Lipid composition and some bioactivities of 3 newly isolated microalgae (*Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp.)

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Abstract

Recently isolated microalgae *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp. were studied. The three novel strains contained relatively high levels of polyunsaturated fatty acids (PUFA) and n3 PUFA. However, highly unsaturated n3 FA contents were relatively low (5.7–13.0% of the total FA). In general, eicosapentaenoic acid (EPA, 20:5 n3) contents were low (< 6.4% of the total FA). However, in *Skeletonema* biomass, EPA levels were higher than 10% of the total FA. α-Linolenic acid (ALA, 18:3 n3) and 16:3 n4 were the main PUFA in *Tetraselmis* strains and *Skeletonema*, respectively. High contents of myristic (14:0) and palmitoleic (16:1 n7) acids were found in *Skeletonema* (exceeding 20% of the total FA in both cases), whereas the *Tetraselmis* strains were rich in palmitic (16:0), 15–27% of the total FA, and oleic (18:1 n9) acids, 12–19% of the total FA. Linoleic acid (18:2 n6) content was low in *Skeletonema* (< 1% of the total FA). This microalga had the highest total polyphenol content, reaching 300–400 mg/100 g dw. Gentisic acid was the main phenolic compound in the aqueous and ethanolic extracts of this microalga. The highest antioxidant activity was displayed by *Skeletonema*. The ferric ion reducing antioxidant power (FRAP) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) methods showed higher antioxidant power for *Skeletonema* sp. extracts, reaching an ABTS reduction of more than 80%. Concerning anti-inflammatory activity, ethanolic extracts of *Skeletonema* sp. exhibited the highest inhibitory capacity of cyclooxygenase-2 (COX-2), $82 \pm 2\%$, which compares to $36 \pm 9\%$ in *Tetraselmis* sp. CTP4 and $45 \pm 5\%$ in *Tetraselmis* sp. IMP3. Aqueous extracts had always a lower anti-inflammatory capacity, 6–30%. Therefore, these microalgae have potential for multiple applications, ranging from bioactive feedstocks to aquaculture and nutraceutical uses.

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Introduction

Microalgae are a relevant and still not fully exploited aquatic resource (Matos et al. 2017). These microorganisms are a promising and valuable natural source of bioactive compounds, such as n3 polyunsaturated fatty acids (n3 PUFA) and polyphenols. Such compounds contribute to antioxidant, anti-inflammatory, and other biological activities. Microalgal biomass can thus be incorporated as a functional ingredient into nutraceutical products due to its high potential to positively influence human health.

In particular, microalgae of the genera *Tetraselmis* (Chlorophyta) and *Skeletonema* (diatom), which have previously been reported to contain a substantial lipid fraction, are potentially promising bioactive feedstocks (Jiang et al. 2016; Patil et al. 2007; Renaud et al. 1999). Moreover, *Tetraselmis* species are commonly used in aquaculture, because of their high nutritional value (Adarme-Vega et al. 2014). Some species, such as *Tetraselmis tetrathele*, have been reported to contain a high total phenolic content and to be a potential antioxidant source, as assessed by 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric ion reducing antioxidant power (FRAP), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays (Farahin et al. 2016). *Skeletonema* species, such as *S. costatum*, are known for their relatively high amounts of PUFA (Blanchemain and Grizeau 1996). More specifically, eicosapentaenoic acid (EPA, 20:5 n3) may account for 20 to more than 50% of their PUFA.

Within n3 PUFA, EPA and docosahexaenoic acid (DHA, 22:6 n3) are associated with decreased morbidity and mortality from cardiovascular and other diseases as well as with fetal development (Simopoulos 2002). Reviewed evidence also pointed out the benefits for the development of the neural system in children (Cardoso et al. 2018) and prevention of mild cognitive decline in elderly (Cardoso et al. 2016). Furthermore, EPA has been claimed to enhance anti-inflammatory properties of high-density lipoprotein among other effects (Tanaka et al. 2014). DHA is an essential FA that has been recognized as a source of anti-inflammatory specialized proresolving mediators: resolvins, protectins, and maresins (Kuda 2017).

Anti-inflammatory activity of microalgae, such as *Isochrysis galbana* (Nuño et al. 2013), has been reported. This activity may be ascribed to n3 PUFA or other compounds, such as peptides, carotenoids, or sulfated polysaccharides (Talero et al. 2015). In the case of the microalga *Chlorella*, evidence linking extracts of this organism to anti-inflammatory outcomes has been stronger (Guzmán et al. 2003; Matos et al. 2017). Moreover, for the microalga *Porphyridium cruentum*, a sulphoglycolipidic fraction has been shown to display anti-inflammatory properties (Bergé et al. 2002). Hence, it is worth testing if any novel microalga displays substantial anti-inflammatory activity.

The main aim of this study was to bioprospect the biomass of three novel microalgae strains, namely *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp. This entailed the determination of the lipid composition, polyphenol content, and relevant bioactivities, such as antioxidant (measured by DPPH, FRAP, and ABTS methods) and anti-inflammatory activities, thereby paving the way for future nutraceutical applications.

Material and methods

Microalgae growth, collection, and preparation

Tetraselmis sp. IMP3 and *Tetraselmis* sp. CTP4 were isolated by fluorescence-activated cell sorting using the methodology described in Pereira et al. (2011) from environmental samples

gathered in Ria Formosa (Portugal) in an aquaculture pond (Estação Piloto de Piscicultura em Olhão; EPPO) and a wastewater stream (Pereira et al. 2016), respectively. *Skeletonema* sp. was isolated by streaking an environmental sample. All strains were identified down to the genus level by means of 18S rDNA sequencing, using the primers and methodology described in Pereira et al. (2011). The biomass of *Tetraselmis* sp. IMP3 used to carry out the present work was cultured indoors in Modified Algal Medium (Table 1), in 70-l plastic airlifts for 15 days (stationary phase) using filtered air (0.2 μm), under continuous light intensity (150 μmol/m²/s, 16 h light–8 h dark) and controlled temperature (20 ± 2 °C). *Tetraselmis* sp. CTP4 was grown in pilot-scale tubular photobioreactors (2.5 m³), as described in Pereira et al. (2018). In particular, Guillard's F2 culture medium adapted to the local water was used in all experiments (Table 1). Both *Tetraselmis* strains were harvested by centrifugation (2500×g for 10 min) and the collected microalgal paste was stored at − 20 °C. The biomass of *Skeletonema* sp. was grown outdoors by Necton S.A. (Necton, Companhia Portuguesa de Culturas Marinhas, S.A., Olhão, Portugal) in 15 m³ tubular flow through photobioreactors between November 2017 and March 2018. The temperature inside the reactor was kept below 25 °C using a water sprinkling system and the pH set point for CO_2 injection was set to pH 8.2. The commercial culture medium of Necton, Nutribloom plus®, was used during all growth at a mean nitrate concentration of 6 mM. Biomass production was carried out using a semi-continuous cultivation approach and cultures were harvested by centrifugation, approximately every 2–3 days and stored at − 20 °C. Cultures were analyzed weekly for the presence of *Vibrio* sp. and other pathogenic bacteria contaminants. *Vibrio* sp. was identified according to ISO/TS 21872-2:2007, by plating the diluted microalgal paste in thiosulphate citrate bile sucrose agar and further incubation at 37 °C for 24 h. All biomasses were freeze-dried before being sent to IPMA laboratory at Lisbon for analysis.

