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RESEARCH

Seasonal variation in the composition and antioxidant potential of *Codium tomentosum* and *Ulva lacinulata* produced in a land-based integrated multi-trophic aquaculture system

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Abstract

In the context of the blue bioeconomy, macroalgae have gained widespread recognition in Europe as a renewable raw material with a wide range of uses, while also providing important ecosystem services. To reach its full potential, the macroalgae industry must diversify the species exploited. The green macroalgae *Ulva lacinulata* and *Codium tomentosum* are among several species currently cultivated at ALGAplus' land-based integrated multi-trophic aquaculture (IMTA) system inserted in Ria de Aveiro, Portugal. This study aims to evaluate seasonal changes in the composition and antioxidant potential of the two cultivated species to assess their value as sea vegetables. To achieve this, samples of *U. lacinulata* and *C. tomentosum* were collected during different seasons and analyzed. The nutritional and mineral composition showed some marked differences across species and seasons. *Ulva lacinulata* contained more protein and dietary fiber, while *C. tomentosum* had a higher lipid content, particularly in polyunsaturated fatty acids. *Codium tomentosum* contained more minerals than *U. lacinulata*, except for magnesium and iron. *Ulva lacinulata* was richer in chlorophylls, carotenoids, and total phenols. Additionally, *U. lacinulata* displayed a higher antioxidant potential against $ABTS^{+\bullet}$ and $O_2^{\bullet-}$. The levels of heavy metals, namely, lead, cadmium, and mercury were below the limit defined for dietary supplements by EU regulations. Moreover, the levels of inorganic arsenic were below the limit recommended for edible macroalgae sold in France. According to current knowledge, recommendations and regulations, both species appear to have good potential as sea vegetables.

Keywords Seaweed · Chlorophyta · Nutritional profile · Minerals · Phytochemicals · Antioxidant activity · Hazardous elements · Inorganic arsenic

Introduction

Macroalgae are regarded as a sustainable raw material for the bioeconomy, as well as a valuable resource to tackle marine ecosystem disturbances (Coleman 2023). As primary producers, macroalgae can provide energy and habitat to higher trophic level organisms, form long-term carbon storage pools in natural beds contributing to carbon sequestration, and can be used directly in food and feed, or as feedstock in a biorefinery to produce, for instance, food additives, cosmetics, pharmaceuticals, ethanol, hydrogen, and bioplastics (Marinho et al. 2013; Angell et al. 2016; Bikker et al. 2016; Margareta et al. 2020; Beaumont et al. 2021; Badmu et al. 2022; Braga & Lens 2023; Hurd et al. 2023). For these purposes, either wild-harvested or cultivated biomass can be used, and it has been acknowledged that, while some algal species will decline due to climate change, others may expand or proliferate (Hurd et al. 2023). Nevertheless, aquaculture production is regarded as having the greatest potential to supply the increasing market demand for macroalgal biomass, as wild stocks are already under pressure due to climate change and disturbance from human activity (Barbier et al. 2019). To reach its full potential, however, the macroalgae industry needs

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to diversify the species that are exploited. Among macroalgae of interest, species from the genera *Ulva* and *Codium* are gaining importance (Mantri et al. 2020; Meinita et al. 2022). Both *Ulva lacinulata* and *Codium tomentosum* are among several species currently cultivated at ALGAplus' land-based integrated multi-trophic aquaculture (IMTA) system inserted in Ria de Aveiro, Portugal.

Ulva spp. are fast-growing and opportunistic macroalgae with a short life cycle that grow all year round and are naturally distributed globally. They can grow attached, sessile, or free-floating, adapting to a wide range of abiotic conditions, such as light, temperature, salinity, nutrients, and pollutants (Eismann et al. 2020; Mantri et al. 2020). *Ulva* spp. exhibit potential ecological significance since they take up large quantities of nutrients from the aquatic environments during rapid growth, thus purifying eutrophic waters. However, their ability for uncontrolled, rapid mass production, termed "green tides" can pose significant ecological and economic hazards (Mantri et al. 2020).

Codium spp. also have a broad distribution thriving in temperate waters. They grow on firm substrates in subtidal habitats having high morphological plasticity (Meinita et al. 2022). Although some species pose concern because of their invasive, bloom-forming nature (Fernández et al. 2014), *C. tomentosum* is in decline in some geographical regions probably due to pollution and competition with *Codium fragile* (Pizzolla 2007).

