Artículo

“Chemical composition and physicochemical properties of Phaeodactylum tricornutum microalgal residual biomass”

1. Contribución al fortalecimiento de la línea de investigación y su relación con los pronaces

El presente artículo fue producto de mi trabajo de tesis de maestría, contribuyo al fortalecimiento de la linea de investigación en el área de biotecnología, con la implementación de ciencia básica y de frontera en la busqueda de alternativas sustentables relacionando algunos programas de pronaces desde energía y cambio climático, agua y soberanía alimentaría. Debido a que, la producción de biocombustibles fotosintéticos a partir de microalgas es una estrategia prometedora para combatir el uso de fuentes de energía no renovables. Además, La biomasa residual de microalgas es un subproducto de desecho de la producción de biocombustibles; sin embargo, podría resultar útil en el desarrollo de nutracéuticos sostenibles y alimentos funcionales. Los resutados publicados han sido utilizados ya, para el uso e implementación de microalgas de manera integral en nuestro país, en zonas costeras y algunas ciudades como Guadalajara existen hoy en día empresas que estan elaborando diversos productos alimenticios, los cuales presentan buenas propiedades nutricionales, por lo qué, son una fuente de compuestos bioáctivos que son benéficos para la salud, además estas empresas una fuente de trabajo novedosa.

2. Índices donde se encuentra incorporado

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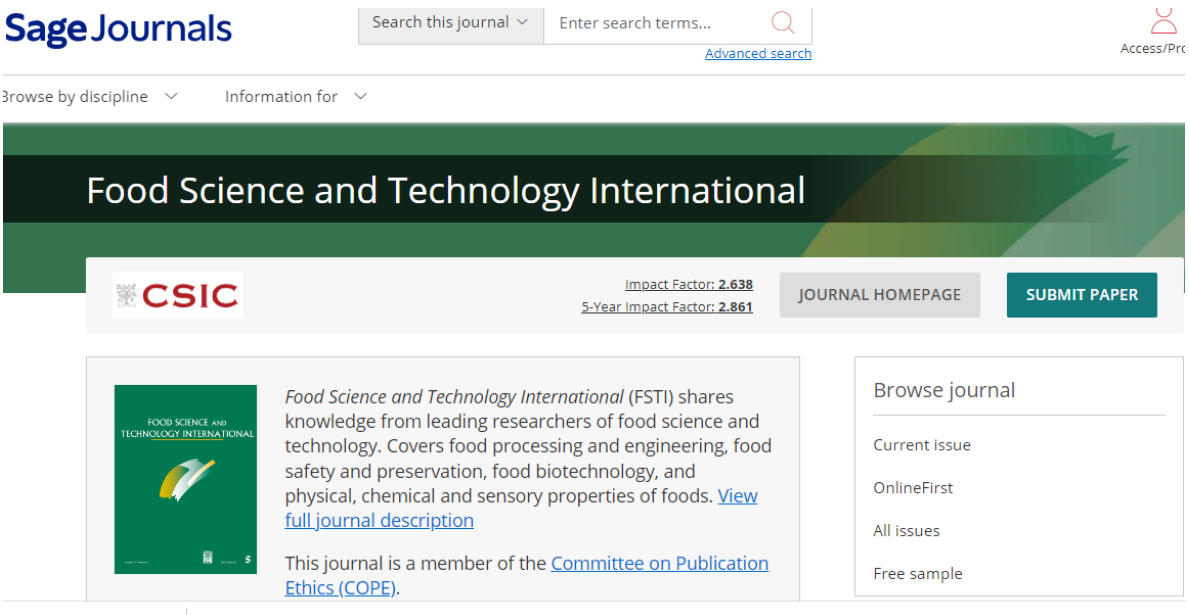

