phase A (phase A - 92% methanol/8% 10 mM ammonium acetate, phase B - 100% methyl tert butyl ether). The extracts (20 µL) were injected onto a YMC C30 Carotenoid Column, 3 µm, 4.6 x 250 mm (Waters, Milford, MA, USA). Using the HPLC conditions described above, an MS scan was undertaken between 530 – 610 mass units in the APCI+ mode (Fu, Magnúsdóttir, Brynjólfson, Palsson, & Paglia, 2012), using the following system and conditions. An Acquity UPLC H-Class system connected to a Quattro Premier triple quad (Micromass MS

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Technologies, Waters Corporation, Milford, MA, USA) was used. Source temperature and probe temperature were 150˚C and 600˚C, respectively, desolvation and cone gas flow were 450 L/h and 50L/h, respectively, and corona, cone and extractor voltages were 5.0 µA, 30V and 3V, respectively.

The carotenoids were identified by comparison of retention times, UV/Vis spectra and mass spectra against authentic standards (Lu *et al.*, 2009), and the concentrations of the identified carotenoids were determined using individual calibration curves. Furthermore, an epoxide test as described by Dugo, Herrero, Giuffrida, Ragonese, Dugo & Mondello (2008) was conducted to confirm the identity of some of the peaks. Three separately-grown cultures were used for each strain (n=3).

2.4. Antioxidant capacity

The freeze-dried samples were crushed using a mortar and pestle and then weighed (10-100 mg) into 50 mL Falcon tubes. The samples were then stored on ice throughout the extraction. Extractions were conducted separately by addition of 10 mL water, hexane or ethyl acetate. The mixture was vortexed and then centrifuged at 3000 x g in 4˚C for 10 min. The supernatants were transferred to clean Falcon tubes. The extraction procedure was repeated until the supernatants were colourless. The supernatant was then dried in a centrifugal evaporator and reconstituted in 5 mL water (water extracts) or 5 mL isopropanol (ethyl acetate and hexane extracts).

For measuring the reducing capacity of the microalgal samples, the total phenolic assay by Folin-Ciocalteu reagent, was used as described previously (Singleton & Rossi, 1965; Schlesier,

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Harwat, Bohm, & Bitsch, 2002). In brief, the extracts (25 µL) were loaded in 96 well-plates and 125 µL of Folin-Ciocalteu reagent and 125 µL sodium carbonate were added. The absorbance was read at 750 nm in a PerkinElmer VICTOR 2030 multilabel counter (PerkinElmer, Waltham, MA, USA). Gallic acid monohydrate was used as a standard for calculating the amount of total phenolics in the samples and was expressed as Gallic Acid Equivalents (GAE)/g DW of microalgae and calculated as mean value ± SD (n=3; from separately-grown cultures).

For measuring the radical absorbance capacity, the oxygen radical absorbance capacity (ORAC) assay as described by Huang, Ou, Hampsch-Woodill, Flanagan, & Prior (2002) was used. In brief, 20 µL of the diluted samples (1:50 with 75 mM phosphate buffer, pH 7.0) was loaded in black 96 well flat bottom plates. The same amount of trolox (6.25 – 100 µM) and phosphate buffer were used in the plates as standard and blank, respectively. Then 200 µL fluorescein solution (92 µM) was added and the mixture was incubated at 37°C for 8 min in a PerkinElmer VICTOR 2030 multilabel counter. Then 25 µL AAPH (2,2-Azobis (2-methylpropionamidine) dihydrochloride; 79.65 mM) was added to the mixture to start the reaction and the fluorescence was recorded every 2 min for 90 min. Samples were assayed at an excitation wavelength of 490 nm and an emission wavelength of 515 nm. The oxygen radical absorbance capacity of the samples was expressed as µmol Trolox Equivalents (TE)/g DW of microalgae and calculated as mean ± SD (n = 3; from separately grown cultures).

2.5 Statistical analyses

The data for total carotenoids, phenolics and antioxidant capacity (ethyl acetate, hexane, and water extracts) of the twelve microalgae strains were compared by one-way ANOVA and Tukey's HSD tests were used to compare differences between strains. Differences were considered significant when p values were below 0.05.