Table 1 Composition of the culture media used to grow the strains of *Tetraselmis* sp. IMP3 and *Tetraselmis* sp. CTP4

Component	*Tetraselmis* sp. IMP3* (mM)	*Tetraselmis* sp. CTP4* (mM)
NaNO₃	2000	882
KH₂PO₄	100	---
Na₂CO₃	---	106
NaH₂PO₄.H₂O	---	36.2
EDTA-Na	26.4	4.36
MgSO₄.7H₂O	2	---
ZnCl₂	1	---
ZnSO₄.H₂O	1	---
ZnSO₄.7H₂O	---	3.15
FeCl₃.6H₂O	---	3.15
MnCl₂.4H₂O	1	0.9
NaMoO₄.H₂O	0.1	---
CoCl₂.6H₂O	0.1	0.05
CuSO₄.5H₂O	0.1	0.04
NaMoO₄.2H₂O	---	0.03
FeCl₃.6H₂O	20	---
Vitamin B1	---	0.296
Vitamin B7	---	0.00205
Vitamin B12	---	0.000369

*Stock solution to be diluted 1:1000

Fatty acid profile

Fatty acid methyl esters (FAME's) were prepared from the freeze-dried microalgae by acid-catalyzed transesterification using the methodology described by Bandarra et al. (1997). More specifically, 300 mg of freeze-dried sample was weighed to a screw cap glass tube. Afterward, 5 ml of a 5% acetyl chloride-methanolic solution (prepared immediately before addition) was added. These glass tubes, after vigorous agitation, were placed in a hot bath (80 °C) and left there for 1 h. After reaction completion, solution was cooled, diluted with 1 ml water and 2 ml n-heptane, and vigorously mixed, the last addition produced an organic phase that was filtered through anhydrous sodium sulfate. The resultant methyl esters were applied to a DB-WAX (Agilent Technologies, Santa Clara, CA, USA) capillary column (film thickness, 0.25 μm), 30 m × 0.25 mm i.d., integrated in a Scion 456-GC gas chromatograph (West Lothian, UK), equipped with an autosampler with a split injector (100:1) and a flame ionization detector, both at 250 °C. The separation of the FAME was carried out with helium as the carrier gas and using a temperature program for the column starting at 180 °C and increasing to 200 °C at 4 °C/min, holding for 10 min at 200 °C, heating to 210 °C at the same rate, and holding at this temperature for 14.5 min. FAME's were identified by comparing their retention time with those of Sigma-Aldrich standard (PUFA-3, Menhaden oil). The limit of detection (LOD) is 1 mg/100 g. Results were calculated in % of total fatty acids on the basis of peak areas and results in mg/100 g were attained through the internal standard (10 mg/ml of heneicosanoic acid, 21:0) method. Analyses were always done in duplicate.

Total polyphenol content

Phenolic compounds were extracted by an appropriate solvent (water or ethanol 96%, w/w) from the freeze-dried microalgal biomass (Siriwoharn et al. 2004)—thus matching the extracts used in the assessment of the antioxidant potential (see sections "Antioxidant activity as measured by the DPPH method," "Antioxidant activity as measured by the FRAP method," and "Antioxidant activity as measured by the ABTS method")—and determined by the Singleton and Rossi (1965) method using the Folin-Ciocalteu reagent. A volume of 100 μl of each microalgal extract was added to a vial. To each vial, 600 μl of MiliQ water plus 150 μl of twice-diluted Folin-Ciocalteu reagent were added and allowed to stand for 5 min at room temperature. Then, 750 μl of a 2% w/v sodium carbonate solution was added. After 1 h 30-min reaction in the dark at room temperature, absorbance at 750 nm was measured in a Helios Alpha model (Unicam, Leeds, UK) UV-Vis spectrophotometer. Gallic acid (GA) was used as standard and phenolic content was expressed as gallic acid equivalents (mg GAE/100 g) through the calibration curve of gallic acid (Sigma, Steinheim, Germany).

Polyphenol composition

The extracts prepared as described in the "Antioxidant activity as measured by the DPPH method" section were analyzed by HPLC-DAD (Agilent 1100 Series LC system, Germany), constituted by the following modules: vacuum degasser (G1322A), quaternary pump (G1311A), autosampler (G1313A), thermostated column compartment (G1316A), and the diode array detector (G1315B). The data acquisition and instrumental control were performed by the software LC3D ChemStation (version Rev.A.10.02(1757), Agilent Technologies). Analyses were performed on a Mediterranea Sea18 column, 15 × 0.21 cm, 5 μm particle size

(Teknokroma, Spain). The mobile phase consisted in a mixture of methanol (solvent A) and 2.5% acetic acid aqueous solution with the following gradient: 0–5 min: 10% A, 5–10 min: 10–30% A, 10–40 min: 30–90% A, 40–45 min: 90% A, 45–55 min: 90–10% A, and 55–60 min: 10% A, using a flow rate of 0.35 ml/min. The injection volume was 20 μl with a draw speed of 200 μl/min. The detector was set at 210, 280 (use for quantification), 320, and 350 nm. For identification, the retention parameters of each assay were compared with the standard controls and the peak purity with the UV-visible spectral reference data. The levels of the different compounds were extrapolated from calibration standard curves. Commercial standards of gallic acid, gentisic acid, *p*-hydroxybenzoic acid, catechin hydrate, 4-hydroxybenzaldehyde, vanillic acid, caffeic acid, chlorogenic acid, epigallocatechin gallate, syringic acid, epicatechin, coumaric acid, ferulic acid, salicylic acid, naringenin-7-glucoside, luteolin-7-*o*-glucoside, rutin, rosmarinic acid, ellagic acid, quercetin, and flavone were prepared in methanol (1.000 mg/l) and diluted with ultrapure water in desired concentration.