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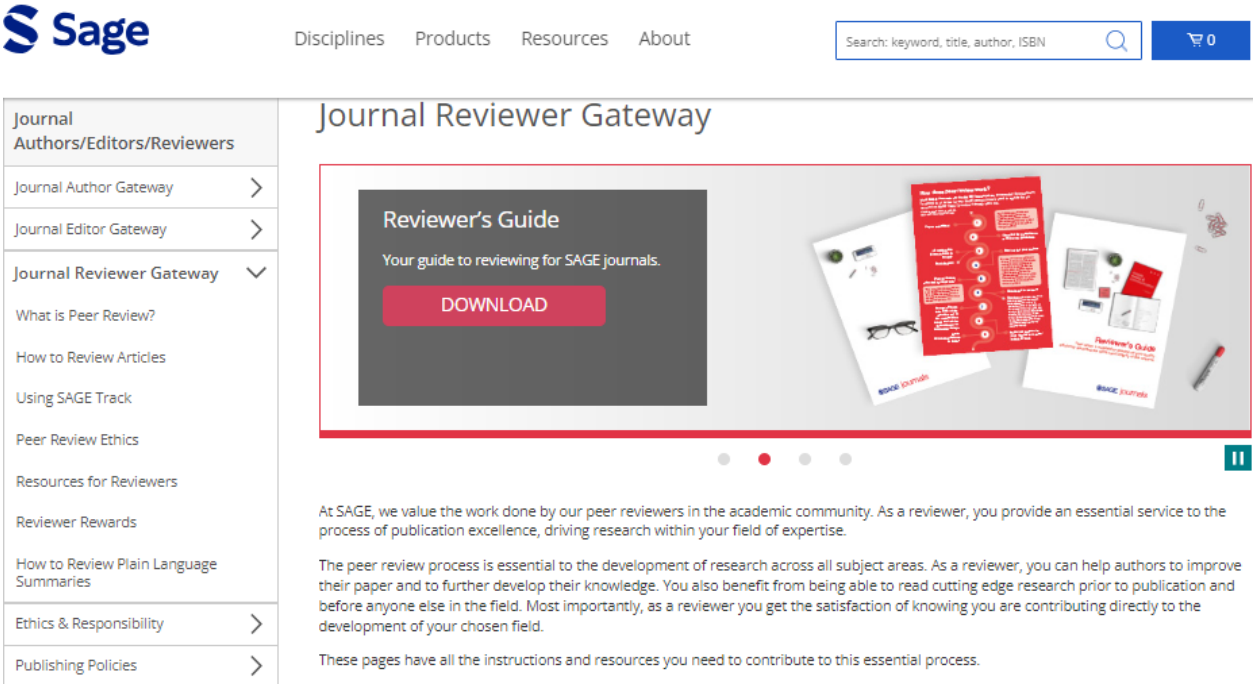

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Article

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Chemical composition and physicochemical properties of *Phaeodactylum tricornutum* microalgal residual biomass

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Abstract

The production of photosynthetic biofuels using microalgae is a promising strategy to combat the use of non-renewable energy sources. The microalgae residual biomass is a waste by-product of biofuel production; however, it could prove to have utility in the development of sustainable nutraceuticals and functional foods. In this study, a comprehensive characterisation of the under-utilised *Phaeodactylum tricornutum* microalgae residual biomass is presented. Proximal composition, antioxidant capacity (using three different antioxidant assays; oxygen radical absorbance capacity; radical cation activity, ABTS; and radical scavenging activity, DPPH), and total phenolic content of free and bound polyphenols were determined. Additionally, the physicochemical properties of water activity, pH, water absorption index, water solubility index, and dispersibility were evaluated. Results revealed that *P. tricornutum* microalgae residual biomass exhibits a relatively high protein and carbohydrate content, with values of 36.67% and 46.78%, respectively; and most carbohydrates were found as total dietary fibre (45.57%), of which insoluble dietary fibre was the most predominant (43.54%). Antioxidant capacity values for total phytochemicals of 106.22, 67.93, 9.54 μM TE g^{-1} dw were determined by oxygen radical absorbance capacity, ABTS, and DPPH assays, respectively. Total phenolic content was found to be 2.90 mg GAE g^{-1} dw. Interestingly, antioxidant capacity and total phenolic content were higher in bound than in free phytochemical extracts. The physicochemical analysis showed *P. tricornutum* microalgae residual biomass to have suitable properties for the generation of a beverage with Aw, pH, water absorption index, water solubility index, and dispersibility values of 0.45, 7.12, 3.40 g gel g^{-1} dw, 2.5 g solids 100 g^{-1} dw, and 90%, respectively. Hence, *P. tricornutum* microalgae residual biomass could be considered a potential source of bioactive compounds suitable for the production of functional food exhibiting antioxidant capacity and high dietary fibre content.