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3. Results and discussion

3.1. Carotenoid profiles of microalgae

The present study reports on carotenoid profiles and contents as well as antioxidant capacity of 12 microalgae species from subtropical coastal and brackish waters. Eight carotenoids, namely trans-violaxanthin, antheraxanthin, astaxanthin, lutein epoxide, lutein, zeaxanthin, α- and β-carotene (Fig. 1) were identified as major carotenoids in some of the various species studied (Table 1). The epoxide test confirmed the identity of lutein epoxide as the peak disappeared after the addition of 0.1 M HCl. There were two other major carotenoids, which were tentatively identified as cis-isomers of violaxanthin or neoxanthin (Table 1). Due to the lack of a cis-violaxanthin or cis-neoxanthin standard and the similarity in the UV/Vis and mass spectra of these compounds there was no basis for further differentiation since an NMR system was not available. However, the acidification of the extract (epoxide test) showed changes in the absorbance spectra of the tentatively identified peaks, with a decrease of around 20 nm, and appearance of neochrome was observed. This is in similarity with the findings of Dugo *et al.* (2008) who reported disappearance of neoxanthin, violaxanthin and antheraxanthin peaks due to acidification of saponified samples of orange juice. Both the carotenoid profile (Table 1) and total carotenoid content (Fig. 2) showed large variation between different strains. β-carotene was present in all of the twelve strains and it was the dominating carotenoid in *Tetraselmis* sp. M8 (49.9%), and *T. chui* (38.1%; Table 1). Trans-violaxanthin was also detected in all strains, except *P. tricornutum* and was the most dominating carotenoid in *Nannochloropsis* sp. BR2 (52.5%). The next abundant carotenoids were lutein and its epoxide dominating the carotenoid levels in *D. salina* (65.2%) and *D.*

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250 *tertiolecta* (lutein: 21.8 and lutein epoxide: 13.3%; Table 1). The tentatively identified cis-
251 isomers of violaxanthin and neoxanthin were the most dominant carotenoids in *C. muelleri*
252 (85%), *P. tricornutum* (82.4%), *C. calcitrans* (78%), *P. salina* (75.9%), *I. galbana* (63.8%),
253 and *P. lutheri* (44.1%; Table 1). Astaxanthin was detected only in two strains (*T. suecica* and
254 *Nannochloropsis* sp. BR2) and was present in substantial quantities in *T. suecica* (38.8%).
255 Statistical tests showed significant differences in total carotenoids among the 12 microalgal
256 strains (one-way ANOVA; $p<0.001$). *D. salina* (6.879 mg/g DW) had the highest total
257 carotenoid content among the 12 strains evaluated (Tukey's test; $p<0.001$), followed by *T.*
258 *suecica* (5.807 mg/g), *I. galbana* (5.035 mg/g) and *P. salina* (4.678 mg/g; Fig. 2) whereas *D.*
259 *tertiolecta* had the lowest content with 1.053 mg/g. Based on the results of Tukey's test, it
260 can be concluded that there were three statistically similar groups: the first containing *D.*
261 *salina* only, the second containing *I. galbana, P. salina* and *C. muelleri*, (*T. suecica*
262 remaining in between these two groups) and the third containing the remaining seven strains
263 (Fig. 2).
264
265 The commercially produced carotenoid, astaxanthin was found in substantial quantities
266 (38.8%; 2.3 mg/g DW) in *T. suecica* confirming its suitability for further studies, that would
267 need to condition it under large-scale cultivation as a potential commercial astaxanthin
268 producer. *T. suecica* has already been used as a source of astaxanthin in feeding trials of the
269 calanoid copepod *Acartia bifilosa* (Holeton, Lindell, Holmborn, Hogfors, & Gorokhova,
270 2009). Zeaxanthin, a carotenoid important in prevention of age-related macular degeneration,
271 age-related cataract formation and ophthalmo-protection in visual processes (Schalch et al.,
272 2007), was found as a minor component only in *D. salina* and *D. tertiolecta*.
273 The present study found *D. salina* to be a rich source of lutein (65.2%; 4.5 mg/g). The
274 content is slightly lower than the Taiwanese *D. salina* strain (6.55 mg/g) reported by Hu *et*

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al., (2008) although the cultivation conditions of the strain were not discussed by the authors. The results are coincident with the findings of Perez-Garcia, Escalante, de-Bashan, & Bashan (2011) that *Dunaliella* sp. can possess lutein up to 14% body weight under autotrophic condition. The only carotenoid that was found across all microalgal species, as a major component, was β-carotene, and as expected, the highest amount was found in *D. salina*. This strain is already well known for its ability to produce high amounts of β-carotene (up to 14% DW; Ibañez & Cifuentes, 2013) under high salinity, high temperature, high light intensity and nitrogen limitation and is used in production plants in Australia, China, India and Israel (Borowitzka, 2013). Several other *Dunaliella* species (e.g. *D. bardawil*) have also been reported to produce high amounts of β-carotene (Mogedas, Casal, Forján, Vílchez, 2009) but *D. tertiolecta* used in this study did not have enough β-carotene to justify its use in further carotenoid studies. Some successful astaxanthin biosynthesis induction techniques, e.g. high irradiance, nutrient deprivation (nitrogen and phosphorus) in *H. pluvialis* have been reviewed (Del Campo, García-González, & Guerrero, 2007) and could be attempted with *T. suecica* to try to make it a commercially suitable alternative to *H. pluvialis*. Lutein and its isomer were found in almost all strains, with the content in *D. salina* being as high as the ones reported in *Muriellopsis* sp., *Scenedesmus almeriensis*, *Chlorella protothecoides* and *Chlorella zofingiensis* (Blanco, Moreno, Del Campo, Rivas, & Guerrero, 2007; Shi, Wu, & Chen 2006; Del Campo et al., 2007). Based on high lutein contents, *I. galbana*, and *P. salina* could also be considered other candidates for further studies for induction of lutein biosynthesis.

