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Antioxidant content and activity of the seaweed *Saccharina latissima*: a seasonal perspective

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Abstract

Seaweeds have been receiving increasing attention as a promising natural source of antioxidants. The present study aimed at evaluating seasonal variations in the antioxidant content and activity of the sugar kelp, *Saccharina latissima*, cultivated at two sites in Denmark from May 2013 to May 2014. The extraction performance of two solvents, methanol and ethyl acetate, was compared, with methanol extracts resulting generally in higher total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC), all determined spectrophotometrically. Phenolic compounds (phloroglucinol, phenolic acids, and flavonoids) were evaluated by HPLC-DAD. TPC was higher in November and January (1.23–2.41 mg GAE g⁻¹ DM) compared to that in September. Contrary, the highest TFC was found in September (4.56–4.83 mg RE g⁻¹ DM; $p < 0.05$). The pigment profile did not change seasonally, whereas fucoxanthin and chlorophyll *a* were the most abundant. Fucoxanthin concentration was lower in September compared to all other months (261–665 µg g⁻¹ DM; $p < 0.05$). TAC was significantly higher in November (3.84–4.05 mg GAE g⁻¹ DM). Regarding the antioxidant activity

(DPPH), there was no significant difference in the IC₅₀ between seasons, although it was tendentially lower in September and November (0.42–0.49 mg mL⁻¹). Statistical analysis revealed a strong positive correlation between TPC and TAC; additionally, TPC and TFC contributed positively for the radical scavenging activity. There was no significant difference for all the analyzed variables between cultivation sites. This study reveals marked seasonal variations, and high biological variability (difference among replicates) in the antioxidant content of *S. latissima*, and substantially lower antioxidant capacity compared to other species such as *Fucus* sp., which needs to be taken into account when considering seaweed as a commercial source of antioxidants.

Keywords

Sugar kelp
Phaeophyta
TPC
Polyphenols
Flavonoids
HPLC
DPPH

Introduction

Seaweeds have been used as a valuable food source in Asian countries since ancient times, while in Europe they constitute a largely unexploited resource, used mainly as raw material for the phycocolloid industry (Arasaki and Arasaki 1983; Bixler and Porse 2011; Mouritsen 2013). With a global seaweed aquaculture production of 30 million tonnes in 2015 (FAO 2018), seaweeds are regarded as a valuable source of vitamins, minerals, polyunsaturated fatty acids, protein, and dietary fibers (Holdt and Kraan 2011; Tabarsa et al. 2012). Lately, seaweeds have been receiving increasing attention in the Western world as a source of bioactive compounds including specific polysaccharides, proteins, lipids, and polyphenols which have reported anti-viral, anti-tumor, anti-inflammatory, and anti-lipidemic activities among many others (Holdt and Kraan 2011; Pal et al. 2014). Particularly, antioxidants have been linked to disease risk reduction and health promotion, due to their ability to attenuate oxidative stress in the body (Shahidi et al. 2012). Antioxidants can also be used to prevent lipid oxidation in lipid-rich or lipid-enriched (fortified) products such as foods and cosmetics, and thus increase their shelf-life. Moreover, seaweed extracts have shown to reduce oxidation in fish oil-enriched emulsion systems (Farvin and

Jacobsen 2015; Hermund et al. 2015; Karadağ et al. 2017; Poyato et al. 2017). While synthetic antioxidants have been widely used in food preservation, their use is currently restricted due to safety concerns (Wattenberg 1986; Sherwin 1990). In this context, there is a great need for natural antioxidant sources with potential to be used as ingredients in the development of new functional foods, cosmetics, and pharmaceuticals. The bioactive substances with antioxidant activity found in seaweeds include phenolic compounds, α -tocopherol (vitamin E), ascorbic acid (vitamin C), carotenoids such as fucoxanthin and β -carotene, and phycobiliproteins (Rodríguez-Bernaldo de Quirós et al. 2010).

Phlorotannins are a subgroup of polyphenolic compounds formed entirely by polymerization of phloroglucinol (Koivikko et al. 2005; Singh and Sidana 2013). Phlorotannins have been isolated for several brown seaweed species belonging to Fucaceae, Alariaceae, Sargassaceae, Cystoseiraceae, Laminariaceae, and Ishigeaceae, and while their concentration is generally above 2%, in some cases it can account for up to 25–30% of dry algal biomass (Singh and Sidana 2013). Reported biological activities of phlorotannins include antioxidant, anti-inflammatory, anti-diabetic, anti-HIV, anti-cancer, and enzyme inhibitory activities (Singh and Sidana 2013; Jacobsen et al. 2018).

Flavonoids are another subgroup of phenolic compounds functioning as UV screen in higher plants, among many other important functions (Rozema et al. 2002), which until recently had only been found in terrestrial plants. Nevertheless, flavonoids have recently been structurally identified in microalgae and seaweeds. Klejdus et al. (2010) detected the presence of isoflavones, a class of isoflavonoids, in brown (*Undaria pinnatifida*, *Sargassum muticum*, and *Sargassum vulgare*) and red seaweed species (*Hypnea spinella*, *Halopytis incurvus*, *Chondrus crispus*, and *Porphyra* sp.) using UHPLC-MS/MS. The same methodology was later used by Goiris et al. (2014) to identify different classes of flavonoids in microalgae. Additionally, Rodríguez-Bernaldo de Quirós et al. (2010) identified catechins (flavanols, a class of flavonoids) in red (*Palmaria palmata* and *Porphyra* spp.) and brown seaweeds (*Laminaria ochroleuca* and *Himanthalia elongata*) confirmed by HPLC-MS.

Saccharina latissima is a North Atlantic kelp species which has been successfully cultivated both in land-based and open-water aquaculture, as monoculture and in integrated multi-trophic aquaculture (IMTA; e.g., Peteiro and Freire 2013; Handå et al. 2013; Marinho et al. 2015c; Azevedo et al. 2016). It contains significant levels of total polyphenols and presents fucoxanthin as major carotenoid (Haugan and Liaaen-Jensen 1994; Rodríguez-Bernaldo de Quirós et al. 2010). Other brown seaweed species such as the fucoid *Fucus vesiculosus* have shown higher content of phenolic compounds and thereby

antioxidant capacity than *S. latissima*. However, the well-established cultivation techniques and relatively high content of antioxidant compounds make *S. latissima* potentially a sustainable natural source of antioxidants (Jacobsen et al. 2018).

Seaweeds present marked seasonal variations in their composition of, e.g., proteins and lipids, (e.g., Marinho et al. 2015a, b) which may also be the case for antioxidants and this needs to be taken into consideration when evaluating their potential as a source of antioxidant compounds and their antioxidant capacity. A seasonal pattern in the concentration of pigments, with generally lower concentrations in summer and higher concentrations in winter, has been reported for a number of seaweed species and linked to changes in light availability and nutrient concentrations in the seawater (Stengel and Dring 1998; Schmid et al. 2017). Increased fucoxanthin content is usually associated with increased light-harvesting efficiency, an adaptation to low-light conditions such as depth or self-shading (Ramus et al. 1977; Fortes and Lüning 1980). Fucoxanthin plays a light-harvesting role but it also likely has a photoprotective function in the seaweed (Fariman et al. 2016). The lipid-soluble carotenoid fucoxanthin, mainly found in the brown seaweed, could be of commercial interest, not only for the antioxidant capacity but also due to other bioactive effects such as anti-obesity, anti-cancer, and UV-B protective effect (Holdt and Kraan 2011).

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Similarly, seasonal changes in the content of polyphenols have been reported to be impacted by irradiance levels, but also temperature, nutrient availability, precipitation, and salinity (Kamiya et al. 2010; Mannino et al. 2014; Fariman et al. 2016). Moreover, biotic factors such as grazing pressure, reproductive state, and stage within the life cycle have been correlated with changes in the polyphenols content (Steinberg 1995; Stiger et al. 2004). More specifically and also in relation to our study, Yates and Peckol (1993) found that the polyphenolic concentrations of *Fucus vesiculosus* were higher (and up to twice as high) in populations from a low-nitrogen site compared to those from a high-nitrogen site (where also the tissue-N content was higher). Cultivating seaweed near a nutrient source (e.g., IMTA) could in that respect possibly change the phenolic content of the seaweed, and thereby also the antioxidant capacity of the biomass.

The present study aimed to (1) evaluate seasonal changes in the content of total phenolics, total flavonoids, and pigments, and the subsequent antioxidant capacity, based on standard assays, of *S. latissima* cultivated in IMTA and at a reference site; (2) tentatively detect selected phenolic acids and flavonoids by HPLC-DAD; and (3) compare the extraction performance of two different solvents, methanol and ethyl acetate, based on the recovery of the target

antioxidant compounds. Finally, the future perspectives of using *S. latissima* as potential source of antioxidant are discussed and compared to other sources such as microalgae and higher plants.

Materials and methods

Chemicals

Sulfuric acid, Folin–Ciocalteu reagent, and aluminum chloride were from Merck (Germany); ammonium molybdate, potassium acetate, DPPH (2,2-diphenyl-1-picrylhydrazyl), and sodium phosphate were from Sigma (Germany).

Gallic acid, rutin, and BHT (butylated hydroxytoluene) used as antioxidant standards were from Sigma-Aldrich (Germany). Likewise, additional standards used for the analysis of phenolic compounds including phenolic acids (caffeic, protocatechuic, gentisic, hydroxybenzoic, chlorogenic, syringic, salicylic, coumaric, and ferulic acids), flavonoids (catechol, quercitrin, morin, hesperidin, myricetin, catechin, quercetin, and naringenin), and phloroglucinol were from Sigma-Aldrich. Standards for pigment analysis were from DHI (Hørsholm, Denmark). All solvents applied were of HPLC grade and from VWR International (Søborg, Denmark). Phosphoric acid and tetrabutylammonium acetate were from Merck and Sigma-Aldrich, respectively. HPLC grade water was prepared using a Milli-Q Advantage A10 water deionizing system from Millipore Corporation (USA).