Keywords

Antioxidant, microalgal biomass, phenolics, functional food

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INTRODUCTION

Dramatic increases in the use of fossil fuels have accompanied an upturn in public awareness of these limited and unrenewable sources of energy in the past few decades. More recently, the use of photosynthetic microorganisms, such as microalgae, for the production of the so-called 'new generation biofuels' has been shown as a promising strategy to combat this issue. However, due to their high processing costs, this process is not yet a commercial reality (Wijffels et al., 2010). Besides oil for biodiesel production, microalgae synthesize diverse phytometabolic contents that are relatively poorly exploited. After microalgae oil extraction, 70% of the whole biomass remains as the microalgae residual biomass (MRB). The MRB has high proportions of proteins and carbohydrates, and owing to the long evolutionary adaptive diversification to multiple extreme environmental conditions, it is considered as an untapped source of bioactive compounds, including phytochemicals. This therefore makes them good candidates for the discovery of new antioxidant components (Norzagaray-Valenzuela et al., 2016b). Thus, microalgae products can serve as highly relevant commercial components of various applications, such as pharmaceuticals, nutraceuticals, and food supplements (Priyadarshani and Rath, 2012); however, they are as yet not fully utilised.

More recently, it is of particular interest to discover new, safe, and powerful antioxidants from natural sources in order to reduce oxidative damage in cells and prevent degenerative chronic conditions, such as cancer, diabetes, and cardiovascular complications (Goiris et al., 2012; Valdez-Flores et al., 2016). In this regard, the feasibility of using microalgae as natural antioxidants is further supported by the relative ease of the extraction steps of target compounds (Goiris et al., 2012).

More than 40 different microalgal species have been isolated from diverse parts of the world and cultured in intensive systems. These include microalgal groups, such as Chlorophyta (green algae), Rhodophyta (red algae), and Phaeophyta (brown algae) (Lavenz and Sorgeloos, 1996; Costa et al., 2010). Although extensive studies exist in exploring microalgae bioactivities, there is limited information about the diatom Phaeophyta, as Chlorophyta is most frequently studied. Given the difference in pigmentation between the two species, it may be suggested that Phaeophyta contain different bioactive metabolites, which should be evaluated.

In order to bring new insights into diverse potential applications of MRBs, the Phaeophyta *Phaeodactylum tricornutum* MRB was characterised through determination of proximal composition, antioxidant capacity (AOXC) with three different antioxidant assays, total phenolic content (TPC), and various physicochemical properties.

MATERIALS AND METHODS

Materials

P. tricornutum was provided by the Centro de Investigación Científica y Educación Superior de Ensenada (CICESE) culture collection in Baja California, Mexico. The 2,2′-azobis (2-amidinopropane) dihydrochloride (AAPH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid (ABTS), gallic acid, Trolox, Folin–Ciocalteu, HCl, ethanol, and acetic acid reagents were of analytical grade and purchased from Sigma-Aldrich Co. (St Louis, MO, USA).

Culture conditions and biomass processing

P. tricornutum MRB was obtained as previously reported by Norzagaray-Valenzuela et al. (2016b). *P. tricornutum* was grown in sterile natural water enriched with F/2 medium nutrients at 24 °C, 1% CO_2, in continuous light (irradiance of 120–130 µmol photons $m^{-2}s^{-1}$). The culture was harvested in late log-phase of growth using chitosan as a flocculant. Subsequently, microalgae oil was extracted by exposing the sample to a suspension of chloroform:methanol in the ratio of 2:1 with ultrasonication (200 W power, 24 kHz frequency, 40% amplitude) on ice, for 6 h, using a sonicator UP200S (Hielscher Ultrasonics, Germany). MRB was collected, dried at 45 °C for 24 h, ground into powder using a Cyclone Sample Mill (UD Corp., Boulder, CO, USA), and stored at 4 °C.

Proximate composition

Proximate composition of MRB was determined following the Association of the Official Analytical Chemists (AOAC) methods (AOAC, 1999). Drying at 105 °C for 24 h for moisture (method 925.09B), total nitrogen was determined by the MicroKjeldahl method (method 960.52), and protein was calculated using a nitrogen-to-protein conversion factor ($N \times 6.25$). Lipids were determined using a Soxhlet extraction system (method 920.39) using hexane as the solvent, ashes were determined gravimetrically (method 923.03) after heating the samples at 550 °C for 16 h, and carbohydrates were estimated by the difference of total material. All measurements were performed in triplicate and results were expressed as a percentage of dry weight (% dw). Soluble and insoluble dietary fibre were determined following the AOAC (1999) enzymatic-gravimetric method for total dietary fibre (TDF) (method 985.29) using the TDF assay kit from Sigma-Aldrich (TDF 100A).