Antioxidant activity as measured by the DPPH method

The antioxidant activity was measured through the determination of the radical scavenging activity using DPPH (Miliauskas et al. 2004). In order to prepare the extracts, approximately 1.25 g of freeze-dried microalgal biomass was weighed; homogenized with 25 ml of water or ethanol 96%, w/w, using a model Polytron PT 6100 homogenizer (Kinematica, Luzern, Switzerland) at a velocity of 30,000 rpm during 1 min; and agitated for 18 h on an orbital shaker. After centrifugation (5000×*g* at room temperature during 20 min), the supernatant was collected through a filter to a final volume of 25 ml. A volume of 1 ml of the extract was prepared in triplicate for each sample and 2 ml of DPPH (Sigma, Steinheim, Germany) 0.15 mM methanolic solution was added and thoroughly mixed. After 30 min of incubation at room temperature in the dark, absorbance was measured at 517 nm in a Helios Alpha model (Unicam, Leeds, UK) UV/visible light spectrophotometer. Either water or ethanol 96%, w/w, was used as the blank.

Radical scavenging activity was calculated by the following formula:

% inhibition = (A0 − Asample)/A0 × 100

where A0 is the absorbance of the blank and Asample is the absorbance of the sample.

Antioxidant activity as measured by the FRAP method

The applied FRAP method was a modified technique based on Benzie and Strain (1996). The stock solutions included 300 mM acetate buffer (pH 3.6), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) solution in 40 mM HCl, and 20 mM $FeCl_3 \cdot 6H_2O$ solution. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml $FeCl_3 \cdot 6H_2O$. A volume of 100 μl of sample (prepared in 5%, w/v, aqueous and ethanolic extracts attained as described in the "Antioxidant activity as measured by the DPPH method" section) was allowed to react with 3000 μl of the FRAP solution for 30 min in the dark at 37 °C. Readings of the colored product (ferrous-tripyridyltriazine complex) were taken at 595 nm. The standard curve was linear between 250 and 2000 μM $FeSO_4$. Results were expressed in mM Fe^{2+} and compared with an ascorbic acid control.

Antioxidant activity as measured by the ABTS method

ABTS radical scavenging activity was determined using the method described by Re et al. (1999). This required the preparation of the method's specific reagent (7 mM $ABTS^{+\bullet}$ solution)

as follows: 10 mg of ABTS was dissolved in 2.6 ml of a 2.45-mM potassium persulfate solution. The solution remained for 16 h in the dark at room temperature before use and the ABTS$^{+\bullet}$ solution was diluted with 5-mM sodium phosphate buffer (pH 7.4) to give an absorbance value of 0.70 ± 0.02 at 734 nm. At a later stage, 20 μl of sample solutions (prepared in 5%, w/v, aqueous and ethanolic extracts attained as described in the "Antioxidant activity as measured by the DPPH method" section) were added to 2 ml of the diluted ABTS$^{+\bullet}$ solution and the mixture was homogenized and incubated in the dark at 30 °C for 6 min. The absorbance of the samples was measured at 734 nm. The ABTS radical scavenging activity of the samples was expressed as a percentage of inhibition:

% inhibition = (A0 − Asample)/A0 × 100

where A0 is the absorbance of the blank and Asample is the absorbance of the sample.

Anti-inflammatory activity

The anti-inflammatory activity of the microalgae *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp. was determined in 10%, w/v, aqueous and ethanolic extracts, attained from approximately 200 mg of freeze-dried microalgae homogenized with 2 ml of water or ethanol 96%, w/w, using a model Polytron PT 6100 homogenizer (Kinematica, Luzern, Switzerland) at a velocity of 30,000 rpm during 1 min. The samples were then subjected to heat bath (80 °C, 1 h) and then centrifuged (3000×*g* at 4 °C for 10 min). The supernatant was collected and the solvent was evaporated using vacuum rotary evaporator with the water bath temperature at 65 °C. The residue was directly dissolved in 100% dimethyl sulfoxide (DMSO) to prepare a stock preparation with a concentration of 10 mg/ml. The extract was tested at 1 mg/ml using a commercial cyclooxygenase (COX) inhibitory screening assay kit, Cayman test kit-560131 (Cayman Chemical Company, Ann Arbor, MI, USA). The COX inhibitor screening assay directly measures the amount of Prostaglandin F2α generated from arachidonic acid (AA, 20:4 ω6) in the cyclooxygenase reaction. A volume of 10 μl of each test extract or DMSO was used. The reaction was initiated by addition of 10 μl 10 mM AA and each reaction tube was incubated at 37 °C for 2 min. Reaction was terminated by the addition of 50 μl 1 N HCl and saturated stannous chloride. Assays were performed using 100 units of human recombinant COX-2. An aliquot was removed and the prostanoid produced was quantified spectrophotometrically (412 nm) via enzyme immunoassay (ELISA) after 18 h incubation, washing, addition of Ellman's reagent, and further 90-min incubation. Results were expressed as a percentage of inhibition of COX-2.

Statistical analysis

To test the normality and the homogeneity of variance of data, the Kolmogorov-Smirnov's test and Levene's *F*-test, respectively, were used. Data, which corroborated these assumptions, were analyzed by one-way ANOVA distribution using the Tukey HSD post hoc test to determine the difference in the constituents' contents between microalgae. Alternatively, a factorial ANOVA using the Tukey HSD to determine the difference in biological activities between microalgae and between aqueous and ethanolic extracts was used. When normality and/or homogeneity of variance were not verified (14:0, 16:1 n9, 16:1 n7, 16:2 n4, 16:3 n4, 16:4 n3, 18:4 n3, and 20:5 n3 contents), data were tested non-parametrically with Kruskal-Wallis test (analysis of variance) followed by nonparametric multiple comparisons test (Zar

1999). For all statistical tests, the significance level (α) was 0.05. All data analysis was performed using STATISTICA 6 (Stat-sof, Inc. USA, 2003).

Results

Fatty acid profile

The fatty acid composition (in % of total fatty acids and in mg/100 g dry weight) of the studied microalgae *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp. is displayed in Table 2.