Experimental design, sampling, and sample preparation

Samples were harvested at two commercial cultivation areas by Hjarnø Havbrug A/S in vicinity of Horsens Fjord, in the inner Danish waters. The integrated multi-trophic aquaculture (IMTA) facility was located in the area As Vig (55° 47.529' N, 10° 03.027' E); seaweeds cultivated here were approximately 100 m away from a blue mussel SmartFarm™ (35 tubes including nets) and 500 m from rainbow trout (*Oncorhynchus mykiss*) open net-pen farm (175 t year⁻¹). The reference site (REF) seaweed cultivation area (100 ha; 55° 49.045' N, 10° 06.824' E) was located at approximately 2 km away from the IMTA, and therefore not impacted by nutrient derived from the production of fish and mussels (remineralization). Seaweed commercial production consisted of 7 km and 90 km of cultivation lines (seeded with seedlings of *S. latissima*) deployed at the IMTA and REF site, respectively, during 2011–2013. On 21 May 2013, prior to the experimental period, some ropes were moved from the REF site to the IMTA site for comparison. For this reason, in May 2013, biomass samples were taken solely at the REF site. For more details, see Marinho et al. (2015c).

The cultivated biomass was sampled bimonthly in triplicates (each replicate consisting of at least 10 individual seaweeds randomly selected from each dropper; vertical cultivation line), except in May 2013 ($n = 2$). Samples were freeze-dried, ground to fine powder using a Siebtechnik Screening disc mill TS 250, and hereafter stored at $-80\text{ }^{\circ}\text{C}$ until further analyses. Bimonthly analyses were performed for most of the chemical composition (Marinho et al. 2015a, b, c), and samples covering different seasons were chosen for the antioxidant analyses performed in the present study (May 2013, September 2013, November 2013, January 2014, and May 2014).

Dry matter (DM) was determined after drying the biomass in an oven at $105\text{ }^{\circ}\text{C}$ until constant weight. Prior to the analysis, bioactive compounds, i.e., phenolics, flavonoids, and pigments, were extracted with solvent from the freeze-dried seaweed samples. Methanol and ethyl acetate were tested for the extraction of bioactive compounds from the REF samples for total phenolic content (TPC), total flavonoid content (TFC), and total antioxidant capacity (TAC) assays. Based on the extraction performance, methanol was selected and used in the subsequent extractions.

Extractions of antioxidants

The extraction was performed according to Safafar et al. (2015), however, with some modifications. Briefly, dried powder of seaweed (0.2 g) was weighed into centrifuge tubes, 5 mL solvent (either methanol or ethyl acetate) was added, and the tubes were placed in a sonicator for 30 min. Thereafter, the samples were centrifuged (3500 rpm, ca. 2164g for 10 min) and supernatant was collected in a new tube. The pellets were resuspended and extractions repeated twice. The solvent was evaporated under nitrogen flow. When the extracts were completely dried, they were stored in the freezer ($-18\text{ }^{\circ}\text{C}$). Prior to the analyses, the dried powders were dissolved in 1 mL solvent (either methanol or ethyl acetate). One extraction was performed for each of the three sampling replicates for all sampling points (except REF in May 2013, $n = 2$).

Total antioxidant capacity

This method was performed according to Prieto et al. (1999). Extracts (100 μL) were mixed with 1 mL TAC reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The samples were then left in a water bath ($95\text{ }^{\circ}\text{C}$, 90 min). Thereafter, the samples were cooled to room temperature and then the absorbance was measured at 695 nm. Sample blank (only solvent without sample) was included. Gallic acid was used as standard and the measured absorbance was converted to gallic acid equivalents from a

calibration curve with gallic acid. For the calibration curve, gallic acid was dissolved in the same solvent as the extracts (either methanol or ethyl acetate) in the concentration range from 10 to 100 $\mu\text{g mL}^{-1}$.

Total phenolic content

Extracts (100 μL) were mixed with Folin–Ciocalteu's reagent (0.75 mL, ratio 1:10). The mixture was left for 5 min at room temperature. Thereafter, 6% sodium carbonate (0.75 mL) was added. The samples were placed in the dark for 90 min at room temperature, and the absorbance was measured at 725 nm (Singleton and Rossi 1965; Farvin and Jacobsen 2013). Sample blank (only solvent without sample) was included. Gallic acid was used as standard, and the measured absorbance was converted to gallic acid equivalents from a calibration curve with gallic acid. For the calibration curve, gallic acid was dissolved in the same solvent as the extracts (either methanol or ethyl acetate) in the concentration range from 10 to 100 $\mu\text{g mL}^{-1}$.

Total flavonoid content

This method was performed according to Safafar et al. (2015). Extracts (100 μL) were mixed with 10% aluminum chloride (100 μL), 1 M potassium acetate (100 μL), and distilled water (900 μL). The samples were left for 30 min (room temperature, darkness) and the absorbance was measured at 415 nm. For extracts with ethyl acetate, acetone (200 μL) was added prior to the absorbance measurements, due to the abrupt two phases that were present. Sample blank (only solvent without sample) was included. Rutin was used as standard and the measured absorbance was converted to rutin equivalents from a calibration curve with rutin. For the calibration curve, rutin was dissolved in the same solvent as the extracts (either methanol or ethyl acetate) in the concentration range from 30 to 180 $\mu\text{g mL}^{-1}$.

Radical scavenging activity (DPPH)

Different dilutions of the extracts (100 μL) were transferred to wells in a microplate and 0.1 mM DPPH in methanol (100 μL) was added (Farvin et al. 2014). The absorbance (517 nm) was measured after 30 min (room temperature, darkness) in a microplate reader (Synergy 2 BioTek, USA). Results are expressed as IC_{50} , i.e., the concentration of extract needed to obtain 50% inhibition. BHT was included in the assay as a positive control since a concentration of 0.91 mM of BHT is giving approximately 70% inhibition.

Phenolic acids and flavonoids evaluated by HPLC

The extracts were filtered using 0.45- μm filters (Millipore) before analysis. Chlorophylls and carotenoids were largely removed from the extracts by adsorbing these pigments on an Oasis MCX ion exchange column (60- μm particle size, 6 mL, 150 mg sorbent, Waters, USA), in order to reduce noise (unwanted peaks) in the chromatograms. The analyses were performed by reverse-phase HPLC using an Agilent 1100 Liquid Chromatograph (Agilent Technologies, USA) equipped with a DAD (Agilent G13158). The separation was carried out on a Prodigy ODS-3 column 250 mm \times 46 mm with 5- μm particle size from Phenomenex (USA). The analysis was performed in the gradient mode using phosphoric acid in deionized water (pH = 3) as mobile phase A and methanol-acetonitrile (50:50, v/v) as mobile phase B. The gradient elution program was as follows: 0 min (95% A + 5% B), 2 min (60% A + 40% B), 20 min (100% B), 35 min (95% A + 5% B). Total acquisition time was 40 min. Separation was performed at 25 °C. The flow rate was set at 0.9 mL min⁻¹ and the injection volume was 20 μL . Detection was performed at 235, 255, 280, and 320 nm. Spectral recording for identification purposes was facilitated by using a photodiode detector with a spectral range from 200 to 400 nm.

Pigments

Extraction and analysis of the pigments was done by a method described by Safafar et al. (2015). Samples were extracted by pure methanol containing BHT (butylated hydroxytoluene) in a sonication bath (Branson Ultrasonics, USA) in the dark, at a temperature lower than 5 °C and for 15 min. The separation was carried out by HPLC on a Zorbax Eclipse C8 column 150 mm \times 46 mm with 3.5- μm particle size from Phenomenex. The mobile phase was a mixture of solvent A (70% methanol + 30% of 0.028 M tetrabutylammonium acetate in water) and solvent B (methanol) at a flow rate of 1.1 mL min⁻¹. The gradient program was started with 5% of B and then increased to 95% in 27 min, kept constant for 7 min and then changed to 100% in 1 min and kept constant for 5 min. Total acquisition time was 40 min. Identification of peaks and calibration was done by individual standard for each pigment. Detection of pigments and internal standard (BHT) was done at 440 nm and 280 nm, respectively.

Data treatment

All results are presented as mean \pm standard deviation, corresponding to three experimental replicates ($n = 3$), except in May 2013 ($n = 2$). Analyses were run in duplicate to evaluate analytical precision.

A permutational ANOVA (using Euclidean distances) was used to test the effect of time (random) and site (REF and IMTA; fixed), and time and solvent (methanol and ethyl acetate; fixed) on various response parameters: TPC, TFC, and TAC assays, and concentration of fucoxanthin. Whenever a significant difference between sample means or interaction of factors was revealed by PERMANOVA ($p < 0.05$), a posteriori analysis (pairwise test) was performed. Pairwise comparisons were performed among levels of factors, including within individual levels of other factors in the case of a significant interaction. A one-way PERMANOVA was used to test the effect of harvest time on DPPH radical scavenging activity (PERMANOVA package in PRIMER+; Anderson et al. 2008; type III sum of squares and unrestricted permutation (9999) on raw data; $\alpha = 0.05$).

Multiple regression and multivariate data analysis as partial least squares coefficient method were carried out using STATGRAPHICS software, version Centurion XVI (StatPoint Technologies Inc., USA).