Extraction of free and bound phenolic compounds

Free and bound phenolic compound extractions were performed as described by Mora-Rochin et al. (2010), with minor modifications. Free phenolic compounds were extracted from 0.25 g of sample, and this was homogenised with 5 mL of ice-cooled ethanol 80% for 10 min and then centrifuged at 2500 × *g* for 10 min (centrifuge model 5804R, Eppendorf, AG, Hamburg, Germany). The supernatant was taken and concentrated under vacuum at 45 °C, and phenolic residues were reconstituted in methanol–water (1:1, v/v) and stored at −20 °C until use. The pellet was used for bound phenolic compound extraction. Consequently, the pellet was hydrolyzed with 5 mL of 2 N NaOH for 30 and 60 min at 95 °C and 25 °C, respectively, in a water bath (Labnet W1106A, Labnet International, Inc., NJ, USA), and then agitated in a rotator for 1 h (OVAN Noria R, USA). Finally, the mixture was acidified (pH < 2.0) with 2 N HCl and exposed to 5 mL of hexane to remove lipids. The sample was extracted five times with 10 mL of ethyl acetate, and the five fractions were pooled and dried under vacuum at 35 °C. The final sample was reconstituted in 2 mL of methanol–water (1:1, v/v) and stored at −20 °C until further use.

Total phenolic content

Total phenolic content of free and bound extracts were determined using the Folin–Ciocalteu colorimetric method as described by Singleton et al. (1999). Twenty microlitres of extract was added to 180 μL of Folin–Ciocalteu reagent (Sigma Chemical Co., St Louis, MO, USA). After 20 min incubation at room temperature in the dark, the absorbance of the reaction mixture was measured at 750 nm using a microplate reader (SynergyTM Multi-Detection, BioTek, Inc., Winooski, VT, USA). Gallic acid (Sigma Chemical Co., St Louis, MO, USA) was used for a standard calibration curve, and the total phenolic content was expressed as milligrams of gallic acid equivalents (mg GAE) per gram of sample dry weight (g^{-1} dw). All measurements were made in triplicate, and results were calculated as mean value ± standard deviation (SD) ($n = 3$).

Determination of AOXC

The AOXC of the different MRB extracts was determined in vitro using oxygen radical absorbance capacity (ORAC), DPPH, and ABTS assays. In each assay, a Trolox standard curve was made with fluorescein and used as a reference (Ou et al., 2001). All results were expressed as μmol of Trolox equivalent per gram of dry weight sample (μmol TE g^{-1} dw). All measurements were made in triplicate, and results were calculated as mean value ± standard deviation (SD) ($n = 3$).

ORAC assay was performed using the methodology of Ou et al. (2001). In this method, the antioxidant action of hydrogen atom transfer as well as single electron transfer is quantified through monitoring fluorescent degradation. Peroxyl radicals were generated by 2-2'-azabis (2-amidinopropane) dihydrochloride, and fluorescein degeneration was monitored in a Microplate reader (Synergy^{TM}, BioTek, Inc., Winoosgki, VT, USA). The excitation and emission wavelengths were set at 485 and 538 nm, respectively.

ABTS assay was performed using the methodology of Li et al. (2007). The ABTS*$^{+}$ radical cation was generated by reaction of ABTS with potassium persulphate. The subsequent reduction of the ABTS*$^{+}$ radical by MRB extracts is indicative of the AOXC of the sample. The reaction mixture was allowed to stand in the dark for 16 h at room temperature and was used within two days. The ABTS*$^{+}$ solution was diluted with ethanol, to give an absorbance of 0.700 ± 0.050 at 734 nm. Fifty microlitres of diluted sample were mixed with 1.9 mL of diluted ABTS*$^{+}$ solution and the absorbance of the resulting mixture was recorded at 734 nm after 6 min of incubation at room temperature.

DPPH assay AOXC values were determined following the method of Fukumoto and Mazza (2000). The DPPH reagent was prepared in 80% methanol to a final concentration of 150 μM. Twenty-two microlitres of each extract were added to 200 μL of DPPH radical solution (150 μM) in 96-well plates. After 30 min of incubation at room temperature, absorbance was measured at 520 nm, using methanol as blank. A Trolox standard curve was prepared with methanol 80% (0–200 μM).