3.2. Antioxidant capacity of microalgae

The total phenolic content varied from 0.068 mg GAE/g DW (*D. tertiolecta* hexane extract) to 1.54 mg GAE/g DW (*D. salina* ethyl acetate extract) for different microalgal species and

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also for different extracts. Among the three solvents used for extraction, ethyl acetate extracts had the highest yields for all microalgal species (Fig. 3). *D. salina* (ethyl acetate: 1.54 mg GAE/g DW; hexane: 0.32 mg GAE/g DW) and *T. suecica* (ethyl acetate: 0.77 mg GAE/g DW; hexane: 0.3 mg GAE/g DW) had the highest total phenolic content among all species in both, ethyl acetate and hexane extracts (Tukey's test; $p<0.001$; Fig. 3). However, in water extracts, the highest values were obtained for *I. galbana* (0.235 mg GAE/g DW) and *P. lutheri* (0.225 mg GAE/g DW; Tukey's test; $p<0.001$; Fig. 3). The results of Tukey's test for ethyl acetate and hexane extracts clearly indicate statistical differences of *D. salina* and *T. suecica* to the other strains, however, for water extracts such distinctive conclusions could not be reached.

The ORAC values varied from 45 (*T. suecica* hexane extract) to 577 µmol TE/g DW (*D. salina* ethyl acetate extract) (Fig. 4). Among the three solvents used for this study, the ethyl acetate extracts had the highest ORAC values for all species except *I. galbana* (ethyl acetate: 169 µmol TE/g DW; hexane: 207 µmol TE/g DW; water: 149 µmol TE/g DW), and *P. lutheri* (ethyl acetate: 176 µmol TE/g DW; hexane: 126 µmol TE/g DW; water: 273 µmol TE/g DW). Among all microalgae tested, *D. salina* had the highest ORAC values in both ethyl acetate (577 µmol TE/g DW) and hexane (288 µmol TE/g DW) extracts (Tukey's test; $p<0.001$; Fig. 4). In the comparison of ORAC values, the top five species in ethyl acetate extracts were *D. salina*, *P. tricornutum*, *C. muelleri*, *P. salina* and *T. suecica*, in hexane extracts were *D. salina*, *I. galbana*, *P. salina* and *Nannochloropsis* sp. BR2 and in water extracts were *P. lutheri, P. tricornutum, P. salina, C. muelleri* and *T. suecica* (Fig. 4). Similar to total phenolics, the results of Tukey's test for ethyl acetate and hexane extracts for ORAC assays clearly indicated statistical differences of *D. salina* compared to the other strains, while for water extracts such distinctive conclusions could not be found.

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Phenolic/polyphenolic compounds are secondary metabolites and stress compounds that are involved in chemical protective mechanisms against different factors of biotic (e.g. grazing, settlement of bacteria or other fouling organisms) and abiotic (e.g. UV-radiation, metal contamination) stresses (Connan & Stengel, 2011). Unlike the findings of Li et al., (2007) and Hajimahmoodi, Faramarzi, Mohammadi, Soltani, Oveisi, & Nafissi-Varcheh (2010), the highest total phenolic contents in the current study were found in the ethyl acetate extracts. The current study, however, confirmed very low total phenolic levels (<5 mg GAE/g DW), similar to the 23 microalgal strains studied by the previously mentioned authors. The low total phenolic contents might be due to the culture conditions as no oxidative or other stress was provided that might trigger the production of more phenolic compounds as described in *Spirulina platensis* (Kepekçi & Saygideger, 2012).

The ORAC assay is considered more biologically relevant than diphenylpicrylhydrazyl (DPPH) and other similar protocols and is especially useful for extracts when multiple constituents co-exist and complex reaction mechanisms are involved (Huang *et al*., 2005). The antioxidant capacity in the species studied was higher than the ones reported previously (Blanco *et al.*, 2007). The results also differ from the findings of Blanco *et al.*, (2007) who reported higher antioxidant capacities in water extracts. This discrepancy might be due to the differences of the chemical nature of the compounds that contribute to antioxidant responses within the cellular structure of these species. The ORAC values reported in the current study (45-577 µmol TE/g DW) are comparable to or higher than those reported for various fruits and spice extracts which include blueberry (46 µmol TE/g DW; Prior *et al.*, 1998) and strawberry (540 µmol TE/g DW; Huang *et al.*, 2002), but lower than cinnamon (1256 µmol of TE/g DW; Su, Yin, Charles, Zhou, Moore, & Yu, 2007). This confirms the suitability of microalgae as a good source of natural antioxidants.