Regarding the relative FA profile (as a percentage of the total FA), three main aspects deserve to be highlighted. Firstly, across all microalgae, PUFA were the main FA group, ranging from $37.1 \pm 2.8\%$ in *Skeletonema* sp. to $49.4 \pm 0.4\%$ in *Tetraselmis* sp. CTP4. Saturated FA (SFA), in the case of *Tetraselmis* sp. IMP3 and *Skeletonema* sp., and monounsaturated FA (MUFA), in the case of *Tetraselmis* sp. CTP4, were the second most abundant

Table 2 Fatty acid profile (in % of total fatty acids and in mg/100 g dry weight) of the studied microalgae *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp.

Fatty acid	*Tetraselmis* sp. IMP3		*Tetraselmis* sp. CTP4		*Skeletonema* sp.	
	(% total fatty acids)	(mg/100 g dw)	(% total fatty acids)	(mg/100 g dw)	(% total fatty acids)	(mg/100 g dw)
14:0	0.7 ± 0.0^a	80 ± 1^A	1.2 ± 0.0^a	90 ± 1^A	21.5 ± 3.9^b	1414 ± 109^B
16:0	27.1 ± 0.1^a	3010 ± 47^A	15.3 ± 0.1^b	1090 ± 10^B	6.0 ± 1.1^c	415 ± 156^C
18:0	0.2 ± 0.0^a	20 ± 1^A	0.5 ± 0.0^b	40 ± 0^B	0.1 ± 0.1^a	8 ± 7^A
Σ SFA	29.8 ± 0.1^a	3310 ± 52^A	20.2 ± 0.2^b	1440 ± 3^C	31.4 ± 3.0^a	2087 ± 300^B
16:1 n9	0.3 ± 0.0^a	30 ± 0^A	1.3 ± 0.0^a	90 ± 0^B	1.0 ± 0.8^a	58 ± 36^A
16:1 n7	0.7 ± 0.0^a	80 ± 1^A	0.6 ± 0.0^a	40 ± 0^A	20.9 ± 5.8^b	1465 ± 649^B
18:1 n9	18.7 ± 0.1^a	2080 ± 30^A	11.8 ± 0.1^b	840 ± 1^B	0.3 ± 0.1^c	21 ± 5^C
18:1 n7	3.1 ± 0.1^b	340 ± 1^A	3.7 ± 0.0^a	270 ± 1^B	0.6 ± 0.2^c	38 ± 11^C
20:1 n9	2.2 ± 0.1^a	250 ± 2^A	1.2 ± 0.0^b	80 ± 1^B	ND^c	ND^C
Σ MUFA	25.9 ± 0.1^a	2880 ± 36^A	21.4 ± 0.2^a	1530 ± 3^B	23.6 ± 5.5^a	1646 ± 667^B
18:2 n6	6.2 ± 0.0^a	690 ± 10^A	5.3 ± 0.1^b	380 ± 1^B	0.8 ± 0.1^c	51 ± 13^C
16:2 n4	0.7 ± 0.0^a	80 ± 1^B	0.6 ± 0.0^a	50 ± 0^A	5.3 ± 2.0^b	338 ± 44^C
16:3 n4	0.1 ± 0.0^a	10 ± 0^A	ND^a	ND^A	11.7 ± 2.1^b	769 ± 42^B
16:3 n3	7.9 ± 0.0^b	880 ± 13^A	12.9 ± 0.1^a	920 ± 0^A	0.4 ± 0.3^c	28 ± 25^B
16:4 n3	0.1 ± 0.0^a	10 ± 0^A	ND^a	ND^A	2.0 ± 0.4^b	141 ± 52^B
18:3 n3	13.6 ± 0.0^b	1510 ± 23^A	18.8 ± 0.1^a	1340 ± 4^B	0.1 ± 0.1^c	7 ± 6^C
18:4 n3	2.0 ± 0.0^a	220 ± 3^A	4.9 ± 0.0^c	350 ± 0^B	3.2 ± 0.4^b	213 ± 23^A
20:4 n3	1.2 ± 0.0^b	130 ± 2^A	2.0 ± 0.0^a	140 ± 0^A	ND^c	ND^B
20:5 n3	6.4 ± 0.1^b	710 ± 10^A	3.7 ± 0.0^a	260 ± 1^B	10.8 ± 0.5^c	735 ± 183^A
22:6 n3	ND^a	ND^A	ND^a	ND^A	2.2 ± 0.4^b	154 ± 56^B
Σ PUFA	38.9 ± 0.1^a	4310 ± 71^A	49.4 ± 0.4^b	3530 ± 10^B	37.1 ± 2.8^a	2476 ± 372^C
Σ n3	31.4 ± 0.1^b	3490 ± 41^A	42.7 ± 0.1^a	3040 ± 10^B	18.9 ± 1.3^c	1283 ± 173^C
Σ n6	6.5 ± 0.0^a	730 ± 10^A	5.9 ± 0.1^b	430 ± 10^B	1.3 ± 0.2^c	85 ± 13^C
Σ n3/Σ n6	4.8 ± 0.1^a	4.8 ± 0.1^A	7.2 ± 0.1^a	7.2 ± 0.1^A	14.7 ± 1.7^b	14.7 ± 1.7^B

Values are presented as average ± standard deviation. *ND*, not detected. Different lowercase letters within a row correspond to statistical differences ($p < 0.05$) between the relative (%) FA profiles of microalgae. Different uppercase letters within a row correspond to statistical differences ($p < 0.05$) between the absolute (mg/100 g dw) FA profiles of microalgae

classes of FA. Within the PUFA group, n3 PUFA were clearly more abundant than n6 PUFA—thereby yielding an n3/n6 ratio varying between 4.8 ± 0.1 in *Tetraselmis* sp. IMP3 and 14.7 ± 1.7 in *Skeletonema* sp.—and n4 PUFA. A third main aspect relates to the relatively low contents of the highly unsaturated n3 PUFA, such as EPA and DHA. Indeed, EPA content was low with exception of *Skeletonema* sp., where it surpassed 10% of the total FA, and DHA never exceeded 3% of total FA, being only detected in *Skeletonema* sp.