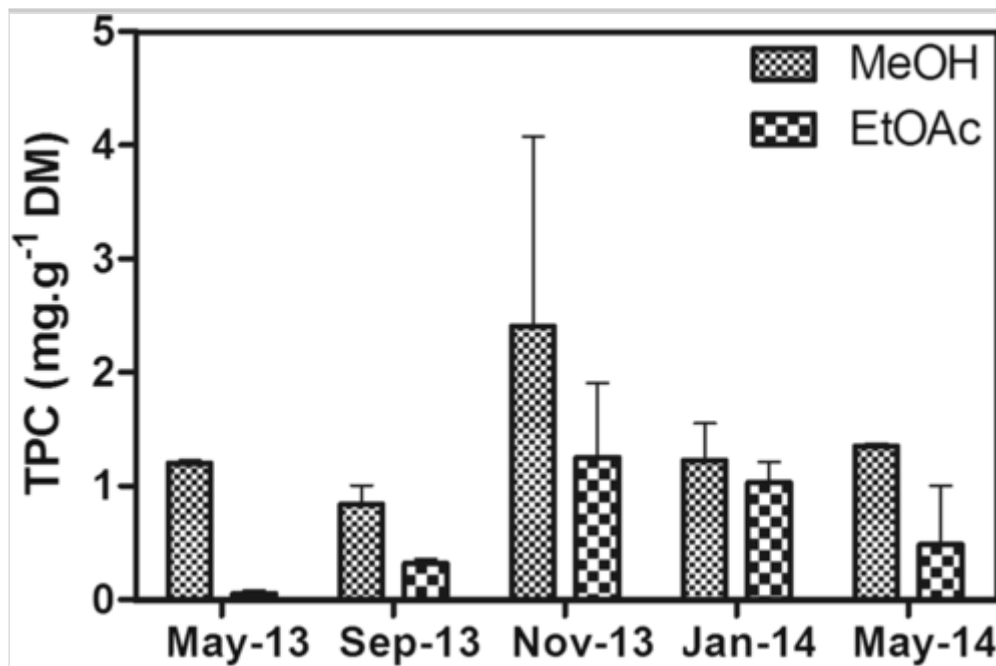
Results

Effect of methanol and ethyl acetate extraction on TPC, TFC, and TAC (REF site)

TPC was significantly higher in *S. latissima* samples extracted by methanol compared with those extracted using ethyl acetate ($F = 18.64$, $p < 0.05$; Fig. 1). TPC ranged from 0.84 to 2.41 mg GAE g⁻¹ algal DM for methanol extraction and 0.056 to 1.253 mg GAE g⁻¹ DM for ethyl acetate extraction. Seasonal variation on TPC was also significant ($F = 3.47$, $p < 0.05$).

Fig. 1

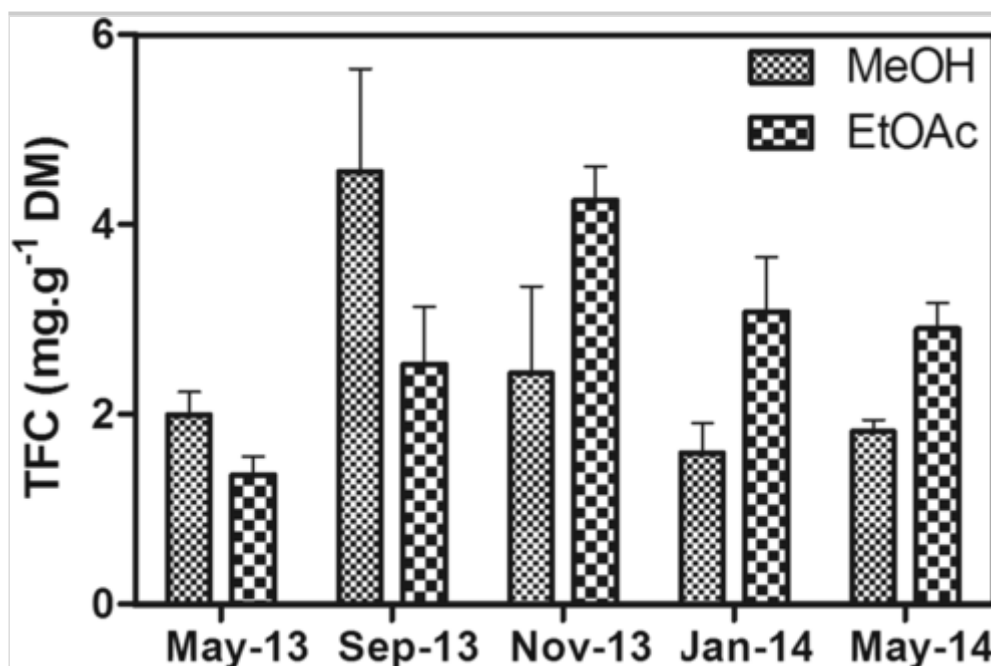
Total phenolic content (TPC; mg GAE g⁻¹ DM) of *Saccharina latissima* harvested in different seasons on the REF site, extracted by methanol or ethyl acetate solvents. Data are mean \pm SD; $n = 3$ except in May 2013 ($n = 2$)



The statistical analysis on TFC revealed a significant interaction between factors (i.e., solvent and harvest time, $p < 0.05$). Methanol extraction yielded higher TFC in May and September 2013 (2.00–4.56 mg RE g⁻¹ DM) compared to ethyl acetate extraction (1.36–2.53 mg RE g⁻¹ DM), while the opposite was observed from November 2013 to May 2014; 1.59–2.43 mg RE g⁻¹ DM compared to 2.90–4.26 mg RE g⁻¹ DM (Fig. 2).

Fig. 2

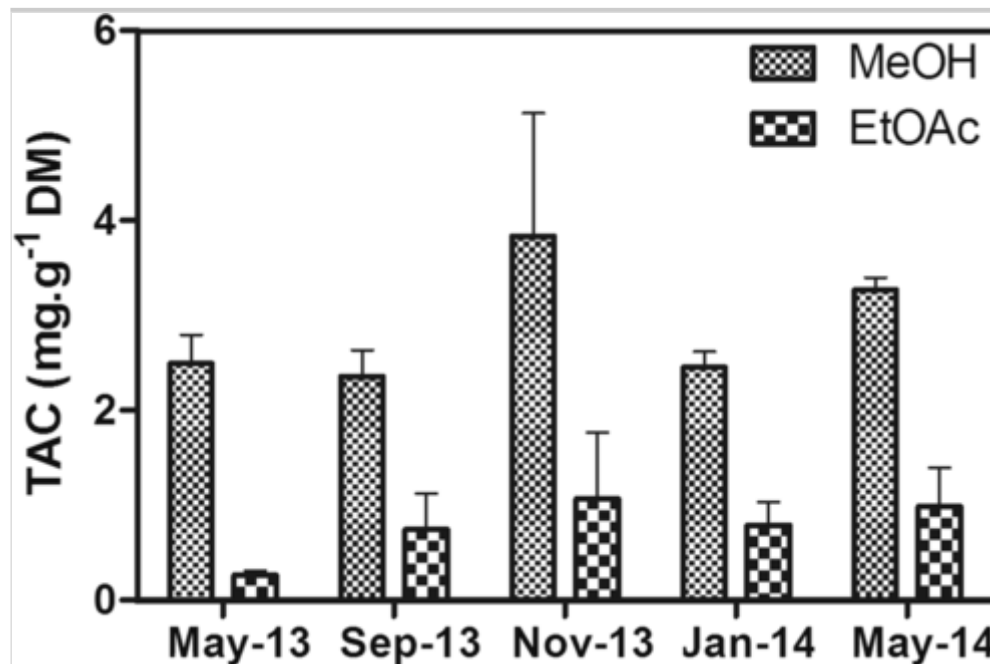
Total flavonoid content (TFC; mg RE g⁻¹ DM) of *Saccharina latissima* harvested in different seasons on the REF site, extracted by methanol or ethyl acetate solvents. Data are mean \pm SD; $n = 3$ except in May 2013 ($n = 2$)



TAC was significantly higher in *S. latissima* samples extracted by methanol, regardless of the harvest time ($F = 65.09$, $p < 0.05$; Fig. 3). TAC ranged from 2.36 to 3.84 mg GAE g⁻¹ DM for methanol extraction and 0.263 to 1.07 mg GAE g⁻¹ DM for ethyl acetate extraction.

Fig. 3

Total antioxidant capacity (TAC; mg GAE g⁻¹ DM) of *Saccharina latissima* harvested in different seasons on the REF site, extracted by methanol or ethyl acetate solvents. Data are mean \pm SD; $n = 3$ except in May 2013 ($n = 2$)



Based on the results from the TPC, TFC, and TAC assays obtained using the two different solvents, methanol was selected to be used in the subsequent extractions.