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4. Conclusion

Most of the commercially important carotenoids were found in microalgae from Australia which also exhibited a high oxygen radical absorbance capacity comparable to some fruits, indicating their potential for further studies to enhance production of bioactive compounds. However, there was significant diversity in the carotenoid profiles and contents between the species. Based on the results presented here, *T. suecica*, *D. salina*, *P. salina* and *I. galbana* are promising candidate species for further studies to increase the production of specific carotenoids through process optimization (e.g. growth conditions, harvesting, extraction, downstream processing) and advanced biotechnology (e.g. genetic or metabolic engineering and metabolic flux modeling).

5. Acknowledgements

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514 **Figure Captions**

515

516 **Figure 1.** Representative HPLC chromatogram of carotenoids extracted from microalgae
517 monitored at 450 nm. Numbered peaks indicate 1. putative violaxanthin/neoxanthin isomer,
518 2. putative violaxanthin/neoxanthin isomer, 3. trans-violaxanthin, 4. antheraxanthin, 5.
519 astaxanthin, 6. lutein epoxide, 7. lutein, 8. zeaxanthin, 9. α-carotene, 10. β-carotene isomers.

520

521 **Figure 2.** Ranking of twelve microalgal strains by total carotenoid contents (sum of
522 identified/tentatively-identified carotenoids). Shown are mean values and SEs from three
523 separately-grown cultures for each strain.

524

525 **Figure 3.** Total phenolic content of ethyl acetate (A), hexane (B) and water (C) extracts of
526 microalgal strains. Shown are mean values and SEs from three separately-grown cultures for
527 each strain.

528

529 **Figure 4.** ORAC values of ethyl acetate (A), hexane (B) and water (C) extracts of microalgal
530 strains. Shown are mean values and SEs from three separately-grown cultures for each strain.

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539 **Tables**

540 **Table 1.** Characteristics and concentration (mean ± SD, µg/g DW and % of total in
541 parenthesis) of carotenoids in 12 microalgae strains from subtropical coastal and brackish
542 waters.

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Table 1. Characteristics and concentration (avg ± sd, µg/g DW and % of total) of carotenoids in 12 microalgae strains from subtropical coastal and brackish water.

Peak	Carotenoid	t_R (min)	λmax (nm)	*m/z*	*Dunaniella salina*	*Dunaniella tertiolecta*	*Tetraselmis* sp. M8	*Isochrysis galbana*	*Tetraselmis chui*	*Tetraselmis suecica*	*Pavlova salina*	*Pavlova lutheri*	*Chaetoceros muelleri*	*Nannochloropsis* sp. BR2	*Phaeodactyllum tricornutum*	*Chaetoceros calcitrans*
1	Putative violaxanthin /neoxanthin isomer	5.6	449	600, 582	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	127±59 (9)	2105±208 (47)	n.d.	n.d.	n.d.
2	Putative violaxanthin /neoxanthin isomer	6.6	449	582	n.d.	360±81 (34)	n.d.	3205±53 (64)	n.d.	n.d.	3545±112 (76)	450±160 (35)	1672±132 (38)	n.d.	2404±100 (82)	1795±10 (78)
3	Trans-violaxanthin	7.2	440	602	619±104 (9)	100±40 (9)	229±21 (11)	244±32 (5)	546±227 (21)	1408±68 (24)	267±69 (6)	147±4 (13)	375±58 (8)	1078±371 (52)	n.d.	338±19 (15)
4	Antheraxanthin	10.2	446	585	279±30 (4)	42±16 (4)	126±19 (6)	n.d.	201±9 (8)	825±45 (14)	n.d.	n.d.	n.d.	165±63 (8)	n.d.	n.d.
5	Astaxanthin	10.6	470	597	n.d.	n.d.	n.d.	n.d.	n.d.	2261±281 (39)	n.d.	n.d.	n.d.	321±107 (16)	n.d.	n.d.
6	Lutein	12.8	445	552	4494±435 (65)	207±66 (22)	665±78 (31)	1194±269 (23)	624±23 (26)	484±121 (8)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
7	Lutein epoxide	11.5	446	583	n.d.	153±88 (13)	n.d.	n.d.	n.d.	n.d.	521±241 (11)	438±227 (32)	146±113 (3)	n.d.	280±25 (10)	n.d.
8	Zeaxanthin	14.1	450	570	122±16 (2)	43±21 (4)	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
9	Alpha carotene	27.2	n/d	n/d	126±8 (2)	12±3 (1)	30±5 (2)	n.d.	174±10 (7)	202±19 (4)	56±19 (1)	n.d.	n.d.	n.d.	n.d.	n.d.
10	Betacarotene	29.5	452	538	1235±11 (18)	136±30 (13)	1057±168 (50)	393±83 (8)	941±23 (38)	626±62 (11)	288±22 (6)	139±33 (11)	159±46 (4)	446±13 (24)	209±39 (8)	169±8 (7)

