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Antioxidant capacities of fucoxanthin-producing algae as influenced by their carotenoid and phenolic contents

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ABSTRACT

Natural antioxidants from sustainable sources are favoured to accommodate worldwide antioxidant demand. In addition to bioprospecting for natural and sustainable antioxidant sources, this study aimed to investigate the relationship between the bioactives (*i.e.* carotenoid and phenolic acids) and the antioxidant capacities in fucoxanthin-producing algae. Total carotenoid, phenolic acid, fucoxanthin contents and fatty acid profile of six species of algae (five microalgae and one macroalga) were quantified followed by bioactivity evaluation using four antioxidant assays. *Chaetoceros calcitrans* and *Isochrysis galbana* displayed the highest antioxidant activity, followed by *Odontella sinensis* and *Skeletonema costatum* which showed moderate bioactivities. *Phaeodactylum tricornutum* and *Saccharina japonica* exhibited the least antioxidant activities amongst the algae species examined. Pearson correlation and multiple linear regression showed that both carotenoids and phenolic acids were significantly correlated ($p < 0.05$) with the antioxidant activities, indicating the influence of these bioactives on the algal antioxidant capacities.

1. Introduction

While the market for antioxidant products is predicted to approach $86 billion in 2016, a steady supply of natural bioactive remains the limiting factor for biotechnology, food and nutraceutical applications (Murray et al., 2013). This problem is further escalated by climate changes; leading to plant diseases and poor yield in terrestrial antioxidant sources (Ruiz-Vera et al., 2015; Van de Perre et al., 2015). An aquatic biomass source that can sustainably supply natural biomolecules without increasing the use of terrestrial resources beyond globally sustainable levels (Elkington, 1997; Lozano, 2008) is urgently needed.

Microalgae are increasingly recognized as the next generation sustainable feedstock (Murray et al., 2013; Pérez-López et al., 2014); especially for their bioactive compounds such as carotenoids, phenolic acids and lipids. Increasing numbers of microalgae-derived products can be found in the form of supplements as microalgae species like *Chlorella* and *Spirulina* are being granted GRAS (generally recognized as safe) status. Production of standardized and quality antioxidants is the key for transition from laboratory proof-of-concept to continuous commercial productions. Prior to that, the composition and characteristic of bioactive responsible for a claimed bioactivity must be known.

Microalgae contain bioactive compounds mainly carotenoids and phenolic acids that are responsible for antioxidant activities. In order to establish the relationship between bioactive and their influence on antioxidant activities, Pearson correlation study is the most common statistical tool to do so (Goh et al., 2010; Hajimahmoodi et al., 2010; Jiménez-Escrig et al., 2001; Li et al., 2007). However, correlation is simply a way to describe how two variables are related to each other. Past microalgae correlation studies have reported carotenoids as the major contributors of antioxidant capacities (Goh et al., 2010; Li et al., 2007) whilst other studies claimed phenolic acids were responsible (Hajimahmoodi et al., 2010; Jiménez-Escrig et al., 2001). This discrepancy needs further inferences on the data hence; multiple linear regression analysis may be added to observe the weight of contribution (*i.e.*

Abbreviations: ABTS, 2,2′azino-bis(3-ethylbenzthiazoline-6-sulphonic acid); BCB, beta carotene bleaching; IC, iron chelating; FRAP, ferric reducing antioxidant properties; TPC, total phenolic content; PUFA, polyunsaturated fatty acids; FAME, fatty acid methyl esters; TAC, total antioxidant capacity; TEAC, trolox equivalent antioxidant capacity; GRAS, generally recognized as safe.

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beta coefficients) of the independent (X) and dependent (Y) variables (Yan, 2009).

Despite the fact that fucoxanthin is the major carotenoid of microalgae in the class Bacillariophyceae and Prymnesiophyceae, scientific investigations on fucoxanthin from microscopic sources and their involvement in antioxidant systems are limited (Goh et al., 2010; Li et al., 2007); as compared to their larger commercialized macroalgae counterparts (Airanthi et al., 2011; Martins et al., 2013; Sachindra et al., 2010). In addition, the exclusive marine carotenoid, fucoxanthin is produced in a higher quantity in the microalgae (*e.g. Chaetoceros calcitrans*: 2.33 ± 0.44 mg. g^{-1} DW; (Goiris et al., 2012)) as compared to the macroalgae (*e.g. Laminaria digitata*: 0.468 mg. g^{-1} DW; (Holdt and Kraan, 2011)). In this regard, the commercialized brown macroalga, *Saccharina japonica* (Kanazawa et al., 2008) was included in our investigation for comparison purposes. The five microalgae species were selected because they have gained widespread uses in aquaculture due to their rapid and stable growth rates in hatchery systems, good nutrient composition and absence of toxins that may be transferred up the food chain (Brown, 2002).

In this study, carotenoid and phenolic acid of methanolic extracts were quantified followed by antioxidant evaluations. The aim of this study was to identify lead compounds from each bioactive type and to examine statistical relationship between bioactives and antioxidant activities in the investigated species. The fatty acid and PUFA profile of each species was also reported in this study.