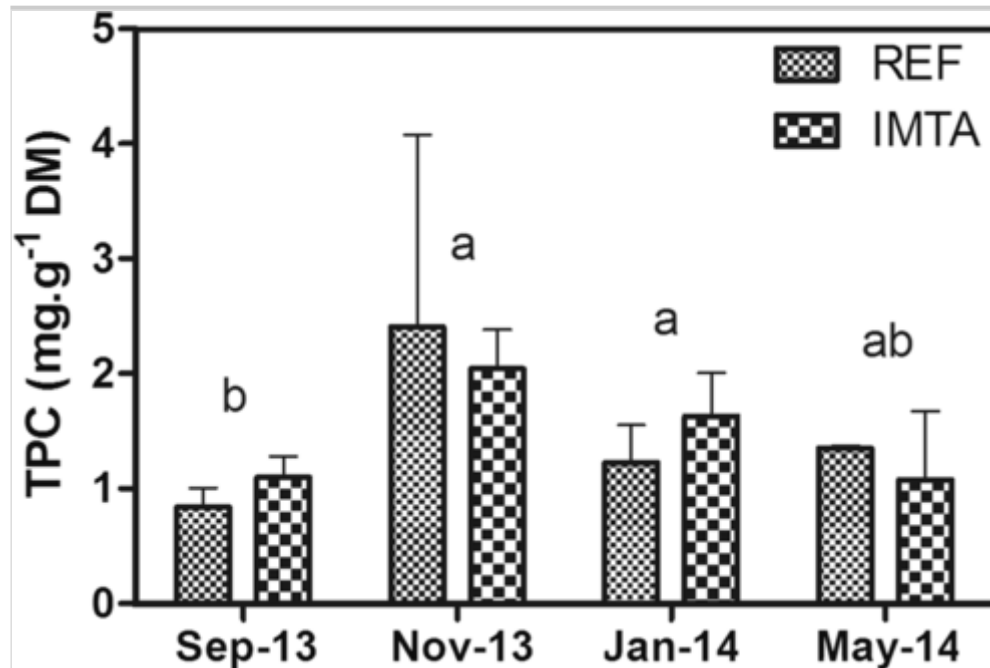
Effect of season and cultivation site on the pigment concentration, antioxidant content, and activity (REF and IMTA sites)

Generally, cultivation site did not result in significant difference on the tested parameters (TPC, $F = 0.002$, $p = 0.97$; TFC, $F = 1.44$, $p = 0.31$; and TAC, $F = 0.04$, $p = 0.86$), while significant seasonal differences were identified for the TPC, TFC, and TAC assays.

TPC was higher in November and January (1.23–2.41 mg GAE g⁻¹ DM) compared to September (0.84–1.10 mg GAE g⁻¹ DM; $p < 0.05$), but it was not significantly different from May 2014 (1.08–1.35 mg GAE g⁻¹ DM; Fig. 4).

Fig. 4

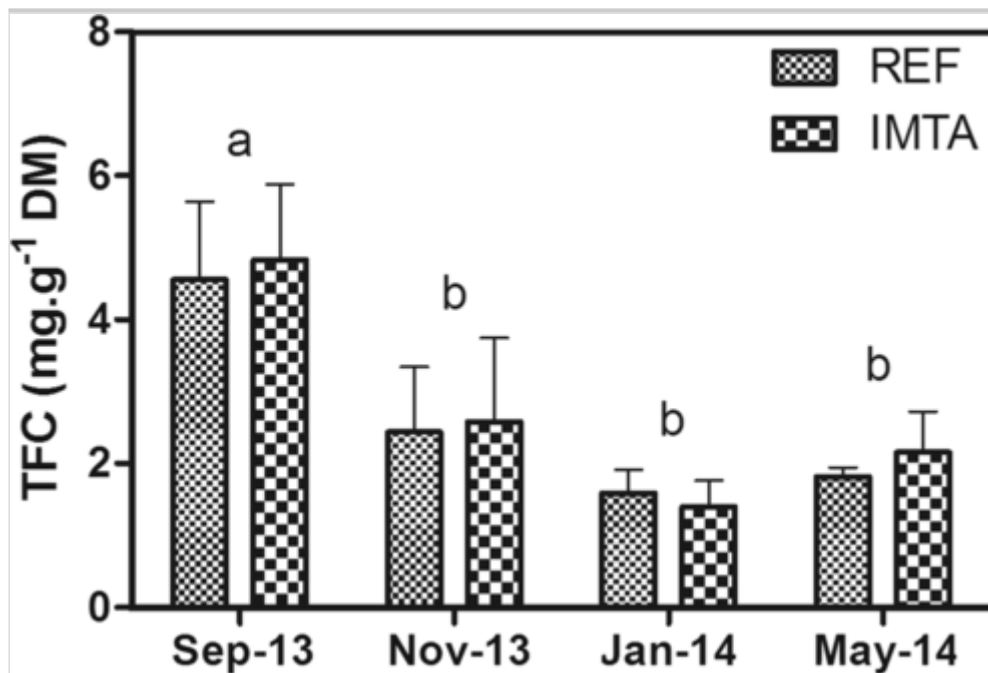
Total phenolic ~~content~~ compounds (TPCs) in *Saccharina latissima* harvested in different seasons, and cultivated both at the IMTA and REF sites. Different letters represent significant difference ($p < 0.05$) between months. Data are mean \pm SD; $n = 3$



The highest TFC was found in September (4.56–4.83 mg RE g⁻¹ DM; $p < 0.05$), whereas there was no significant difference between all other harvest times (1.40–2.59 mg RE g⁻¹ DM; Fig. 5).

Fig. 5

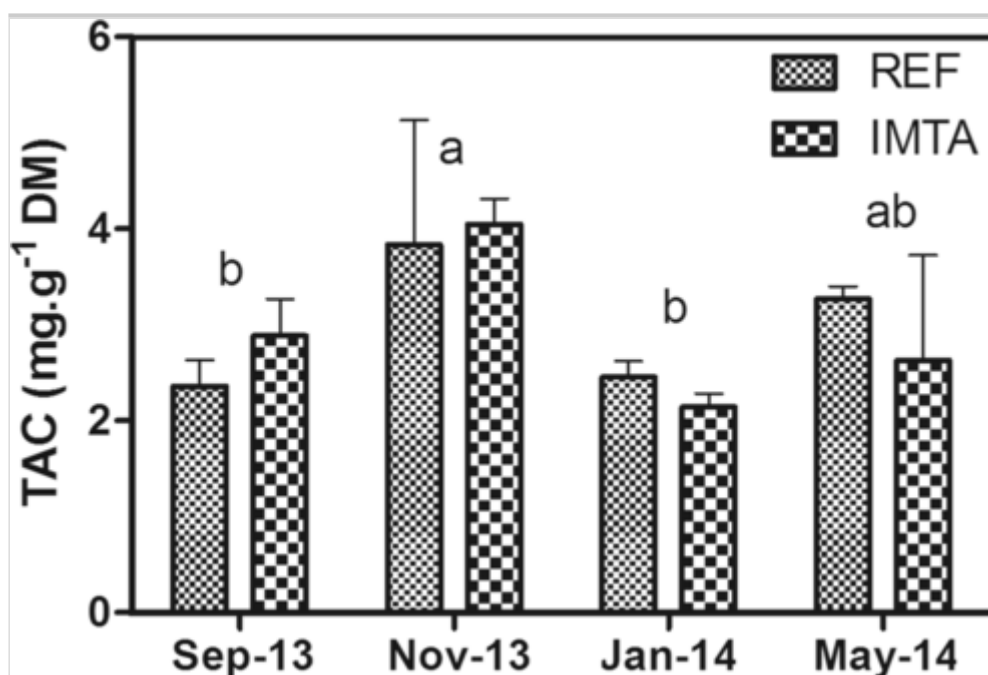
Total flavonoid content (TFC) of *Saccharina latissima* harvested in different seasons, and cultivated both at the IMTA and REF sites. Different letters represent significant difference ($p < 0.05$) between months. Data are mean \pm SD; $n = 3$



TAC was significantly higher in November (3.84–4.05 mg GAE g⁻¹ DM), compared to September and January (2.14–2.88 mg GAE g⁻¹ DM), but similar to May 2014 (2.63–3.27 mg GAE g⁻¹ DM; $p > 0.05$; Fig. 6).

Fig. 6

Total antioxidant capacity (TAC) of *Saccharina latissima* harvested in different seasons, and cultivated both at the IMTA and REF sites. Different letters represent significant difference ($p < 0.05$) between months. Data are mean \pm SD; $n = 3$



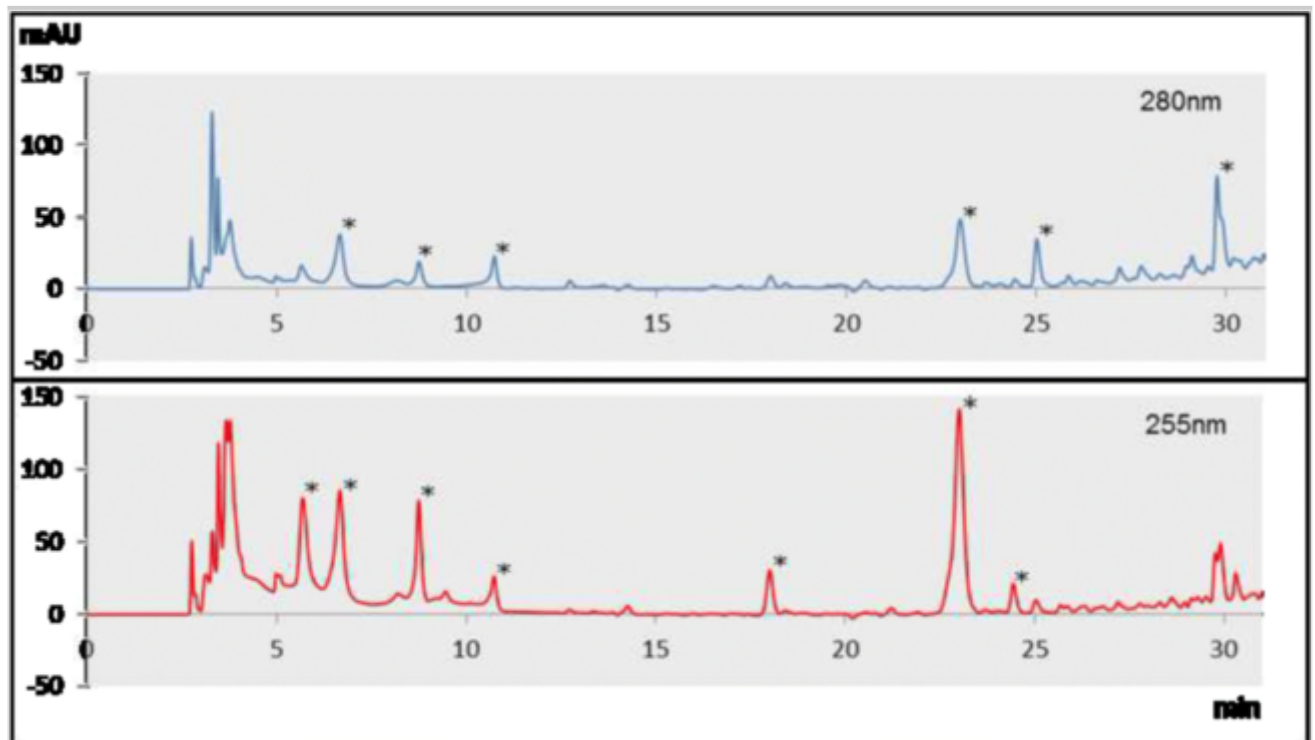
Regarding the HPLC-DAD results, it was not possible to identify any phenolic acids, flavonoids or phloroglucinol. Unidentified peaks were detected in all