Macroalgae are known for their richness in polysaccharides, minerals, and certain vitamins but they also contain other nutrients and bioactive substances like proteins, lipids, carotenoids and polyphenols (Holdt and Kraan 2011). Macroalgae contain many primary and secondary metabolites that allow them to grow in their specific environmental conditions, some of them with potential for human applications. Chlorophylls, the preponderant pigments imparting the characteristic green colour, are good indicators of photosynthetic performance and may reach 2% of algae fresh weight (Strain et al. 1971). Chlorophylls are localized in chloroplasts and are always accompanied by carotenoids as secondary light-absorbing pigments. Some carotenoids are also involved in protection against oxidative stress triggered by radiation. The composition of chlorophylls and auxiliary pigments is tuned to maximize light absorption according to the range of wavelengths that reach the algae in their specific habitat (Lichtenthaler 1987). Besides carotenoids, macroalgae produce an array of secondary metabolites, including bromophenols, which are widespread across algal clades, to protect them from predation (Cotas et al. 2020). The synthesis of bromophenols is favored in seawater, which contains considerable amounts of chloride, bromide, and iodide (Liu et al. 2011). Macroalgae can accumulate hazardous elements from their environment, such as arsenic (As), lead (Pb), mercury (Hg) and cadmium (Cd), in concentrations toxic to other species (Holdt and Kraan 2011; Mantri et al. 2020).

Macroalgae also must cope with reactive oxygen species generated at several cellular sites, including mitochondria, chloroplasts, peroxisomes, and outer plasma membrane. Reactive oxygen species are by-products of metabolic reactions and are part of the arsenal for defense against invaders (e.g., pathogens, epiphytes). Abiotic factors such as excessive light, heavy metals, high salt concentration, extremes of temperature and mechanical and physical stress may also lead to overproduction of oxidants (Collén et al. 1995; Mallick and Mohn 2000; Huang et al. 2019). High intracellular levels of oxidants may be damaging to cell structures and must be counterbalanced by antioxidant mechanisms to protect the cell. Antioxidant defenses generated by macroalgae, specifically small metabolites with radical scavenging capacity, may provide health benefits to consumers and have applications in many industries as natural antioxidants (Mallick and Mohn 2000; Stahl and Sies 2003).

This work aims to evaluate seasonal variation in the composition and antioxidant potential of two cultivated macroalgae species, *U. lacinulata* and *C. tomentosum*, to assess their value and safety as sea vegetables.

Methods

Sampling, sample preparation and extraction

Ulva lacinulata and *Codium tomentosum* were produced by ALGAplus, SA (production site at Ria de Aveiro coastal lagoon, Northern Portugal, 40°36'43.0"N, 8°40'43.0"W). *Ulva* strains domesticated at ALGAplus were recently identified as *U. lacinulata* through DNA barcoding. Macroalgal biomass was collected over four seasons, between fall (November 2022) and summer (August 2023). *Ulva lacinulata* was collected in all four seasons, while *C. tomentosum* could not be collected during the winter due to the seasonality of the species. The experimental replicates (n=3) were collected from different tanks run under the same culture parameters. Abiotic factors such as photoperiod, seawater temperature and salinity were monitored throughout the cultivation period (Table 1). Seawater temperature and salinity in the cultivation systems of the two species were monitored daily. Additionally, satellite data on photosynthetically available radiation (PAR) at the farm area and cultivation period were retrieved from MODIS Aqua using Giovanni online data system, developed and maintained by the NASA GES DISC (MODIS 2022; Acker and Leptoukh 2007). Cultivation conditions such as initial stock density and nutrient

Table 1 Seasonality of abiotic parameters monitored during the cultivation period (September 2022–September 2023)

		Fall	Winter	Spring	Summer
Temperature (ºC)	*U. lacinulata*	16.4 ± 1.2 (13.3–17.7)	13.9 ± 0.6 (13.2–15.1)	21.2 ± 2.0 (17.5–23.9)	22.6 ± 2.1 (18.7–25.0)
	C. tomentosum	16.6 ± 0.7 (15.0–17.5)	–	21.9 ± 2.6 (17.1–25.7)	23.0 ± 1.4 (20.7–24.8)
Salinity (ppt)	*U. lacinulata*	19.6 ± 2.7 (14.0–23.0)	29.0 ± 1.1 (27.6–30.8)	36.1 ± 0.3 (35.5–36.5)	36.7 ± 0.3 (36.2–37.3)
	C. tomentosum	19.6 ± 1.8 (17.2–22.0)	–	36.1 ± 0.2 (35.8–36.3)	36.5 ± 0.3 (36.2–37.0)
PAR (mol photons m^{-2} day^{-1})		18.3 ± 11.9 (0.6–40.8)	20.6 ± 9.1 (7.0–39.9)	50.9 ± 8.5 (40.4–64.0)	52.5 ± 8.7 (38.4–62.6)
Photoperiod (h day^{-1})		9.9 ± 0.1 (9.7–10.1)	10.4 ± 0.2 (10.1–10.6)	14.4 ± 0.4 (13.9–14.8)	14.0 ± 0.2 (13.6–14.3)

Data presented as mean ± SD (minimum – maximum values). Water temperature and salinity measured in the culture systems of *Ulva lacinulata* and *Codium tomentosum*; photosynthetically available radiation (PAR) obtained from satellite data

renewal were similar during these trials and consistent with the internal protocols of the company. Samples were washed with filtered and sterilized (UV and ozone) seawater from Ria de Aveiro, followed by centrifugation to remove excess water. A portion of fresh biomass was used to determine chemical composition (protein, lipids, fibre, and minerals), and the remaining was freeze-dried and used to determine the phytochemical composition and antioxidant potential.