Physicochemical properties

Water activity. Water activity (Aw) was determined using a calibrated Hygrometer Aqua Lab Model CX-2 (Decagon Devices, Inc., Pullman, WA, USA). Approximately 2 g of MRB was tempered at 25 °C and introduced into the Hygrometer. In order to attain headspace equilibrium, measurements started after 1 h. Calibration was performed with a saturated potassium chloride solution (Aw = 0.841 at 25 °C).

pH. The pH of the sample was determined according to the 02-52 AOAC (1999) method using a pH meter (Orion star A111 Benchtop, Thermo Scientific, MA, USA). A 10% (w/v) suspension was prepared with 5 g of MRB and 50 mL of boiled distilled water at 25 °C. The suspension was shaken at 1500 r/min at 25 °C for 20 min using an orbital shaker (Cole Parmer Model

21704-10, Cole Parmer International, Vernon Hills, IL, USA).

Water absorption index and water solubility index. Water absorption index (WAI) and water solubility index (WSI) determination were performed as previously described by Anderson et al. (1969). The sample (2.5 g) was mixed with 30 mL of distilled water and the slurry was stirred with a glass rod, and then cooked at 90 °C in a water bath (Labnet W1106A, Labnet International, Inc., NJ, USA) for 10 min. Subsequently, the cooked paste was cooled to room temperature, centrifuged at $3000 \times g$ for 10 min (centrifuge model 5810, Eppendorf, AG, Hamburg, Germany), and the supernatant was poured carefully into a tared evaporating dish. WAI was calculated from the weight of the remaining gel, and the result was expressed in grams of gel g^{-1} of sample dw. WSI was calculated from the weight of dry solids that were recovered by evaporating the supernatant overnight at 110 °C using an oven (Yamato Scientific American, Inc., IC402, Tokyo, Japan), and expressed in grams of solids 100 g^{-1} of sample dw.

Dispersibility. Dispersibility was determined as described previously by Guzmán-Uriarte et al. (2013) with modifications. A 10% (w/v) suspension was prepared with 1 g of MRB with 10 mL of distilled water in 50 mL graduated conic tube. The mixture was vigorously stirred (Ultra Turrax homogeniser, 10,000 r/min, 5 min) and allowed to settle for 30 min. In order to obtain the dispersibility (%), the volume of settled particles was recorded and subtracted from 10. This value was divided between 10 and multiplied by 100.

Statistical analysis. Analyses were performed in triplicate. Data were analysed using one-way analysis of variance (ANOVA) followed by Fisher's test to determine significant difference between samples ($p < 0.05$). Data were analysed using Statgraphics Centurion XV software (Statpoint Technologies, USA).

RESULTS AND DISCUSSION

Proximal composition of *P. tricornutum* MRB

The proximal composition of *P. tricornutum* MRB is shown in Table 1. This study shows that *P. tricornutum* biomass, after oil extraction, is essentially composed of proteins and carbohydrates, with values of 36.67% and 46.78%, respectively. Lipids (1.07%) and ashes (15.46%) constitute the remaining biomass. The protein content of *P. tricornutum* MRB was higher than that previously reported for MRBs of *Dunaliella tertiolecta* (22.59%), *Tetraselmis suecica* (23.16%), and *Nannochloropsis* sp. (25.46%) (Norzagaray-Valenzuela et al., 2016b). Although there are no studies of the protein quality of *P. tricornutum* MRB, other reports using the complete biomass have found good quality protein with high levels of essential amino acids (Brown, 1991). Brown (1991) evaluated the amino acid profile of *P. tricornutum* and 15 other microalgal species, reporting all to have greater than or similar levels of the same amino acids in oyster larvae, indicating a good protein quality in all assessed microalgal species. Brown (1991) also suggested that other criteria beyond amino acid composition, such as ascorbic acid, riboflavin, and polysaccharide content should be taken into account to describe the nutritional value of a microalgal species.

Table 1. Proximate composition of microalgae residual biomass

Component	MRB (%)
Proteins	36.67 ± 0.43
Lipids	1.07 ± 0.01
Carbohydrates	46.78 ± 0.47
Total dietary fibre	45.57 ± 0.17
Soluble	2.02 ± 0.02
Insoluble	43.54 ± 0.28
Ashes	15.46 ± 0.03

Results are expressed as percentage total MRB and as the mean of triplicates ± standard deviation.
MRB: microalgae residual biomass.