seaweed samples (Fig. 7), but their retention times and spectra did not match those of the ~~1820~~ tested standard Corrected from 18 to 20. Since the total number of standards tested was 20, as stated in other parts of the manuscript. compounds (user-created library).

Fig. 7

HPLC profile of *Saccharina latissima* harvested in November at the REF site registered at 255 and 280 nm. *Unidentified peaks whose concentrations were above limit of detection determined for gallic acid and rutin

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The pigment concentration varied during seasons; however, the profile remained unchanged, which included chlorophyll *a* (ranging from ~~170~~~~169.52~~ to ~~655.12~~ $\mu\text{g g}^{-1}$ DM) and fucoxanthin as dominant pigments, and with minor concentrations of other chlorophylls (*c2*) and other carotenoids (violaxanthin and β -carotene; Table 1). Fucoxanthin concentration was significantly higher in November, January, and May 2014 (261–665 $\mu\text{g g}^{-1}$ DM) compared to that in September (222–370 $\mu\text{g g}^{-1}$ DM; $p < 0.05$; Fig. 8). No significant differences in the concentration of fucoxanthin were found between cultivation sites ($F = 0.014$, $p = 0.92$).

Table 1

Pigment composition ($\mu\text{g g}^{-1}$ DM) of *Saccharina latissima* harvested in different seasons at IMTA and REF sites

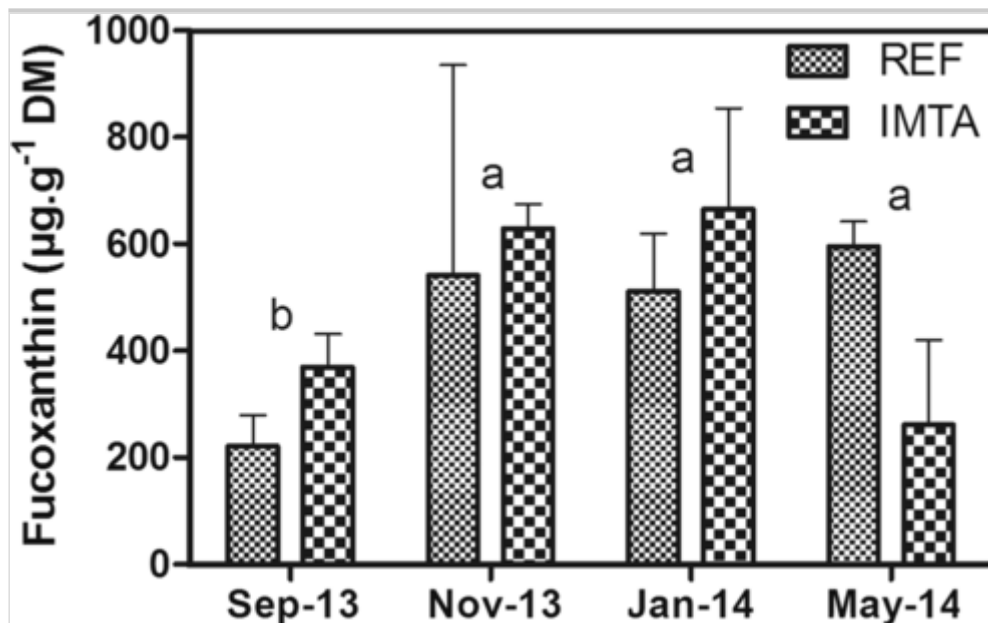
Pigments	May	September		November		January	
	INITIAL	REF	IMTA	REF	IMTA	REF	IM
Chlorophyll <i>c2</i>	n.d.	39.63 ± 12.12	59.49 ± 7.11	93.51 ± 78.56	85.72 ± 8.44	81.12 ± 18.30	10. ± 40
Fucoxanthin	120.3*	221.6 ± 57.7 66	369.9 ± 61.4 +	541.5 ± 393.9	628.9 ± 45.5 0	511.4 ± 108.6	66. ± 18
Violaxanthin	3.79 ± 3.34	16.29 ± 3.39	22.71 ± 3.14	35.88 ± 26.88	33.68 ± 1.87	26.65 ± 14.04	32 ± 14
Chlorophyll <i>a</i>	169.5 ± 62.0 2	282.1 ± 43.4 +	406.2 ± 38.6 2	655.1 ± 503.6	512.9 ± 79.0 0	465.7 ± 323.0	63 ± 23
β-carotene	3.88 ± 0.71	8.76 ± 4.73	5.29 ± 2.14	19.67 ± 3.10	15.90 ± 3.15	19.52 ± 7.24	23 ± 7
Total carotenoids	65.92 ± 86.44	246.7 ± 65.7 0	397.9 ± 66.4 4	590.5 ± 432.3	678.5 ± 42.1 08	557.6 ± 128.7	72 ± 20.
Total chlorophylls	177.9 169.5 ± An error was found here and the numbers were corrected accordingly. This does not have any impact elsewhere in the manuscript. 50.18 62.0	321.7 ± 55.5 +	465.7 ± 41.3 2	748.6 ± 582.2	598.6 ± 87.4 0	546.8 ± 338.0	74 ± 27

Data expressed as mean ± standard deviation ($n = 3$). *Detected in only one experimental *n.d.*, not detected



Fig. 8

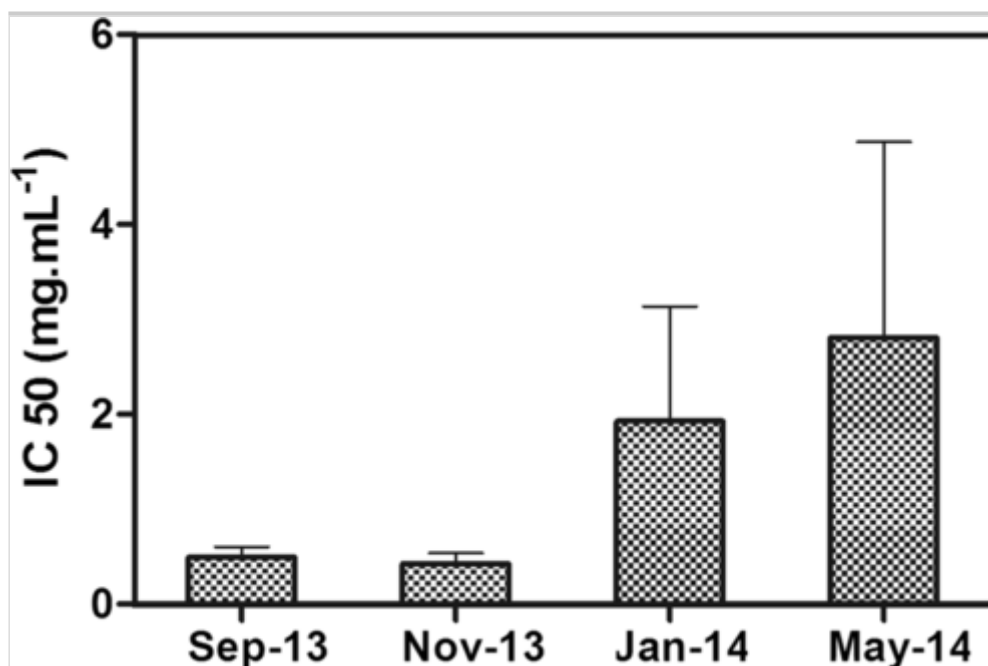
Fucoxanthin content of *Saccharina latissima* harvested in different seasons, and cultivated both at the IMTA and REF sites. Different letters represent significant difference ($p < 0.05$) between months. Data are mean ± SD; $n = 3$



DPPH radical scavenging activity revealed a concentration dependency and increased with increasing concentrations of algal extract (data not shown). IC_{50} ranged from a minimum of 0.42 mg mL^{-1} in September to a maximum of 2.81 mg mL^{-1} in May 2014 (Fig. 9). Nevertheless, difference in the DPPH radical scavenging activity between harvest times was not statistically significant ($F = 2.13$, $p = 0.21$).