n.d.: not detectable

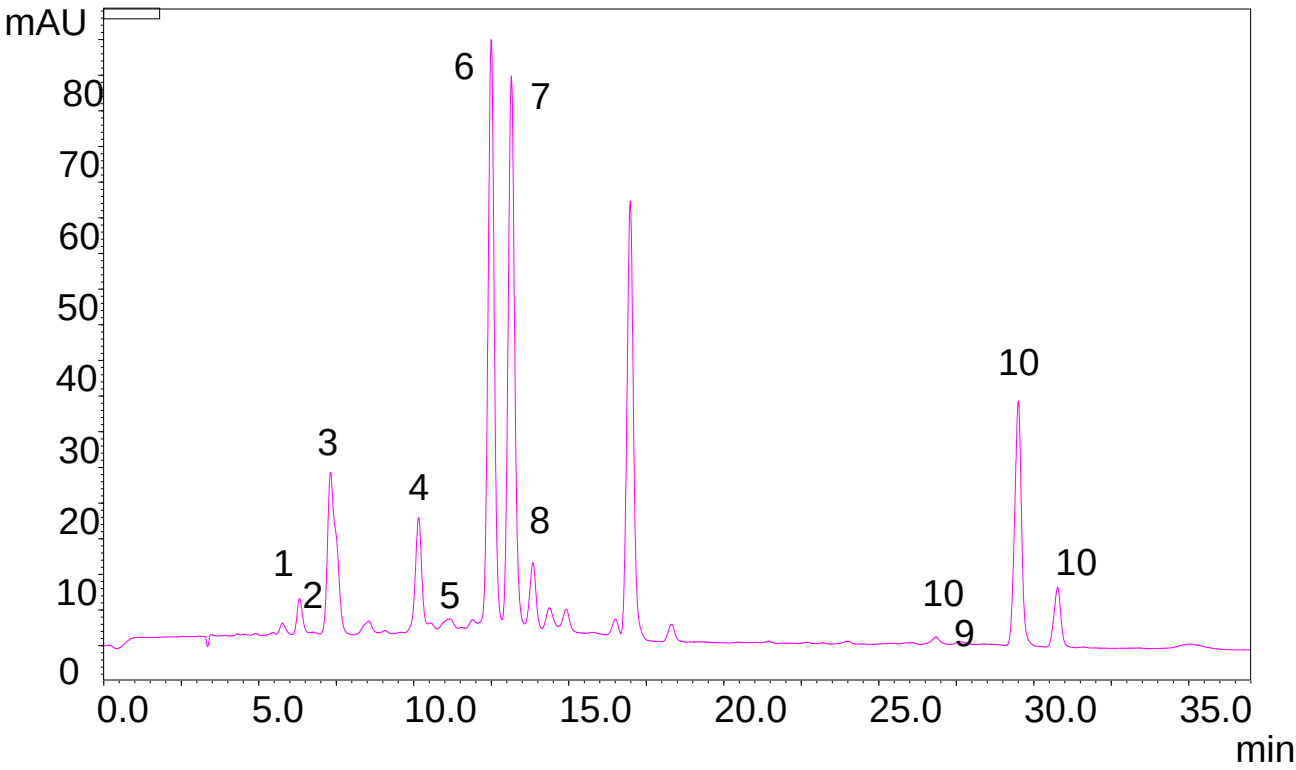
mAU
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Figure 1