In this study, high carbohydrate content was also found in *P. tricornutum* MRB (46.78%). This result is consistent with Kim et al. (2015), who reported high carbohydrate content in *D. tertiolecta* residual biomasses (51.9%). Several studies have suggested that the major constituents of carbohydrates in microalgae are polysaccharides, which are involved in ionic, osmotic, and mechanical functions (Guil-Guerrero et al., 2004). Polysaccharides, such as agar, carrageenans, and alginates are isolated from microalgae and used in food, pharmaceuticals, cosmetics, paper, and textiles industries as stabilisers, emulsifying, and gelling agents (Laurienzo 2010). Additionally, algal carbohydrates are easily extractable, and are considered as potential bioactive ingredients in functional food (Laurienzo, 2010). For example, microalgal carbohydrates have been shown to have a variety of bioactivities, such as hyaluronidase, AOXC inhibition (Norzagaray-Valenzuela et al., 2016b), and antibacterial activity (Raposo et al., 2014). Moreover, for biotechnological conversion technologies, carbohydrates are the desirable or the principal substrate for several important biofuel products (bioethanol, biobutanol, and biohydrogen) (Markou et al., 2012). Therefore, besides microalgae

oil being used for the production of biodiesel, carbohydrate-rich MRB may be utilised for other highly relevant biofuels, for textiles industries, and for the generation of nutraceuticals and functional food (Hammed et al., 2015; Kim et al., 2015).

As expected, the lipid content was determined as low, which is an indicator of the efficiency of the oil extraction process, and ashes content was similar to that reported previously for *D. tertiolecta* residual biomass (16.76%) (Norzagaray-Valenzuela et al., 2016b). The ashes content is an indicator of the presence of certain minerals, likely to be inorganic constituent elements, such as silicon, potassium, sodium, magnesium, iron, manganese, and aluminium, as all are commonly found in other marine microorganisms. Concentrations of these inorganic constituents vary according to species and growth conditions (Kim et al., 2015).

To further analyse the proximal composition of *P. tricornutum* MRB, total, soluble, and insoluble dietary fibre content was determined. It can be observed from Table 1 that MRB exhibits 45.57% TDF, of which 43.54% is insoluble dietary fibre and the remaining 2.02% is soluble. To our knowledge, this is the first report showing dietary fibre content of MRB. The result of TDF was found to be higher than some brown seaweeds previously reported, such as *Fucus spiralis* and *Undaria pinnatifida*, as well as foods such as apples, beans, brown rice, and rye that are considered high in dietary fibre (Raposo et al., 2016).

Dietary fibre consists of polysaccharides that are resistant to hydrolysis by human digestive enzymes, including cellulose, hemicellulose, lignin, gums, pectin, amongst others (McCleary et al., 2012). Polysaccharides can be found in microalgae primarily as cell-wall constituents and storage products (Raposo et al., 2016). Brown algae (Phaeophyta), such as *P. tricornutum*, contain a variety of carbohydrates including alginates, fucans, and laminarans. Red seaweeds (Rhodophyta) contain sulphated galactans, xilans, floridean, and starch, whereas green algae (Chlorophyta) have starch, xilans, mannans, uronic acids, rhamnose, xylose, galactose, arabinose, and ionic polysaccharides with sulphate groups (Laurienzo, 2010). Insoluble dietary fibre, like cellulose, is found in all algal species (Hammed et al., 2015). Dietary fibre intake, particularly TDF or insoluble dietary fibre has been previously suggested as a method of controlling blood pressure. Such changes in blood pressure can be attributed to the enhanced insulin sensitivity resulting from the inhibition of macronutrient reabsorption, the reduction of post-prandial glucose responses and modulation of blood lipid constitution (Weickert and Pfeiffer, 2008). This positively influences endothelial function and may improve the pathology of other cardiovascular disease risk factors, such as blood pressure and low-density-lipoprotein (LDL) levels (Aljuraiban et al., 2015). Thus, the high dietary fibre content of *P. tricornutum* MRB is indicative of favourable nutraceutical properties in food; however, the amount of dietary fibre intake has to be considered prior to formulate a functional food.

To summarize, the natural dietary fibre content (and particularly insoluble dietary fibre content) of *P. tricornutum* MRB suggests this brown alga to have favourable physiological properties. However, purification and further analysis of potential beneficial effects is warranted to understand the potential applications of the MRB and to derive new uses for the commercially less-favoured by-product of oil extraction.