Fig. 9

DPPH radical scavenging activity (IC_{50} ; mg mL^{-1}) of methanol extracts of *Saccharina latissima* harvested in different seasons at the REF site. Data are mean \pm SD; $n = 3$

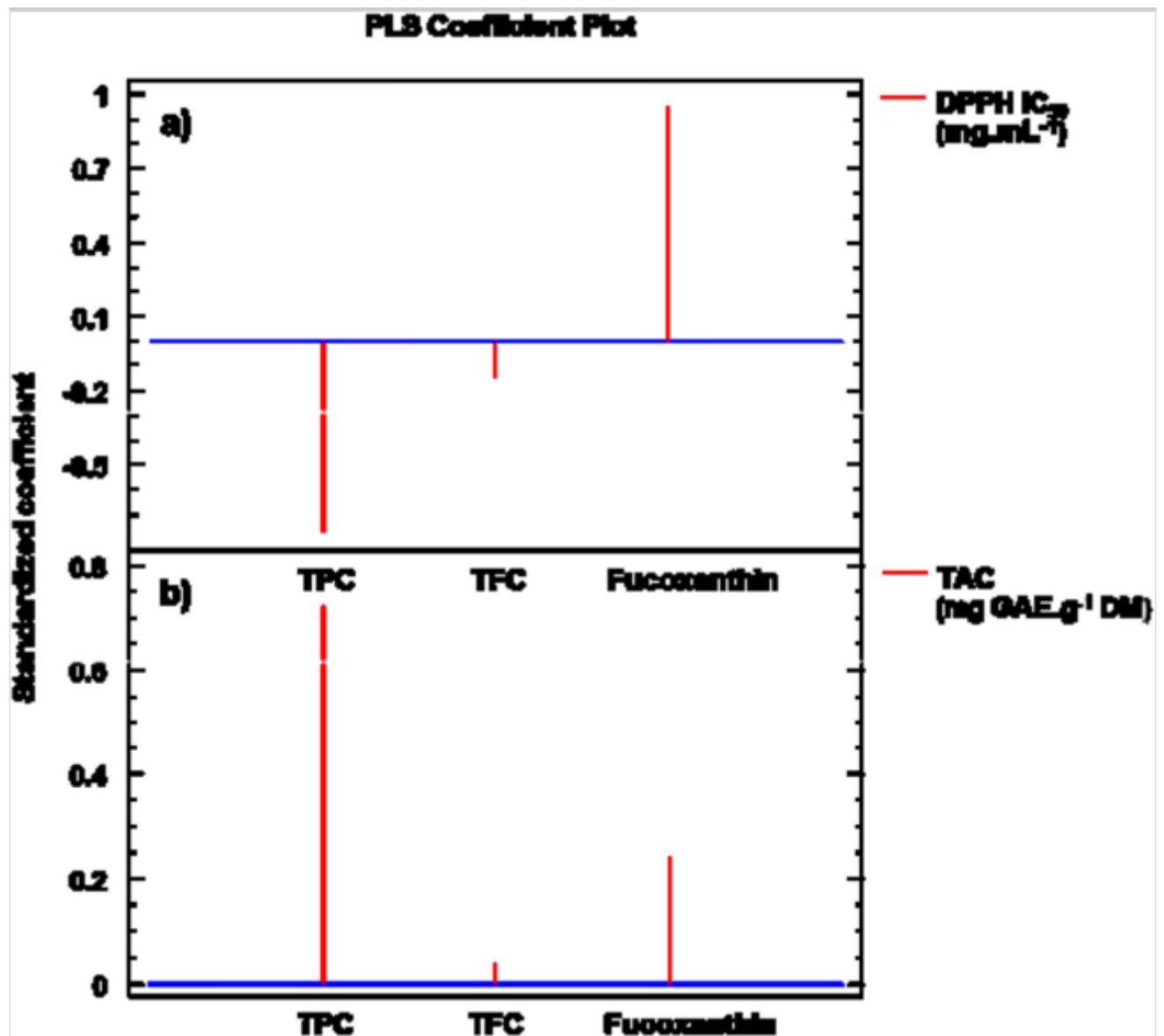


Correlation between antioxidant content and activity

Effect of variations of TPC, TFC, and fucoxanthin contents as independent variables on the variation of IC_{50} has been tested by a multiple least square regression model. Analyses of variances results showed weak correlation between the parameters ($F = 2.82$ and $p = 0.12$ at the 5.0% significance level). Partial least square plot showed that fucoxanthin had the main effect on the variations of IC_{50} , with fucoxanthin increase leading to a reduction in anti-radical activity (Fig. 10(a)). On the other hand, both TPC and TFC were negatively correlated to IC_{50} , suggesting that these variables had a positive effect on the radical scavenging activity, especially TPC. Moreover, the combination of TPC and fucoxanthin contents showed strong correlation with TAC as dependent variable ($F = 11.67$, $p < 0.005$). Partial least square model showed that both independent variables positively contributed to the variations in TAC, with TPC as the main contributing factor (Fig. 10(b)).

Fig. 10

Partial least square (PLS) coefficient plots showing the standardized coefficient for effect of the independent variables TPC (mg GAE g^{-1} DM), TFC (mg RE g^{-1} DM), and fucoxanthin (μg g^{-1} DM) on (a) IC_{50} values for the DPPH scavenging activity, and (b) TAC



Discussion

The results obtained for the TPC, TFC, and TAC assays were affected by the solvent applied for the extraction process. Methanol extraction resulted in higher values than ethyl acetate except for few months in the TFC assay. Our observations are most likely due to the polarity of the solvents applied (methanol is a more polar solvent than ethyl acetate) and polarity of the antioxidative compounds present in the seaweed. Several studies have been performed on the antioxidative properties of seaweed extracts, where different extraction solvents resulted in different results for the antioxidant properties and effects (Cho et al. 2007; Farvin and Jacobsen 2013; Hermund et al. 2015; Honold et al. 2016). Koivikko et al. (2005) reported a general increase in the extraction efficiency of phenolic compounds (phlorotannins) from the brown seaweed *Fucus vesiculosus* with increasing solvent polarity. Furthermore, our findings are in accordance with the results reported on pomegranate fruit, where methanol extraction resulted in much higher total phenolic and total flavonoid contents than ethyl

acetate. The differences observed were in that study also ascribed to the differences in the polarity of the applied solvents (Ali et al. 2014).

Generally, cultivation site did not result in significant difference on the tested variables, while significant seasonal differences were identified for the TPC, TFC, and TAC assays. Similarity in these variables between cultivation sites may be explained by the similar environmental conditions found in the two cultivation areas including water temperature, salinity, irradiance, and inorganic nitrogen and phosphorus. Moreover, the inorganic nutrients released from the fish farm at the IMTA site were considered negligible compared to the naturally occurring background concentrations of nitrogen (Marinho et al. 2015c).

The TPC found in this study was several times higher than that previously reported for water (209 $\mu\text{g GAE g}^{-1}$ DM) and ethanol extracts (354 $\mu\text{g GAE g}^{-1}$ DM) of *S. latissima* (Farvin and Jacobsen 2013). Likely, this difference may be related to the different polarity of the solvents applied. On the other hand, the concentrations reported here for *S. latissima* (0.84–2.41 mg GAE g^{-1} DM) are similar to those reported for *L. digitata* harvested monthly in a sheltered rocky shore in Brittany (France), ranging seasonally from 0.09 to 0.19% DM (using phloroglucinol as standard; Connan et al. 2004). Likewise, the seasonal variation on the TPC followed the pattern reported for *L. digitata* in the same study with the highest values found in winter and lowest values found in summer/autumn. On the other hand, Yates and Peckol (1993) observed an opposite trend in natural populations of *F. vesiculosus*, where the lowest concentrations of polyphenols were found from November to February. Moreover, the authors found that the concentration of polyphenols was negatively correlated with the ambient N concentration and tissue-N content (Yates and Peckol 1993). These results are contrary to those reported in our study, since both ambient nutrient concentrations and tissue-N content of *S. latissima* are highest during winter in the inner Danish waters (Marinho et al. 2015c). The highest concentrations found for *S. latissima* are at least 10 times lower compared with the values reported by Connan et al. (2004) for seven species from the order Fucales. Our results further support that species belonging to the order Laminariales present substantially lower content of total phenolics compared with those belonging to the order Fucales (e.g., *Fucus* sp.).

To our knowledge, this is the first report on TFC of *S. latissima*. TFC varied seasonally reaching a maximum in summer and a minimum in winter. Flavonoids are UV-absorbing compounds functioning as UV screen in terrestrial plants, among many other important functions. For instance, quercetin can be induced by UV-B and is primarily found in the epidermal cells. This role is assumed to be played by simpler UV-absorbing compounds such as

mycosporine-like amino acids in the aquatic environment (Rozema et al. 2002). Nevertheless, structural identification of flavonoids in red and brown seaweeds, and microalgae, has recently been reported, contradicting the general assumption that these compounds could be exclusively present in terrestrial plants (Klejduš et al. 2010; Rodríguez-Bernaldo de Quirós et al. 2010; Goiris et al. 2014). TFC found in the present study (1.40–4.83 mg RE g⁻¹ DM over all seasons) is much higher than that found in a number of brown and green seaweed species originated from the Black Sea (6.5–229 µg RE g⁻¹ DM), and higher but comparable to the values found in brown and green species from Rameshwaram coast of India (1.35–2.02 mg GAE/g; Meenakshi et al. 2009; Sava and Sîrbu 2010). On the other hand, our values are considerably lower compared with those reported in another study testing seaweed species belonging to the three major groups: green, red, and brown seaweeds, harvested from the Visakhapatnam coast of India (6.03–33.439 mg quercetin equivalents g⁻¹ DM; Sarojini et al. 2012). Nevertheless, because of the differences in the extraction methods, solvents applied, and species tested comparisons among studies, should be carefully considered.