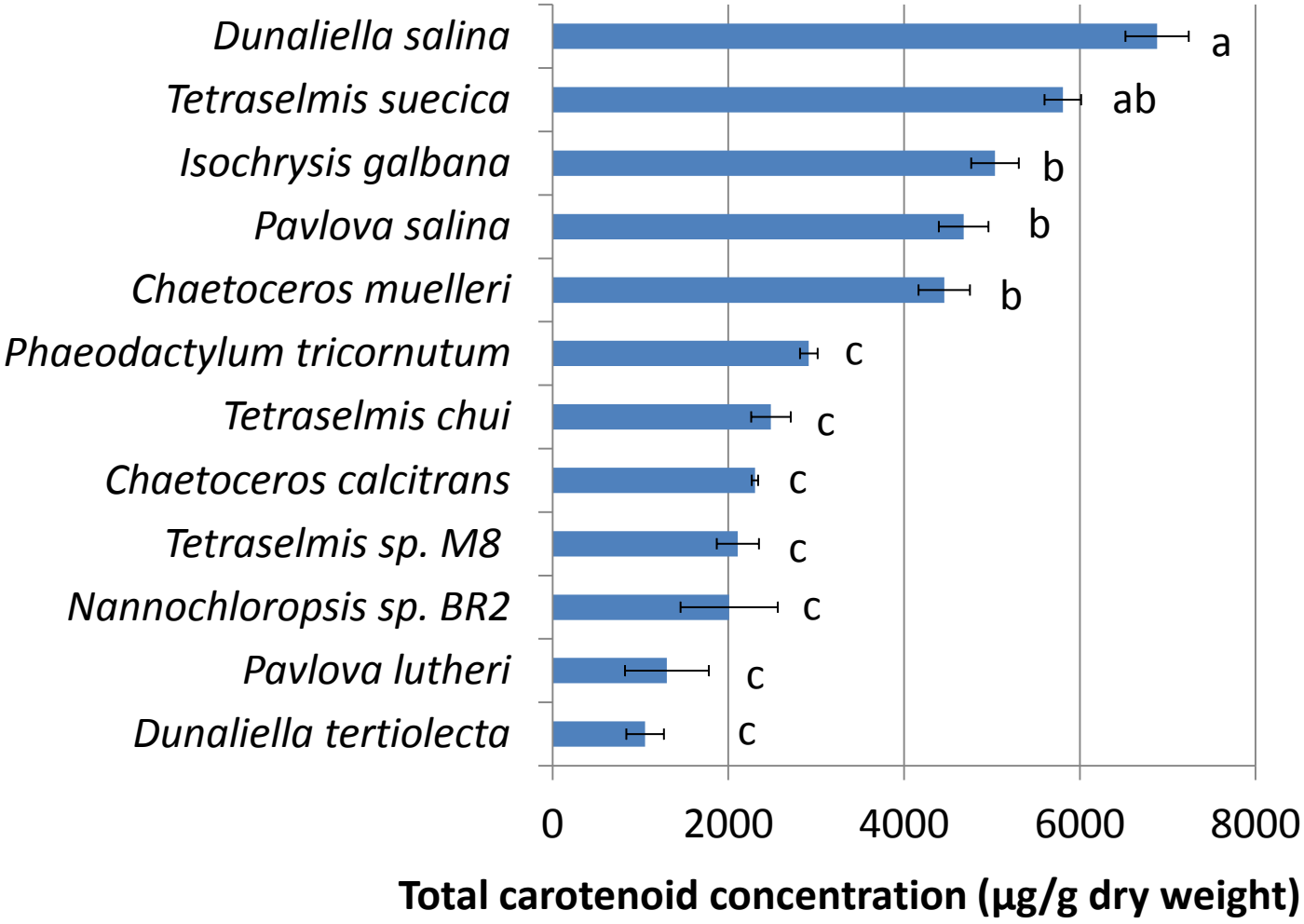

Dunaliella salina
Tetraselmis suecica
Isochrysis galbana
Pavlova salina
Chaetoceros muelleri
Phaeodactylum tricornutum
Tetraselmis chui
Chaetoceros calcitrans
Tetraselmis sp. M8
Nannochloropsis sp. BR2
Pavlova lutheri
Dunaliella tertiolecta
a
ab
b
b
b
c
c
c
c
c
c
0
2000
4000
6000
8000
Total carotenoid concentration (µg/g dry weight)