A more painstaking analysis of the FA profiles reveals other aspects also worth mentioning. Namely, main PUFA was α-linolenic acid (ALA, 18:3 n3) in microalgae of the *Tetraselmis* genus, reaching 18.8 ± 0.1 % (*Tetraselmis* sp. CTP4). However, in *Skeletonema*, the most abundant PUFA was 16:3 n4, 11.7 ± 2.1%. Moreover, high contents of myristic (14:0) and palmitoleic (16:1 n7) FA were found in *Skeletonema*, whereas the *Tetraselmis* strains were rich in palmitic (16:0) and oleic (18:1 n9) FA. Linoleic acid (18:2 n6) content was very low in *Skeletonema* sp., 0.8 ± 0.1%, clearly different from content range in both *Tetraselmis* microalgae, 5.3–6.2%. On the other hand, 16:2 n4 content was higher in *Skeletonema* sp., 5.3 ± 2.0%, than in the *Tetraselmis* microalgae, 0.6–0.7%, thus contributing for a very substantial total amount of n4 PUFA in the diatom under study.

With respect to FA concentrations in mg/100 g of the microalgae dw, a different picture emerged since the biomass of *Tetraselmis* sp. IMP3 was fatter than that of *Tetraselmis* sp. CTP4 and *Skeletonema* sp. Accordingly, SFA, 18:1 n7, MUFA, 18:3 n3, n3 PUFA, and PUFA contents became higher in *Tetraselmis* sp. IMP3 than in the other microalgae. Furthermore, EPA absolute content in *Tetraselmis* sp. IMP3 was not lower than that in *Skeletonema* sp., lying in the same range of 710–735 mg/100 g dw.

Total polyphenol content

The total polyphenol content of *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp. is shown in Table 3.

The microalga *Skeletonema* sp. had the highest total polyphenol content, at least, on the basis of an alcoholic extraction of the biomass, thereby yielding a total of 368 ± 54 mg GAE/100 g dw. The lowest value was found in an alcoholic extract of *Tetraselmis* sp. CTP4, 24 ± 17 mg GAE/100 g dw. For *Tetraselmis*, polyphenol levels attained by alcoholic extraction were not higher than those achieved by aqueous extraction.

Polyphenol composition

Polyphenol composition of the aqueous and ethanol extracts of *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp. is shown in Table 4.

Table 3 Total polyphenol content (in mg GAE/100 g dry weight) of the studied microalgae *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp.

Extract	*Tetraselmis* sp. IMP3 (mg GAE/100 g dw)	*Tetraselmis* sp. CTP4 (mg GAE/100 g dw)	*Skeletonema* sp. (mg GAE/100 g dw)
Aqueous	231 ± 5^{ab}	76 ± 22^{a}	282 ± 20^{b}
Ethanolic	172 ± 18^{b}	24 ± 17^{a}	368 ± 54^{c}

Values are presented as average ± standard deviation. Different lowercase letters within a row correspond to statistical differences ($p < 0.05$) between microalgae

Table 4 Polyphenol composition (concentration in mg/l) of the aqueous and ethanol extracts of the studied microalgae *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp.

Phenolic compound	*Tetraselmis* sp. IMP3		*Tetraselmis* sp. CTP4		*Skeletonema* sp.	
	Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract	Aqueous extract	Ethanol extract
Gallic acid	4.1	nd	1.8	1.2	2.2	14.6
Gentisic acid	3.5	nd	nd	nd	93.4	41.0
p-Hydroxybenzoic acid	nd	nd	1.2	nd	1.8	nd
Catechin hydrate	6.1	nd	3.1	nd	5.5	1.7
4-Hydroxybenzaldehyde	2.7	nd	< 0.1	nd	nd	nd
Vanillic acid	0.8	nd	nd	nd	0.4	0.1
Caffeic acid	53.3	nd	nd	nd	6.8	7.4
Chlorogenic acid	nd	nd	nd	0.2	3.7	3.5
Epigallocatechin gallate	< 0.1	nd	nd	nd	< 0.1	< 0.1
Syringic acid	nd	nd	nd	nd	0.3	0.1
Epicatechin	2.9	nd	1.0	0.6	8.1	4.8
Coumaric acid	< 0.1	< 0.1	< 0.1	< 0.1	5.8	< 0.1
Ferulic acid	0.3	nd	nd	nd	1.0	0.5
Salicylic acid	3.2	nd	nd	nd	6.7	5.5
Naringenin-7-glucoside	0.3	nd	nd	nd	1.5	0.1
Luteolin-7-o-glucoside	9.0	nd	nd	nd	3.0	0.2
Rutin	2.4	nd	nd	nd	nd	nd
Rosmarinic acid	nd	nd	nd	nd	1.0	< 0.1
Ellagic acid	5.3	nd	< 0.1	< 0.1	< 0.1	0.1
Quercetin	10.4	nd	nd	nd	0.5	< 0.1
Flavone	< 0.1	nd	< 0.1	nd	< 0.1	< 0.1

Nd, not detected

A comparison with total polyphenol content (Table 3) shows some agreement with the sum of specific polyphenol components as well as some divergences. Indeed, *Skeletonema* sp. (both extracts) had the highest concentration of the analyzed phenolic compounds, thereby agreeing with the highest total polyphenol contents previously mentioned (see "Total polyphenol content" section). However, contrary to these total results, specific phenolic contents were higher in the aqueous extract than in the ethanolic extract of *Skeletonema* sp.

Regarding phenolic profile itself, gallic acid, gentisic acid, catechin hydrate, caffeic acid, epicatechin, salicylic acid, luteolin-7-*o*-glucoside, and quercetin were the most abundant compounds. Particularly, it is noteworthy the very high gentisic acid contents in the aqueous and ethanolic extracts of *Skeletonema* sp., 93.4 and 41.0 mg/l, respectively, and the high caffeic acid content in the aqueous extract of *Tetraselmis* sp. IMP3, 53.3 mg/l. Both extracts of *Tetraselmis* sp. CTP4 were very poor in the analyzed phenolic compounds.

Microalgal antioxidant activity

The antioxidant activity results for aqueous and ethanolic extracts of *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp. are presented in Fig. 1. The applied methods, DPPH, FRAP, and ABTS are presented in panels A, B, and C, respectively.