Freeze-dried samples were ground in a coffee mill to obtain powder (< 1 mm). Powdered samples were kept protected from light in a dry atmosphere until analysis. To extract bioactive compounds, cell disruption was performed by shear force in a Precellys® homogenizer with zirconia beads using water and absolute ethanol (saturated with sodium carbonate) as solvents to lyse *U. lacinulata* (1/20 w/v) and *C. tomentosum* (1/50 w/v). It was necessary to use water to ensure cell disruption of *U. lacinulata* before the extraction of phytochemical compounds with ethanol. Samples were subjected to 6 homogenization cycles, 30 s long, at a speed of 8000 rpm. A break of 45 s was included between cycles to avoid excessive heating. Afterwards, absolute ethanol was added to *U. lacinulata* (to obtain 80% ethanol concentration) and the homogenates were incubated for 30 min at 4°C. The ethanolic or hydro-ethanolic supernatants were decanted and two more extractions were performed with absolute ethanol ensuring that neither pigments nor phenols remained in the pellet (they were not detected in a subsequent last extraction). All supernatants were combined and analyzed in 24 hours.

Chemical composition

The chemical composition of the fresh algae was determined according to standard procedures. Samples were analyzed for moisture (ISO 6496 1999a), crude protein (Dumas method, ISO 16634-1 2008), total fat (after Soxhlet extraction, ISO 6492 1999b), ash (gravimetry, ISO 5984 2002), and fiber (enzymatic-gravimetric method; Bobin-Dubigeon et al. 1997). Saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids were determined by GC-FID (Pinheiro et al. 2019). Minerals including sodium, magnesium, iron, iodine, potassium, and calcium were determined by ICP-MS (Rodrigues et al. 2015). The hazardous elements arsenic, cadmium, lead, and mercury were also determined by ICP-MS (NEN-EN 15763 2010). Speciation of arsenic was also performed by ICP-MS after species separation by ionic chromatography (IC-ICP-MS; Lai et al. 2022).

Phytochemical composition

Total phenolic compounds were quantified by the Folin–Ciocalteu colorimetric assay, following Morone et al. (2022). A calibration curve with gallic acid (12.5 to 100 µg mL^{-1}) was obtained under the same conditions as the extracts. The results were expressed as mg of gallic acid equivalents per g of dry weight (dw) algae.

Chlorophylls were determined by measuring the absorbance (A) of the crude extract prepared for phytochemical composition as previously described, at 665 nm (chlorophyll *a*) and 649 nm (chlorophyll *b*) corresponding to the maximum absorption in the red peak of the pigment under study, setting the baseline correction at 750 nm. The concentrations of pigments were calculated using the equations for chlorophyte extracts in ethanol developed by Ritchie (2006). Using a light path of 1 cm the equations are:

$$\text{Chl } a\left(\mu\text{g mL}^{-1}\right) = -5.2007 \times \left(A_{649} - A_{750}\right) + 13.5275 \times \left(A_{665} - A_{750}\right)$$
$$\text{Chl } b\left(\mu\text{g mL}^{-1}\right) = 22.4327 \times \left(A_{649} - A_{750}\right) - 7.0741 \times \left(A_{649} - A_{750}\right)$$

Total carotenoids were determined by measuring the absorbance (A) at 480 nm, since absorption at this wavelength is mainly due to carotenoids (Lichtenthaler 1987; Connan 2015). The concentration was calculated using a molar absorption coefficient of 2500 $M^{-1}cm^{-1}$. Using a 1 cm light path the equation is:

$$\text{Total Carotenoids}\left(\mu\text{g mL}^{-1}\right) = 4 \times \left(A_{480} - A_{750}\right)$$