AQ4

The pigment qualitative composition did not change seasonally, whereas chlorophyll *a* and fucoxanthin were the dominant pigments. Fucoxanthin has been previously identified as a major pigment found in brown seaweed species (Holdt and Kraan 2011), which may account to 79% of total carotenoids (Haugan and Liaaen-Jensen 1994). On the other hand, the quantity of specific pigments changed seasonally, and with fucoxanthin concentrations that could be of commercial interest, varying seasonally from 222 to 665 µg g⁻¹ DM of *S. latissima*. Although, the concentrations of fucoxanthin in *S. latissima* are in the lower end of the range of values reported for fucoxanthin-rich microalgae such as diatoms (700–21,670 µg g⁻¹ DM; Petrushkina et al. 2017), efficient commercial-scale extraction of fucoxanthin from processing discards of seaweed (*Saccharina japonica*) has already been established, reaching a recovery ratio of 82%. Moreover, the fucoxanthin obtained was stable and reduced by only 2% after a 6-month storage at 4 °C (Kanazawa et al. 2008). Concentration of fucoxanthin found in a number of Norwegian brown seaweed species ranged from 172 to 720 µg g⁻¹ DM (Jensen 1966), which matches the range of values reported here for *S. latissima* harvested at different seasons. Our results reveal a seasonal pattern in the concentration of fucoxanthin which ranged from a summer minimum to an autumn/winter maximum. A similar seasonal pattern on the concentration of fucoxanthin was also observed in the brown seaweed *Ascophyllum nodosum* (Stengel and Dring 1998). Furthermore, the increased fucoxanthin content observed during autumn/winter in this study is supported by studies showing that this increase can be a response to, i.e., shade and/or depth

adaptation in order to increase light-harvesting efficiency (Ramus et al. 1977; Fortes and Lüning 1980). This can be correlated with less irradiance and short day length observed during winter in Denmark.

Seasonal changes in DPPH radical scavenging activity were not of statistical significance, which likely resulted from the high biological variability observed in the samples collected in January and May (very high standard deviation). Nevertheless, the lowest IC₅₀ values, corresponding to the highest radical scavenging activity, were found in September and November, matching the period in which the maximum concentrations of TFC and TPC were found, respectively. This data suggested a possible correlation between DPPH scavenging activity and TFC and TPC. This assumption was further supported by the partial least square coefficient plot revealing that both TPC and TFC were negatively correlated with IC₅₀, suggesting that these variables had a positive effect on the radical scavenging activity. These results are in accordance with previous studies, which directly correlated DPPH scavenging activity with the TPC found in the extracts both in seaweeds (e.g., Cho et al. 2007; Wang et al. 2009, 2012; Hermund et al. 2015) and in plants (e.g., Ghasemzadeh et al. 2010; Ali et al. 2014). On the other hand, the analysis revealed a strong positive correlation between fucoxanthin and IC₅₀, which could suggest a negative impact of fucoxanthin in the radical scavenging activity. However, fucoxanthin has been reported to have strong radical scavenging activity in a number of studies (e.g., Sachindra et al. 2007; Peng et al. 2011; Farvin et al. 2018). Thus, it is likely that our results are impacted by the presence of other compounds in the seaweed extracts such as specific phenolic compounds, low-molecular-weight polysaccharides, proteins, or peptides which may contribute to free radical scavenging activity (Farvin and Jacobsen 2013). Additionally, the regression analysis revealed a strong positive correlation between TPC and TAC assays. This result supports previous studies suggesting that polyphenols are a main contributing factor for TAC, which act by donating electrons and reacting with free radicals to convert them into more stable product and terminate free radical chain reaction (Jayaprakasha et al. 2003; Kasangana et al. 2015).

Using both retention times and spectral matching (DAD), it was not possible to find a positive match between the tested 20 phenolic standards and the peaks detected in the seaweed samples. It can be hypothesized that phenolic compounds may be oxidized during prolonged storage, and/or their concentrations may be below the limit of detection of the HPLC-DAD method, or the selected phenolic compounds are not present in the seaweed. On the other hand, considering that more than 8000 phenolic compounds have been identified so far (Guo et al. 2009), it is possible that seaweed may contain other phenolic compounds than the ones evaluated in this study. In this context, there is a great

need for further research to investigate the composition of phenolic compounds in seaweed, particularly using analytical methods suitable for structural identification such as NMR and/or HPLC-MS.

Conclusion

Methanol seems to be more suitable than ethyl acetate for extracting antioxidants from seaweed.

This study did not find any differences in biomass between the IMTA and the reference site, but demonstrates seasonal changes in the content of total phenolics, total flavonoids, and pigments, and the subsequent total antioxidant capacity of cultivated *S. latissima*, which needs to be taken into consideration when evaluating this species as a source of antioxidants. Overall, biomass harvested in November presented a combination of higher TPC, TAC, fucoxanthin, and radical scavenging activity, and thus this seems to be the prime harvest time when targeting antioxidant content and activity. TPC and TFC had a positive effect on the radical scavenging activity, especially TPC, which likewise contributed most for TAC.

Saccharina latissima had fucoxanthin as a major pigment, which may be of commercial interest due to its numerous reported bioactivities and established efficient commercial-scale extraction process. *Saccharina latissima* is not the species with the highest antioxidant capacity, but accounting that species from the order Fucales such as *Fucus* sp. are not yet in cultivation, the harvestable volumes, and thereby overall yields of antioxidants from *S. latissima*, may be higher and of commercial interest.

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References

Ali SI, El-Baz FK, El-Emary GAE, Khan EA, Mohamed AA (2014) HPLC-analysis of polyphenolic compounds and free radical scavenging activity of

pomegranate fruit (*Punica granatum* L.). Int J Pharm Clin Res 6:348–355

Anderson M, Gorley R, Clarke K (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth

Arasaki S, Arasaki T (1983) Low calorie, high nutrition vegetables from the sea to help you look and feel better. Japan Publications Inc., Tokyo

Azevedo IC, Marinho GS, Silva DM, Sousa-Pinto I (2016) Pilot scale land-based cultivation of *Saccharina latissima* Linnaeus at southern European climate conditions: growth and nutrient uptake at high temperatures. Aquaculture 459:166–172

Bixler HJ, Porse H (2011) A decade of change in the seaweed hydrocolloids industry. J Appl Phycol 23:321–335

Cho S, Kang S, Cho J, Kim AR, Park SM, Hong YK, Ahn DH (2007) The antioxidant properties of brown seaweed (*Sargassum siliquastrum*) extracts. J Med Food 10:479–485

Connan S, Goulard F, Stiger V, Deslandes E, Ar Gall E (2004) Interspecific and temporal variation in phlorotannin levels in an assemblage of brown algae. Bot Mar 47:410–416

FAO (2018) Fishery statistical collections. Global Aquaculture Production. <http://www.fao.org/fishery/statistics/global-aquaculture-production/en> . Accessed 16 Feb 2018

Fariman GA, Shastan SJ, Zahedi MM (2016) Seasonal variation of total lipid, fatty acids, fucoxanthin content, and antioxidant properties of two tropical brown algae (*Nizamuddinia zanardinii* and *Cystoseira indica*) from Iran. J Appl Phycol 28:1323–1331

Farvin KHS, Jacobsen C (2013) Phenolic compounds and antioxidant activities of selected species of seaweeds from Danish coast. Food Chem 138:1670–1681

Farvin KHS, Jacobsen C (2015) Antioxidant activity of seaweed extracts: *in vitro* assays, evaluation in 5% fish oil-in-water emulsions and characterization. J Am Oil Chem Soc 92:571–587

Farvin KHS, Andersen LL, Nielsen HH, Jacobsen C, Jakobsen G, Johansson I, Jessen F (2014) Antioxidant activity of cod (*Gadus morhua*) protein hydrolysates: *In vitro* assays and evaluation in 5% fish oil-in-water emulsion. *Food Chem* 149:326–334

Farvin KHS, Surendraraj A, Jacobsen C (2018) Isolation of fucoxanthin from brown algae and its antioxidant activity: *in vitro* and 5% fish oil-in-water emulsion. *J Am Oil Chem Soc* 95:835–843

Fortes MD, Lüning K (1980) Growth rates of North Sea macroalgae in relation to temperature, irradiance and photoperiod. *Helgol Meeresunters* 34:15–29

Ghasemzadeh A, Jaafar HZE, Rahmat A (2010) Antioxidant activities, total phenolics and flavonoids content in two varieties of Malaysia young ginger (*Zingiber officinale* Roscoe). *Molecules* 15:4324–4333

Goiris K, Muylaert K, Voorspoels S, Noten B, De Paepe D, E Baart GJ, De Cooman L (2014) Detection of flavonoids in microalgae from different evolutionary lineages. *J Phycol* 50:483–492

Guo W, Kong E, Meydani M (2009) Dietary

Handå A, Forbord S, Wang X, Broch OJ, Dahle SW, Størseth TR, Reitan KI, Olsen Y, Skjermo J (2013) Seasonal- and depth-dependent growth of cultivated kelp (*Saccharina latissima*) in close proximity to salmon (*Salmo salar*) aquaculture in Norway. *Aquaculture* 414–415:191–201

Haugan JA, Liaaen-Jensen S (1994) Algal carotenoids 54. Carotenoids of brown algae (Phaeophyceae). *Biochem Syst Ecol* 22:31–41

Hermund DB, Yeşiltaş B, Honold P, Jónsdóttir R, Kristinsson HG, Jacobsen C (2015) Characterisation and antioxidant evaluation of Icelandic *F. vesiculosus* extracts *in vitro* and in fish-oil-enriched milk and mayonnaise. *J Funct Foods* 19:828–841

Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. *J Appl Phycol* 23:543–597