Concerning DPPH, the highest values were measured for the aqueous extract of *Skeletonema* sp. and the ethanolic extract of *Tetraselmis* sp. IMP3 since their inhibition percentages lay clearly above 70%. For *Tetraselmis* sp. CTP4, antioxidant activity was higher for the aqueous extract than the ethanolic extract, $21 \pm 1\%$ vs $0 \pm 0\%$, respectively. Regarding
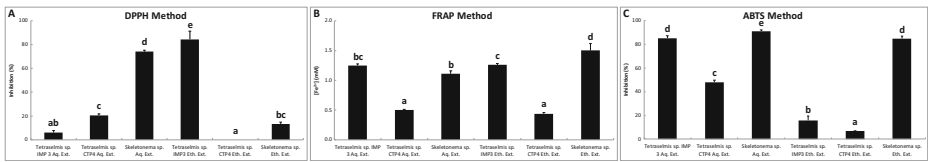
Fig. 1 Antioxidant activity as measured by **a** DPPH (% inhibition), **b** FRAP (mM Fe^{2+} eq.), and **c** ABTS (% inhibition) methods in aqueous (Aq.) and ethanolic (Eth.) extracts of the studied microalgae *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp.

FRAP, the ethanolic extract of *Skeletonema* sp. produced the highest value (1.5 ± 0.1 mM Fe^{2+}, which corresponds to 300 mM Fe^{2+}/g dw microalgal biomass). The aqueous extract of the same microalga showed a lower antioxidant power, 1.1 ± 0.1 mM Fe^{2+}. Again, as with DPPH, *Tetraselmis* sp. CTP4 extracts had a low FRAP level, 0.4–0.50 mM Fe^{2+}, regardless of being aqueous or ethanolic. Finally, ABTS results also indicated *Skeletonema* sp. as the most antioxidant biomass, but with the aqueous extracts yielding the highest inhibitory value, $91 \pm 1\%$. Indeed, aqueous extracts generated always higher antioxidant activities (as measured by ABTS) than ethanolic extracts. Within each group of extracts, *Tetraselmis* sp. CTP4 presented the lowest inhibitory values, $48 \pm 2\%$ and $7 \pm 0\%$, for extraction with water and ethanol, respectively.

Microalgal anti-inflammatory activity

The anti-inflammatory activity values of *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp. biomass measured as a percentage of inhibition of the enzyme COX-2 are presented in Table 5. Two different extracts of the freeze-dried biomass were tested, an aqueous and an ethanolic extract. Extract concentration was 1 mg/ml in DMSO.

Anti-inflammatory activity was detected in all microalgal extracts but to a different extent. Ethanolic extracts yielded higher values of COX-2 inhibition than aqueous extracts. The highest value of anti-inflammatory activity ($82 \pm 2\%$) was measured for the ethanolic extract of *Skeletonema* sp. Regarding aqueous extracts, the inhibitory effect did not surpass $30 \pm 6\%$ (*Tetraselmis* sp. IMP3), which was higher than that of *Tetraselmis* sp. CTP4, $6 \pm 5\%$. Indeed, the latter microalga showed the lowest anti-inflammatory activities.

Discussion

Fatty acid profile

A thorough analysis of the FA profiles, especially the relative proportions between FA, may provide some valuable glimpses into the metabolic paths in each microalga species. Indeed, results suggest some peculiarities of the FA elongation and desaturation machinery of these microalgae. While *Tetraselmis* microalgae accumulate 16:0 and, after elongation, desaturate 18:0 to 18:1 n9, 18:2 n6, and 18:3 n3, *Skeletonema* sp. apparently accumulates 14:0 and, after elongation, desaturates 16:0 to 16:1 n7, 16:2 n4, and 16:3 n4.

As these microalgae are newly isolated strains, all their biochemical aspects are poorly studied. Nevertheless, there are studies on other strains, such as *Tetraselmis* sp. M8 (Adarme-

Table 5 Anti-inflammatory activity (% inhibition of COX-2) in aqueous and ethanolic extracts of the studied microalgae *Tetraselmis* sp. IMP3, *Tetraselmis* sp. CTP4, and *Skeletonema* sp.

Extract	*Tetraselmis* sp. IMP3 (% inhibition)	*Tetraselmis* sp. CTP4 (% inhibition)	*Skeletonema* sp. (% inhibition)
Aqueous	30 ± 6[b]	6 ± 5[a]	17 ± 1[ab]
Ethanolic	45 ± 5[a]	36 ± 9[a]	82 ± 2[b]

Values are presented as average ± standard deviation. Different lowercase letters within a row correspond to statistical differences ($p < 0.05$) between microalgae

Vega et al. 2014). A comparison with this strain's FA profile shows great similarity in the SFA, in the proportion between myristic and palmitic FA and their relative importance in the total FA. There is also similarity in the MUFA, with a higher proportion of 18:1 than 16:1, 6.2–16.3% vs 1.3–4.7%, in ALA content, 11.3–20.5%, 18:4 n3, 1.4-6.0%, 20:4 n3, 0.9–2.8%, and EPA, 4.4–7.2% (Adarme-Vega et al. 2014). Regarding linoleic acid, strain M8 had higher contents (9.8–15.6%) than the strains in the current study. However, the FA profiles of *Tetraselmis* sp. NT18 and TEQL01 previously reported (Renaud et al. 1999) had a linoleic acid content similar to the contents determined in the current study. Differences between this and literature were most evident in 16:4, which reached very high values in *Tetraselmis* sp. M8, 13.8–22.3% (Adarme-Vega et al. 2014), and in *Tetraselmis* sp. NT18 and TEQL01, 16.1–16.4 % (Renaud et al. 1999). There is also an FA study on *Tetraselmis suecica*, strain NIVA-3/92 (Patil et al. 2007). The same preponderance of palmitic acid to myristic acid and of oleic acid to 16:1 was reported and no important levels of 16:4 were determined. In addition, total FA in mg/100 g dw was lower in *T. suecica* and EPA was 480 mg/100 g dw (Patil et al. 2007), which is within the range of both *Tetraselmis* strains here reported, 260–710 mg EPA/100 g dw (Table 2). This succinct overview demonstrates the existence of conserved patterns in the FA profile of *Tetraselmis* microalgae, but also very substantial variations.