Figure 2

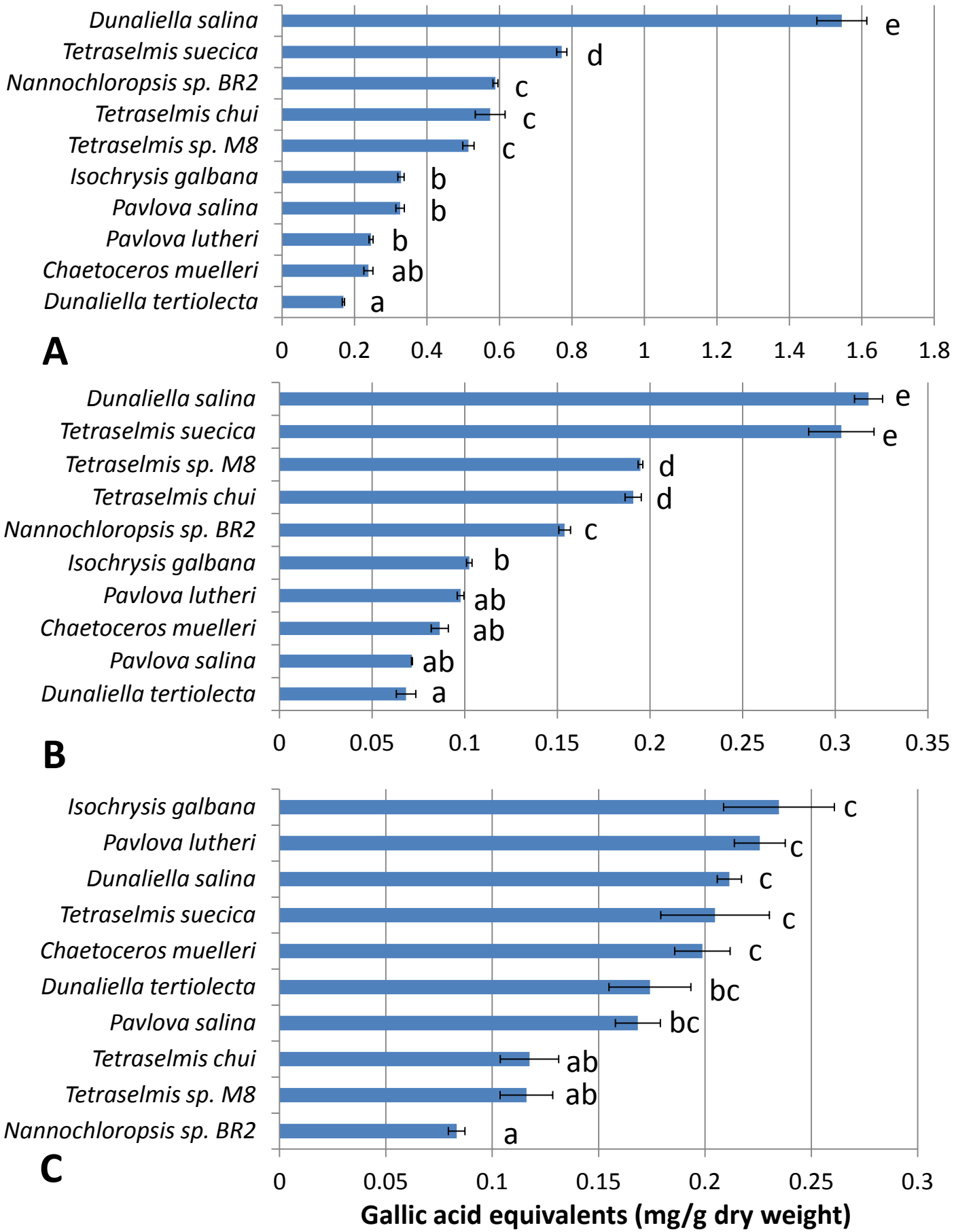
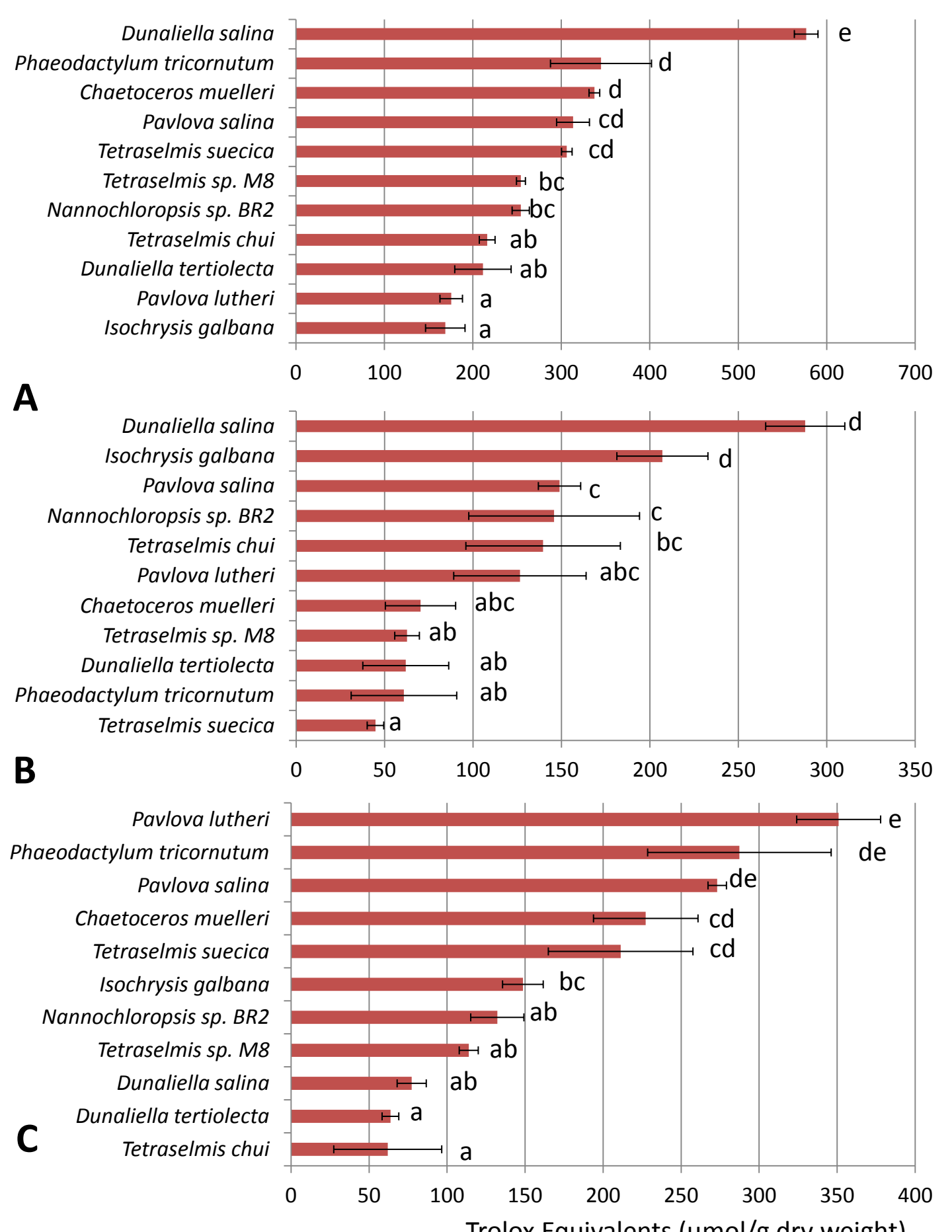