2. Materials and methods

2.1. Chemicals

All chemicals and reagents were of analytical or HPLC grade. Methanol and chloroform were purchased from Merck KGaA (Darmstadt, Germany). Fucoxanthin, boron trifluoride (BF_3), potassium hydroxide (KOH), henicosanoic acid (C21:0), vanillin (4-hydroxy-3-methoxybenzaldehyde), tripalmitin, concentrated sulphuric acid, 3,4,5-trihydroxybenzoic acid (gallic acid), 2,5- dihydroxybenzoic acid, cinnamic acid, protocatechuic acid, sinapic acid, caffeic acid, vanilic acid, chlorogenic acid, ferulic acid, syringic acid, 4-hydroxybenzoic acid, rosmarinic acid, sodium hydrogen carbonate ($NaHCO_3$), potassium persulfate ($K_2S_2O_8$), iron (II) chloride ($FeCl_2$), 3-(2-pyridyl)-5,6-diphenyl-1,2-4-triazine-4′4″ disulphonic acid monosodium salt (ferrozine), 2,4,6-tripyridyl-s-triazine (TPTZ), 0.1 M acetate buffer, linoleic acid, beta carotene (type II, synthetic), Tween 20, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin-Ciocalteu phenol reagent, sodium nitrate ($NaNO_3$), silica ($Na_2SiO_3{\cdot}9H_2O$), ethylenediaminetetraacetic acid disodium (Na-EDTA), boric acid (H_3BO_3), sodium hydrogen phosphate ($NaH_2PO_4{\cdot}2H_2O$), zinc chloride ($ZnCl_2$), cobalt chloride ($CoCl_2{\cdot}6H_2O$), copper sulphate ($CuSO_4{\cdot}5H_2O$), iron (III) chloride ($FeCl_3{\cdot}6H_2O$), ammonium molybdate ($(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$), manganese chloride ($MnCl_2{\cdot}4H_2O$), cobalamin (vitamin B_{12}) and ammonium formate (NH_4HCO_2) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA).

2.2. Algae biomass collection

Tropical microalgae species, *C. calcitrans* (UPMC-A0010), *Isochrysis galbana* (UPMC-A0009), *Skeletonema costatum* (UPMC-A0019) and *Odontella sinensis* (UPMC-A0050) were isolated from local waters. The diatom, *Phaeodactylum tricornutum* was a gift from Dr. Graziella Chini Zittelli from Italy. All microalgae species were maintained in the Microalgae Culture Collection Unit, Laboratory of Marine Biotechnology, Institute of Bioscience, Universiti Putra Malaysia, Serdang, Selangor, Malaysia. Detailed culture conditions and biomass collection were described in our previous study (Foo et al., 2015a). At least 5.0 g of biomass was collected for each species and stored at −80 °C prior to analysis. The commercial macroalga biomass, *S. japonica* Aresch was procured from Changsha NutraMax Inc., China for comparison purposes in terms of bioactive content and bioactivity.

2.3. Preparation of algae methanolic extracts

The common solvents used to extract algal antioxidants is methanol (Goh et al., 2010; Kim et al., 2012; Natrah et al., 2007). Solvent comparison study also found that methanol has the polarity most suitable in extracting antioxidants; especially from brown microalgae. This was reflected by the good radical scavenging and chelating activities (Foo et al., 2015a). As such, approximately 1.0 g of lyophilised biomass from each species was added with 250 ml methanol and homogenized (WiseTis® HG–15 s digital homogenizer, Daihan Scientific, Korea) at $14125.93 \times g$ for 15mins. Filtrates from three extractions were pooled and methanol was removed under reduced pressure using a rotary evaporator (RotaVapor R210, Buchi, Poshfach, Flawil, Switzerland). Extracts were then placed in a 40 °C oven followed by a desiccator at room temperature to completely remove residual solvents. Dry weight was taken and this procedure was repeated until a constant weight of the extract was obtained. The percentage extraction yield (%) was calculated according to the formula; $yield\ (\%) = \frac{weight\ of\ extract\ (g)}{weight\ of\ dry\ biomass\ (g)} \times 100\%$.

All extracts were stored in −80 °C freezer at minimal light exposure.

2.4. Antioxidant activities

The total amount of antioxidant of algal biomass was determined using total antioxidant capacity (TAC) assay; which included the trolox equivalent antioxidant capacity (TEAC) and ferric reducing antioxidant properties (FRAP) assays (Rubio et al., 2016). TEAC assay is also known as ABTS radical cation scavenging assay (Aminjafari et al., 2016).

The $ABTS^{\cdot+}$ scavenging activity of algae extracts was determined according to Foo et al. (2015b). Extracts from each species were prepared in triplicates by fully dissolving 10 mg of dried extracts each in 1000 μl methanol. $ABTS^{\cdot+}$ solution was produced by adding 50 ml of 7 mM ABTS stock solution with 50 ml of 2.45 mM potassium persulfate in the dark at room temperature. After 24 h of incubation, $ABTS^{\cdot+}$ solution was diluted to an absorbance of 0.70 ± 0.02 at 734 nm. Then, 200 μl of the adjusted solution was added to 20 μl sample in the dark and left to react for 10mins in a 96-well microtitre plate. The absorbance of the radicals-sample mixture was measured at 734 nm (Multiskan™ GO UV/Vis microplate spectrophotometer, Thermo Fischer Scientific, USA). Trolox was used as the standard (3.125–100 μg. ml^{-1}; 2 fold dilution) and antioxidant activity was expressed in milligram trolox equivalent per gram dry weight biomass (mg TE. g^{-1} DW).

The iron chelating (IC) properties of microalgae extracts were determined according to Decker and Welch (1990). To 100 μl of sample extract, 135 μl distilled water and 5 μl of 2 mM $FeCl_2$ were added. After addition of 10 μl of 5 mM ferrozine, mixtures were left for 10 min and measured at 562 nm (Multiskan™ GO UV/Vis microplate spectrophotometer, Thermo Fischer Scientific, USA). Na-EDTA was used as standard (3.125–100 μg. ml^{-1}; 2 fold dilution) and methanolic extract iron chelation activity was expressed in milligram EDTA equivalent per gram dry weight biomass (mg Na-EDTA .g^{-1} DW).

FRAP of microalgae methanolic extracts was determined according to Nilsson et al. (2005). To 50 μl extract, 150 μl of FRAP reagent