Concerning *Skeletonema*, FA studies are also relatively scarce (Blanchemain and Grizeau 1996; Jiang et al. 2016; Renaud et al. 1999). It has been reported that *Skeletonema* sp. GOC27 and GOC36 contain moderate levels of EPA, approximately 13% of the total FA (Renaud et al. 1999). A higher level of myristic acid with respect to palmitic acid as well as of palmitoleic acid in comparison to oleic acid was reported by these authors. Content of 16:3 n4 ranged between 10.0 and 11.3% (Renaud et al. 1999). Precisely, all these traits were also observed in the current study. Furthermore, low linoleic and DHA contents in the 1.2–2.2% range were determined for strains GOC27 and GOC36 (Renaud et al. 1999), as observed in the current study. Likewise, for *S. costatum*, a myristic acid concentration much larger than that of palmitic acid and a palmitoleic acid content exceeding that of oleic acid were determined (Blanchemain and Grizeau 1996). A more recent study on *Skeletonema menzelii* has reported higher EPA contents, reaching 18% of total FA under high nitrogen and phosphorus supplement, moderate available iron, and deprivation of silicon (Jiang et al. 2016). Under these conditions, other aspects, such as myristic acid and palmitoleic acid abundance were also observed.

In fact, the FA profile of microalgae can vary widely as a function of cultivation conditions and growth phase at the harvest time, as is the case of the well-studied strain *I. galbana* (Durmaz et al. 2008) or other strains *Tetraselmis*, such as *Tetraselmis* sp. M8 (Adarme-Vega et al. 2014). Namely, it has been measured an inverse relation between EPA and DHA levels and growth temperature in *Isochrysis* as well as in other microalgae (Tasselli and Doimi 1990). In particular, EPA contents may experience wide variations between batches with different

cultivation conditions (Durmaz et al. 2008). For *Tetraselmis* sp. M8, correlations were found for specific FA biosynthesis and gene expression according to salinity and the growth phase (Adarme-Vega et al. 2014). Moreover, EPA levels in *Skeletonema costatum* were related to growth and irradiance (Blanchemain and Grizeau 1996). Some other FA concentrations are less prone to wide variations, for instance, the abundance of myristic acid (Durmaz et al. 2008; Fidalgo et al. 1998; Fradique et al. 2013).

Concerning absolute contents, if EPA and DHA are taken together, *Skeletonema* sp. is richer in these highly unsaturated n3 PUFA with 889 mg/100 g dw. This makes this diatom a potential source of EPA + DHA. In fact, taking into account human EPA + DHA requirements and considering the possibility of using *Skeletonema* sp. in food applications, it may be calculated how much freeze-dried microalga is needed to meet the recommended daily intake of EPA + DHA (500 mg/day) according to the American Heart Association (Kris-Etherton et al. 2002). This yields an estimated amount of 56.2 g of freeze-dried *Skeletonema* sp., which is not so low if hydration is taken into account, but even so it is worth exploring from a nutraceutical point of view.

Total polyphenol content

The obtained total polyphenol content values for *Tetraselmis* sp. IMP3 and *Skeletonema* sp. are within the range reported in the relevant literature for biomass rich in polyphenols, 100–500 mg GAE/100 g dw (Farasat et al. 2013). In particular, a study on *T. tetrathele* (Farahin et al. 2016) determined a maximum phenolic content of approximately 300 mg GAE/100 g dw. Other study comprising *Tetraselmis* sp. and *T. suecica* (Goiris et al. 2012) measured phenolic contents ranging between 171 and 374 mg GAE/100 g dw in ethanol/water extracts, thus largely matching the value range observed in *Tetraselmis* sp. IMP3. Only *Tetraselmis* sp. CTP4 seems to be relatively poor in phenolic substances in comparison to the values previously reported in the literature. Concerning *Skeletonema*, high total phenolic concentrations of up to 1000 mg GAE/100 g dw have been found in methanol/ethanol/hexane extracts of *S. costatum* (Sushanth and Rajashekhar 2015), thereby surpassing the high values obtained for *Skeletonema* in the current study. However, other authors (Foo et al. 2017) working with methanolic extracts of *S. costatum* found a total phenolic content (< 20 mg GAE/100 g dw) significantly lower than the one obtained in this study.

The relatively high total polyphenol content in *Skeletonema* sp. may be related to the relative richness of this microalga in highly unsaturated n3 PUFA (Table 2). These FA are very prone to oxidation (Tao 2015), thereby generating radicals and other substances with a potentially harmful effect on the cell. Accordingly, living organisms must defend themselves against such compounds. The accumulation of polyphenols, given their antioxidant properties, is apparently a suitable response in order to efficiently scavenge radicals. Indeed, with exception of ethanolic extracts assessed by DPPH method, *Skeletonema* sp. showed a stronger antioxidant activity (see "Polyphenol composition" section) than the other microalgae not so rich in highly unsaturated n3 PUFA. In addition, the low phenolic content reported by Foo et al. (2017) in *S. costatum* was matched by an EPA content of approximately 4% of total FA.

Polyphenol composition

There are only few published studies regarding the identification and quantification of phenolic composition in microalgae species. Comparison to literature values (Foo et al. 2017; Goiris

et al. 2014) shows that phenolic profiles may be very different, even when comparing to microalgal species of the same genus, such as *S. costatum* (Foo et al. 2017). In this case of a methanolic extraction, gallic acid was the dominant compound and its content was much higher than caffeic acid's content (Foo et al. 2017). The ethanolic extract of *Skeletonema* sp. in the current study also had a high content of gallic acid, but gentisic acid was more abundant and caffeic acid was the third most abundant component, thus differing from *S. costatum*. Regarding *Tetraselmis* genus, a recent UPLC-MS/MS study detected simple phenolics and hydroxycinnamic acids (ferulic acid and *p*-coumaric acid) in *T. suecica* (Goiris et al. 2014). However, studied *Tetraselmis* sp. (IMP3 and CTP4) did not present high contents of these hydroxycinnamic acids.

Microalgal antioxidant activity

There was a substantial degree of convergence in the antioxidant activity as determined by different methodologies. Since these methodologies are not equivalent and address different aspects of antioxidant activity, this convergence is relevant. Namely, while FRAP assay detects antioxidants that act through single-electron transfer but is unable to detect compounds that act as radical quenchers by hydrogen atom transfer, ABTS quantifies antioxidant activity by single-electron transfer (direct reduction of $ABTS^{+\bullet}$) or radical quenching by hydrogen atom transfer (Prior et al. 2005).