Dunaliella salina
Tetraselmis suecica
Nannochloropsis sp. BR2
Tetraselmis chui
Tetraselmis sp. M8
Isochrysis galbana
Pavlova salina
Pavlova lutheri
Chaetoceros muelleri
Dunaliella tertiolecta
e
d
c
c
c
b
b
b
ab
a
A
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
1.8
Dunaliella salina
Tetraselmis suecica
Tetraselmis sp. M8
Tetraselmis chui
Nannochloropsis sp. BR2
Isochrysis galbana
Pavlova lutheri
Chaetoceros muelleri
Pavlova salina
Dunaliella tertiolecta
e
e
d
d
c
b
ab
ab
ab
a
B
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
Isochrysis galbana
Pavlova lutheri
Dunaliella salina
Tetraselmis suecica
Chaetoceros muelleri
Dunaliella tertiolecta
Pavlova salina
Tetraselmis chui
Tetraselmis sp. M8
Nannochloropsis sp. BR2
c
c
c
c
c
bc
bc
ab
ab
a
C
0
0.05
0.1
0.15
0.2
0.25
0.3
Gallic acid equivalents (mg/g dry weight)

Figure 3


A
Dunaliella salina
Phaeodactylum tricornutum
Chaetoceros muelleri
Pavlova salina
Tetraselmis suecica
Tetraselmis sp. M8
Nannochloropsis sp. BR2
Tetraselmis chui
Dunaliella tertiolecta
Pavlova lutheri
Isochrysis galbana
e
d
d
cd
cd
bc
bc
ab
ab
a
a
0
100
200
300
400
500
600
700
B
Dunaliella salina
Isochrysis galbana
Pavlova salina
Nannochloropsis sp. BR2
Tetraselmis chui
Pavlova lutheri
Chaetoceros muelleri
Tetraselmis sp. M8
Dunaliella tertiolecta
Phaeodactylum tricornutum
Tetraselmis suecica
d
d
c
c
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abc
abc
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ab
a
0
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150
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350
C
Pavlova lutheri
Phaeodactylum tricornutum
Pavlova salina
Chaetoceros muelleri
Tetraselmis suecica
Isochrysis galbana
Nannochloropsis sp. BR2
Tetraselmis sp. M8
Dunaliella salina
Dunaliella tertiolecta
Tetraselmis chui
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cd
bc
ab
ab
ab
a
a
0
50
100
150
200
250
300
350
400
Trolox Equivalents (µmol/g dry weight)

Figure 4

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Highlights

- Carotenoid profiles and antioxidant capacity data of microalgae are presented.
- *Tetraselmis suecica* and *Dunaniella salina* were the highest carotenoid producers.
- Total phenolics contents in microalgae were relatively low.
- Antioxidant capacity was comparable or higher than in plants.
- Carotenoid accumulation should be further induced by biotic or abiotic stress.

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