Honold PJ, Jacobsen C, Jónsdóttir R, Kristinsson HG, Hermund DB (2016) Potential seaweed-based food ingredients to inhibit lipid oxidation in fish-oil-enriched mayonnaise. *Eur Food Res Technol* 242:571–584

Jacobsen C, Sørensen A-DM, Holdt SL, et al (2018) Novel antioxidants from seaweed. Manuscript submitted to *Annu Rev Food Sci Technol*

Jayaprakasha GK, Selvi T, Sakariah KK (2003) Antibacterial and antioxidant activities of grape (*Vitis vinifera*) seed extracts. *Food Res Int* 36:117–122

Jensen A (1966) Carotenoids of Norwegian brown seaweeds and of seaweed meals. Norwegian Institute of Seaweed Research, Report No. 31. Tapir, Trondheim

Kamiya M, Nishio T, Yokoyama A, Yatsuya K, Nishigaki T, Yoshikawa S, Ohki K (2010) Seasonal variation of phlorotannin in sargassacean species from the coast of the sea of Japan. *Phycol Res* 58:53–61

Kanazawa K, Ozaki Y, Hashimoto T, Das SK, Matsushita S, Hirano M, Okada T, Komoto A, Mori N, Nakatsuka M (2008) Commercial-scale preparation of biofunctional fucoxanthin from waste parts of brown sea algae *Laminaria japonica*. *Food Sci Technol Res* 14:573–582

Karadağ A, Hermund DB, Jensen LHS, Andersen U, Jónsdóttir R, Kristinsson HG, Alasalvar C, Jacobsen C (2017) Oxidative stability and microstructure of 5% fish-oil-enriched granola bars added natural antioxidants derived from brown alga *Fucus vesiculosus*. *Eur J Lipid Sci Technol* 119:1500578

Kasangana P, Haddad P, Stevanovic T (2015) Study of polyphenol content and antioxidant capacity of *Myrianthus Arboreus* (Cecropiaceae) root bark extracts. *Antioxidants* 4:410–426

Klejdus B, Lojková L, Plaza M, Snóblová M, Stěrbová D (2010) Hyphenated technique for the extraction and determination of isoflavones in algae: ultrasound-assisted supercritical fluid extraction followed by fast chromatography with tandem mass spectrometry. *J Chromatogr A* 1217:7956–7965

Koivikko R, Lopenen J, Honkanen T, Jormalainen V (2005) Contents of soluble, cell-wall-bound and exuded phlorotannins in the brown alga *Fucus vesiculosus*, with implications on their ecological functions. *J Chem Ecol* 31:195–212

Mannino AM, Vaglica V, Oddo E (2014) Seasonal variation in total phenolic content of *Dictyopteris polypodioides* (Dictyotaceae) and *Cystoseira amentacea* (Sargassaceae) from the Sicilian coast. *Flora Mediterr* 24:39–50

Marinho G, Holdt S, Jacobsen C, Angelidaki I (2015a) Lipids and composition of fatty acids of *Saccharina latissima* cultivated year-round in integrated multi-trophic aquaculture. *Mar Drugs* 13:4357–4374

Marinho GS, Holdt SL, Angelidaki I (2015b) Seasonal variations in the amino acid profile and protein nutritional value of *Saccharina latissima* cultivated in a commercial IMTA system. *J Appl Phycol* 27:1991–2000

Marinho GS, Holdt SL, Birkeland MJ, Angelidaki I (2015c) Commercial cultivation and bioremediation potential of sugar kelp, *Saccharina latissima*, in Danish waters. *J Appl Phycol* 27:1963–1973

Meenakshi S, Gnanambigai DM, Tamil Mozhi S, Arumugam M, Balasubramanian T (2009) Total flavanoid and *in vitro* antioxidant activity of two seaweeds of Rameshwaram coast. *Glob J Pharmacol* 3:59–62

Mouritsen OG (2013) *Seaweeds: edible, available & sustainable*. University of Chicago Press, Chicago

Pal A, Kamthania MC, Kumar A (2014) Bioactive compounds and properties of seaweeds—a review. *OALib* 01:1–17

Peng J, Yuan J-P, Wu C-F, Wang J-H (2011) Fucoxanthin, a marine carotenoid present in brown seaweeds and diatoms: metabolism and bioactivities relevant to human health. *Mar Drugs* 9:1806–1828

Peteiro C, Freire Ó (2013) Biomass yield and morphological features of the seaweed *Saccharina latissima* cultivated at two different sites in a coastal bay in the Atlantic coast of Spain. *J Appl Phycol* 25:205–213

Petrushkina M, Gusev E, Sorokin B, Zotko N, Mamaeva A, Filimonova A, Kulikovskiy M, Maltsev Y, Yampolsky I, Guglya E, Vinokurov V, Namsaraev Z, Kuzmin D (2017) Fucoxanthin production by heterokont microalgae. *Algal Res* 24:387–393

Poyato C, Thomsen BR, Hermund DB, Ansorena D, Astiasarán I, Jónsdóttir R, Kristinsson HG, Jacobsen C (2017) Antioxidant effect of water and acetone extracts of *Fucus vesiculosus* on oxidative stability of skin care emulsions. *Eur J Lipid Sci Technol* 119:1600072

Prieto P, Pineda M, Aguilar M (1999) Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem* 269:337–341

Ramus J, Lemons F, Zimmerman C (1977) Adaptation of light-harvesting pigments to downwelling light and the consequent photosynthetic performance of the eulittoral rockweeds *Ascophyllum nodosum* and *Fucus vesiculosus*. *Mar Biol* 42:293–303

Rodríguez-Bernaldo de Quirós A, Lage-Yusty MA, López-Hernández J (2010) Determination of phenolic compounds in macroalgae for human consumption. *Food Chem* 121:634–638

Rozema J, Björn LO, Bornman JF, Gaberscik A, Häder DP, Trost T, Germ M, Klisch M, Gröniger A, Sinha RP, Lebert M, He YY, Buffoni-Hall R, de Bakker NV, van de Staaij J, Meijkamp BB (2002) The role of UV-B radiation in aquatic and terrestrial ecosystems—an experimental and functional analysis of the evolution of UV-absorbing compounds. *J Photochem Photobiol B* 66:2–12

Sachindra NM, Sato E, Maeda H, Hosokawa M, Niwano Y, Kohno M, Miyashita K (2007) Radical scavenging and singlet oxygen quenching activity of marine carotenoid fucoxanthin and its metabolites. *J Agric Food Chem* 55:8516–8522

Safafar H, van Wagenen J, Møller P, Jacobsen C (2015) Carotenoids, phenolic compounds and tocopherols contribute to the antioxidative properties of some microalgae species grown on industrial wastewater. *Mar Drugs* 13:7339–7356

Sarojini Y, Lakshminarayana K, Rao PS (2012) Variations in distribution of flavonoids in some seaweed of Visakhapatnam coast of India. *Der Pharma Chem* 4:1481–1484

Sava C, Sîrbu R (2010) Analytical study of the determination of flavonoids in Black Sea algae. *Ovidius Univ Ann Chem* 21:29–34

Schmid M, Guihéneuf F, Stengel DB (2017) Ecological and commercial implications of temporal and spatial variability in the composition of pigments and fatty acids in five Irish macroalgae. *Mar Biol* 164:1–18

- Shahidi F, Zhong Y, Chandrasekara A (2012) Antioxidants and human health. In: Yu LL, Tsao R, Shahidi F (eds) *Cereals and Pulses: Nutraceutical Properties and Health Benefits*, 1st edn. John Wiley & Sons, Inc., Hoboken, pp 273–308
- Sherwin ER (1990) Antioxidants. In: Branen L, Davidson PM, Salminen S (eds) *Food additives*. Marcel Dekker, New York, pp 139–193
- Singh IP, Sidana J (2013) Phlorotannins. In: *Functional ingredients from algae for foods and nutraceuticals*. Elsevier, pp 181–204
- Singleton VL, Rossi JA (1965) Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am J Enol Vitic* 16:144–158
- Steinberg PD (1995) Seasonal variation in the relationship between growth rate and phlorotannin production in the kelp *Ecklonia radiata*. *Oecologia* 102:169–173
- Stengel DB, Dring MJ (1998) Seasonal variation in the pigment content and photosynthesis of different thallus regions of *Ascophyllum nodosum* (Fucales, Phaeophyta) in relation to position in the canopy. *Phycologia* 37:259–268
- Stiger V, Deslandes E, Payri CE (2004) Phenolic contents of two brown algae, *Turbinaria ornata* and *Sargassum mangarevense* on Tahiti (French Polynesia): interspecific, ontogenic and spatio-temporal variations. *Bot Mar* 47:402–409
- Tabarsa M, Rezaei M, Ramezanpour Z, Waaland R, Rabiei R (2012) Fatty acids, amino acids, mineral contents, and proximate composition of some brown seaweeds. *J Phycol* 48:285–292
- Wang T, Jónsdóttir R, Ólafsdóttir G (2009) Total phenolic compounds, radical scavenging and metal chelation of extracts from Icelandic seaweeds. *Food Chem* 116:240–248
- Wang T, Jónsdóttir R, Liu H, Gu L, Kristinsson HG, Raghavan S, Ólafsdóttir G (2012) Antioxidant capacities of phlorotannins extracted from the brown algae *Fucus vesiculosus*. *J Agric Food Chem* 60:5874–5883
- Wattenberg LW (1986) Protective effects of 2(3)-tert-butyl-4-hydroxyanisole on chemical carcinogenesis. *Food Chem Toxicol* 24:1099–1102

Yates JL, Peckol P (1993) Effects of nutrient availability and herbivory on polyphenolics in the seaweed *Fucus vesiculosus*. *Ecology* 74:1757–1766