AOXC of *P. tricornutum* MRB

The AOXC of *P. tricornutum* MRB was determined for the polyphenols (free and bound) using three different antioxidant assays. As expected, the results of AOXC varied depending on the determination assay used. *P. tricornutum* MRB overall exhibits a good AOXC, with values for total phytochemicals of 106.22, 67.93, 9.54 μM TE g^{-1} dw determined by ORAC, ABTS, and DPPH assays, respectively. TPC was found to be 2.90 mg GAE g^{-1} dw. Interestingly, the bound polyphenols showed significantly higher TPC and AOXC in comparison to free fractions (Table 2).

It is suggested to be cautious when comparing AOXC data with other reports, as AOXC varies depending on the microalgae species and growth conditions used (Goiris et al., 2015). In the current study, *P. tricornutum* was cultured under normal conditions (as stated in the Materials and methods section), and AOXC results were found to be in the range of AOXC values for Chlorophyta MRBs (*D. tertiolecta, T. suecica*, and *Nannochloropsis* sp.) cultured under the same conditions (Norzagaray-Valenzuela et al., 2016b). Additionally, both AOXC and TPC values determined in this study were in the range of those derived from hydrophilic extracts of 15 medicinal plants when using the DPPH assay (Tukun et al., 2014).

Microalgae, like antioxidant food products, have a large variety of biologically active compounds with antioxidant activity that differ in their chemical composition and availability. For example, in microalgae, hydrophilic- and lipophilic-like compounds with antioxidant activity have been reported (Goiris et al., 2012; Li et al., 2007). This large variety of antioxidant compounds complicates determination of total AOXC due to differences in mechanisms of antioxidant action. Therefore, it is recommended that determination of total AOXC should be performed using multiple in vitro methods of quantification (Thaipong et al., 2006).

Table 2. Antioxidant capacity and total phenolic content of microalgae residual biomass

	AOXC			
Phytochemicals	ORAC	ABTS	DPPH	TPC
Free extract	36.20 ± 0.30^{b}	23.02 ± 0.75^{b}	2.42 ± 0.05^{b}	1.10 ± 0.04^{b}
Bound extract	70.02 ± 0.62^{a}	44.90 ± 0.90^{a}	7.11 ± 0.17^{a}	1.79 ± 0.06^{a}
Total	106.22 ± 0.59	67.93 ± 0.36	9.54 ± 0.13	2.90 ± 0.02

Results are expressed as the mean of triplicates ± standard deviation. Means with different letters in each column are significantly different by Fisher test ($p < 0.05$, $n = 3$).
AOXC: antioxidant capacity (oxygen radical absorbance capacity (ORAC), ABTS, and DDPH; μM TE g^{-1} dw); MRB: microalgae residual biomass; TPC: total phenolic content (mg GAE g^{-1} dw).

To more accurately estimate the AOXC of *P. tricornutum* MRB, ORAC (involving hydrogen atom transfer), DPPH (involving single electron transfer) (Cao and Prior, 1999), and ABTS (involving both hydrogen atom transfer as well as single electron transfer) assays were chosen based on their divergent methods of determining AOXC (Goiris et al., 2012; Prior et al., 2005). As observed in Table 2, AOXC value by ORAC was found to be higher than ABTS and DPPH assays. This phenomenon has been shown before by Norzagaray-Valenzuela et al. (2016b), which may be explained by the ORAC assay exhibiting greater specificity and being more capable of responding to a greater number of antioxidant compounds than the ABTS and DPPH assays (Thaipong et al., 2006).

Additionally, the type of extraction solvent greatly affects quantification of AOXC, as the variety of antioxidant compounds present in microalgae have different polarities and activities after extraction (Goiris et al., 2012). When screening 32 microalgae species for AOXC, Goiris et al (2012) found an ethanolic/water extract to demonstrate higher AOXC than hexane, ethyl acetate, and water extracts. Conversely, Maadane et al. (2015) reported higher antioxidant capacities in the ethanolic extract of nine microalgae species than that found in water and water/ethanol extracts. Thus, ethanolic extraction was chosen in the present study.

Table 2 demonstrates the difference in free and bound MRB extracted using ethanol. Phenolic compounds of MRB are present in both free and bound forms; however, they are more abundant in the bound fraction, contributing to more than 61% with respect to total phytochemicals. As aforementioned, AOXC was found to be higher in the bound fraction, determined with the three AOXC assays, indicating the possible contribution of MRB phenolic content to the antioxidant activity. This result is in general agreement with other studies in which bound fractions are shown to have higher TPC and AOXC than free fractions (Adom and Liu, 2002).