For *S. costatum*, there was also significant agreement between different assays focused on different antioxidant properties (Sushanth and Rajashekhar 2015). Regarding the *Tetraselmis* genus, other studies (Farahin et al. 2016) have concluded for being a potential feedstock for antioxidants after testing with DPPH, FRAP, and ABTS methods. Significant antioxidant activity as measured by DPPH was also observed with *T. chuii* (Rahman et al. 2017). Goiris et al. (2012) have determined the antioxidant activity of *Tetraselmis* sp. and *T. suecica* through ABTS and found high antioxidant capacity. These authors also studied other microalgae and concluded that phenolic compounds (along with carotenoids) contribute significantly to the antioxidant capacity of these organisms. Though correlation is not strong, this seems to occur in the microalgae studied here since *Skeletonema* had the highest phenolic content and overall showed the best antioxidant properties. Conversely, *Tetraselmis* sp. CTP4 was poor in phenolic substances and displayed the lowest antioxidant capacity.

Microalgal anti-inflammatory activity

There are some studies on the anti-inflammatory activity of microalgae, but used methodologies differ significantly, ranging from *in vitro* assays to *in vivo* models (Bonfanti et al. 2018; Jensen et al. 2015; Renju et al. 2013). This makes comparison among studies difficult. Nevertheless, several studies point to the existence of anti-inflammatory activity in microalgae (Banskota et al. 2013; Bonfanti et al. 2018; Jensen et al. 2015; Reyes et al. 2016). Some authors found significant activity in aqueous extracts, for instance, in *Arthrospira platensis* (Jensen et al. 2015), whereas other authors have reported anti-inflammatory properties of lipid components, for instance, in *I. galbana* (Reyes et al. 2016). Banskota et al. (2013) reported anti-inflammatory effects of lipid extracts of *T. chuii*. In contrast, Lauritano et al. (2016) did not find an anti-inflammatory activity in aqueous extracts of *S. marinoi*. However, these studies on species of the same genera analyzed in the current work used different methodologies. Using a similar methodology, inhibition of COX-2 enzyme, Bonfanti et al. (2018)

observed a 79 ± 7% inhibitory effect with an *I. galbana* lipid extract residue dissolved in DMSO to a final concentration of 5 mg/ml. Accordingly, a *Skeletonema* ethanolic extract residue dissolved at a concentration of 1 mg/ml in DMSO compares positively to results reported in the literature. This is reinforced by comparing COX-2 inhibition due to the aqueous extracts of the microalgae studied here (6–30%, Table 5) with the absence of anti-inflammatory activity in an aqueous extract of *I. galbana* (Bonfanti et al. 2018). DMSO concentration and aqueous extract solution concentration (10%, w/v) were identical in the cases of *Tetraselmis* and *Skeletonema* novel strains as well as in that of *I. galbana* (see "Anti-inflammatory activity" section).

In general, different components may be involved in this biological activity, such as phenolic compounds, carotenoids, phytosterols, alkaloids, polysaccharides, or proteins, such as phycocyanin (Jensen et al. 2015). Some lipids and lipophilic substances seem to be the causative factor for the observed anti-inflammatory activity (Bonfanti et al. 2018). Namely, EPA is associated to anti-inflammatory properties (Tanaka et al. 2014). However, it should be noticed that though EPA and n3 PUFA have anti-inflammatory effects (Calder 2010), their action usually requires *in vivo* systems, different from the enzymatic COX-2 system used in the current study. Accordingly, the anti-inflammatory activity of novel microalgae strains may be the result of substances more soluble in ethanol than water, but not necessarily hydrophobic. This largely excludes polysaccharides, given their typical low solubility in ethanol. On the other hand, with exception of *Skeletonema*'s phenolic compounds, which were more soluble in ethanol, there seems to be no correlation between polyphenol content in the extracts and their anti-inflammatory activity (Table 3). Therefore, two possible hypotheses may be put forward. A first possibility is that anti-inflammatory bioactives might belong to an ethanol-soluble class as yet to be identified in these microalgae. Alternatively, different bioactives generated the COX-2 inhibition in aqueous and ethanolic extracts. Polyphenols might be important in the former extracts (and in *Skeletonema* sp.). Conversely, less polar compounds (e.g., carotenoids and phytosterols), that are known to be soluble in alcohols, might be crucial for the latter (at least, for both *Tetraselmis* strains). Another possible option is that, due to different polyphenols being extracted differentially by water and ethanol (see Table 4), polyphenols in the ethanolic extract were more effective at inhibiting COX-2 enzyme. For instance, this would link gallic acid with anti-inflammatory properties. Indeed, there are some reports linking this compound (along with others) to anti-inflammatory potential (BenSaad et al. 2017). Moreover, the role of gentisic acid in the case of the ethanolic extract of *Skeletonema* sp. is also worth research, though its high content in the aqueous extract of this microalga did not generate a strong COX-2 inhibitory effect. In fact, experimental results by other authors (Hinz et al. 2000) suggest that certain metabolites of salicylic acid, such as gentisic acid, may contribute to the pharmacological action of its parent compound by inhibiting COX-2-dependent prostaglandin E(2) formation at sites of inflammation.

Conclusions

The FA profile of the three novel microalgal strains was characterized by abundance of PUFA and, within PUFA, n3 PUFA were the most abundant. However, EPA and DHA contents were relatively low. In fact, EPA (20:5 n3) content was low with exception of *Skeletonema* sp., where it surpassed 10% of the total FA and 500 mg/100 g of biomass dw, and DHA (22:6 n3) never exceeded 3% of total FA. Main PUFA were ALA (18:3 n3) in *Tetraselmis* genus and

16:3 n4 in *Skeletonema* sp. Results suggest some peculiarities of the FA elongation and desaturation machinery of these microalgae. It seems that while *Tetraselmis* microalgae accumulate 16:0 and, after elongation, desaturate 18:0 to 18:1 n9, 18:2 n6, and 18:3 n3, *Skeletonema* sp. accumulates 14:0 and, after elongation, desaturates 16:0 to 16:1 n7, 16:2 n4, and 16:3 n4. Moreover, *Skeletonema* sp. had the highest total polyphenol content, reaching 300–400 mg/100 g dw. Gentisic acid was the main phenolic compound in the aqueous and ethanolic extracts of this microalga. The highest antioxidant activity was also displayed by *Skeletonema* sp. Concerning anti-inflammatory activity, ethanolic extracts of *Skeletonema* sp. exhibited the highest inhibitory capacity of COX-2. Aqueous extracts had always a lower inhibitory capacity. Therefore, these microalgae have the potential for multiple applications, ranging from bioactive feedstocks to pharmaceutical uses. Future work should also focus on preparing extracts for nutraceutical incorporation and aquaculture feed enrichment as well as on studying the bioaccessibility of lipid components and bioactives in these new products.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This article does not contain any studies with animals performed by any of the authors.

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