Table 3. Physicochemical properties of MRB

Determination	MRB
Aqua activity (Aw)	0.45 ± 0.01
pH	7.12 ± 0.02
WAI	3.40 ± 0.06
WSI	2.50 ± 0.70
Dispersibility	90.00 ± 0.05

Results are expressed as the mean of triplicates ± standard deviation; dispersibility (%).
WAI: water absorption index (g gel g^{-1} dw); WSI: water solubility index (g solids 100 g^{-1} dw).

Physicochemical properties of *P. tricornutum* MRB

Owing to its good AOXC and the relatively high levels of proteins, carbohydrates, and TDF, the physicochemical properties of *P. tricornutum* MRB were analysed to determine potential new uses in the context of application of flours as food, or food ingredients for the generation of functional food (Leonel et al., 2009).

The results of the physicochemical analysis of *P. tricornutum* MRB are shown in Table 3, and revealed values of Aw 0.45 and a pH of 7.12. For WAI and WSI, values of 3.40 g gel g^{-1} dw and 2.50 g solids 100 g^{-1} dw were determined respectively, in addition to a dispersibility of 90%.

Aw is an important parameter in food conservation to judge the quality characteristics of flours, such as texture and flavour. An Aw of 0.45 suggests a good quality MRB, since low Aw decreases enzymatic activity, biochemical reactions, and results in less probable the growth of microorganisms, indicating long shelf life (Concha-Meyer et al., 2016). WAI measures the amount of water absorbed by the sample. This is a characteristic that plays an important role in food preparation processes, because this parameter represents the index of gelatinisation as the ability of a product to interact with water and thus is important for rehydration (Anderson et al., 1969; Guil-Guerrero et al., 2004).

The WAI result of 3.12 g gel g^{-1} dw determined in this study was higher than those reported by Yellavila et al. (2015), who found WAI ranging from 0.89 to 1.12 g gel g^{-1} dw for bean flours from different cultivars. However, the WAI was slightly lower than those observed previously by Norzagaray-Valenzuela (2016a), in which values varied from 4.19 to 5.53 g gel g^{-1} dw for Chlorophyta MRBs (*D. tertiolecta, T. suecica*, and *Nannochloropsis* sp.). This could be due to the generally high carbohydrate content found in MRBs, which correlates with the findings stated from several authors that flours with higher water absorption may have more hydrophilic constituents, such as polysaccharides (Guil-Guerrero et al., 2004). Indeed, exopolysaccharides are commonly reported in microalgal cell walls, serving to protect them from stress conditions, such as desiccation in the natural marine environment (Yellavila et al., 2015).

WSI measures the amount of soluble molecules (particularly polysaccharides and proteins) released from the matrix on addition of excess water, and is used as an indicator of degradation of molecular components; commonly a consequence of processes in which cooking is involved (Seth et al., 2015). A result of 2.50 g solids 100 g^{-1} dw for the selected MRB was slightly lower than the values found by Kaushal et al. (2012) for rice flour with values of 2.66 g solids 100 g^{-1} dw. The low WSI found in this study could be due to the oleaginous nature of microalgae as a negative correlation between lipid content and WSI has been reported (Kaushal et al., 2012).

With respect to dispersibility, this parameter indicates the reconstitution property and denaturalised index of carbohydrates and proteins in a processed product. A high dispersibility results in a better reconstitution (Ghavidel and Davoodi, 2011). In this study, a higher percentage (90%) was found for *P. tricornutum* MRB than that reported by Ghavidel and Davoodi (2011) with values from 72% to 78% for different mixtures of cereals, malted legumes, and vegetable powders. Taken together, this study indicates that *P. tricornutum* MRB have physicochemical properties suitable for use as an ingredient in the production of bakery products, pasta, and soups as functional foods, in addition to a promisingly high dispersibility relevant for the generation of a nutraceutical beverage (Oshodi et al., 1997).

CONCLUSION

P. tricornutum MRB has been shown to have good AOXC, measured through three different AOXC assays and total phenolic content, and a high protein and carbohydrate compositions. The physicochemical properties of MRB suggest its potential use as a nutraceutical component of food and beverages. The physicochemical and compositional properties of *P. tricornutum* MRB suggest potential profitable uses for this previously unexplored residual product of microalgae biodiesel production as a superior source of ingredients for the generation of functional foods, such as pasta, soups, and beverages with AOXC and high dietary fibre content.

DECLARATION OF CONFLICTING INTERESTS

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