Antioxidant potential

The antioxidant potential was assessed with the reactive species superoxide anion radical ($O_2^{-\bullet}$), nitric oxide ($^{\bullet}NO$), DPPH, and ABTS radical cation ($ABTS^{+\bullet}$), using at least four dilutions to evaluate extracts behavior and to calculate IC values. *Codium tomentosum* was tested until the concentration of 3.6 mg mL^{-1} for ABTS, 3.8 mg mL^{-1} for nitric oxide, 12.5 mg mL^{-1} for superoxide, and 18.8 mg mL^{-1} for DPPH scavenging assays. *Ulva lacinulata* was tested until the concentration of 1.4 mg mL^{-1} for ABTS, 3.8 mg mL^{-1} for nitric oxide, and 5.0 mg mL^{-1} for DPPH and superoxide scavenging assays (the concentration range was limited by interference of the color of the extract, making unfeasible determination of IC_{50} for some radicals). Extract scavenging activity of all radicals was monitored with a MultiSkan Go Microplate Reader operated by SkanIt software (Thermo Scientific). Dose-response curves were constructed using the percentage of radical scavenging in comparison with the untreated control, plotted as a function of concentration. IC values were extrapolated from the dose-response curve and expressed as mean ± SD (mg mL^{-1}) of at least three independent assays performed in duplicate. All reactions were performed at room temperature.

The scavenging activity of $O_2^{\bullet-}$ by the extracts was measured in phosphate buffer, pH 7.4 by following the reduction of nitrotetrazolium blue (43 µM) through the action of NADH (166 µM) and phenazine methosulphate (2.7 µM), in kinetic function for 2 min at 562 nm (Morone et al. 2022). Gallic acid was used as a positive control.

$^{\bullet}NO$ scavenging activity was measured in phosphate buffer, pH 7.4, using sodium nitroprusside as a radical donor (10 mM, under light) for 40 min (Baliga et al. 2003). After incubation, the amount of nitrite formed was determined using the Griess reaction. The chromophore was read at 562 nm. Gallic acid was used as a positive control.

DPPH scavenging activity of the extracts was determined in ethanolic solution by monitoring the disappearance (in the dark) of DPPH for 60 min, at 517 nm (Molyneux 2004; Xie & Schaich 2014). Initial absorbance was adjusted to 0.70 ± 0.02, corresponding to 85 µM DPPH radical. Trolox was used as a positive control.

$ABTS^{+\bullet}$ scavenging activity was determined in phosphate buffer, pH 7.4, by monitoring the disappearance of Radical Blue color for 6 min at 734 nm (Re et al. 1999). Initial absorbance was adjusted to 0.70 ± 0.02 (corresponding to a radical concentration of around 60 µM). Trolox was used as a positive control.

Statistical analysis

Results are reported on a dried matter basis and expressed as mean ± standard deviation. Data were analyzed by permutational analysis of variance PERMANOVA using Euclidian distances (PERMANOVA package in PRIMER+; Anderson et al. 2008). The data were tested for homogeneity of variance using PERMDISP. A two-way PERMANOVA was used to test the effect of "Species" (*U. lacinulata* and *C. tomentosum*) and "Season" (fall, winter, spring and summer in *U. lacinulata*, and fall, spring and summer in *C. tomentosum*) as fixed factors on the composition and antioxidant potential of the macroalgae (type III sum of squares and permutation of residuals (9999) under a reduced model (α=0.05)). Whenever a significant difference between sample means or an interaction of factors was revealed by PERMANOVA, a pairwise comparison of levels of factors was performed. A one-way PERMANOVA was applied to test the effect of season on cadmium content, which was quantified only in *U. lacinulata* (type III sum of squares and unrestricted permutation (9999) of raw data (α=0.05)). Whenever a significant difference between sample means was revealed by PERMANOVA, a pairwise comparison of individual means was performed. Means were considered significantly different when levels of $p < 0.05$ were obtained. Pearson's correlation analysis was performed using Prism 8.0 (GraphPad Software, LLC) to analyze the correlation between the level of fiber and phytochemicals, and the antioxidant potential of the macroalgae, and the correlation between the cultivation conditions (abiotic factors) and the composition of the macroalgae.

Results

Phytochemical composition

The variation of chlorophyll and carotenoids along the year, from fall to summer in *U. lacinulata* and *C. tomentosum* is shown in Fig. 1. The concentration of total chlorophyll in *U. lacinulata* was lower in the winter (5.8 mg g^{-1} dry weight (dw) algae), although not significantly different from the spring (7.1 mg g^{-1} dw), and higher in the summer (10.6 mg g^{-1} dw; Fig. 1A). The levels of total chlorophyll in *C. tomentosum* followed the same seasonal trend of *U. lacinulata*, but the actual figures were lower (2.0–4.3 mg chlorophyll g^{-1} dw algae). The chlorophyll *a*/*b* ratio also differs in these species: in *U. lacinulata*, the ratio increased from 1.1 to 1.7 from fall to summer, while in *C. tomentosum* the ratio was 1.1 in fall but decreased in spring (0.8) and summer (0.4).

Like chlorophylls, the levels of carotenoids were much higher in *U. lacinulata* (0.9 to 1.8 mg g^{-1} dw) than in *C.*

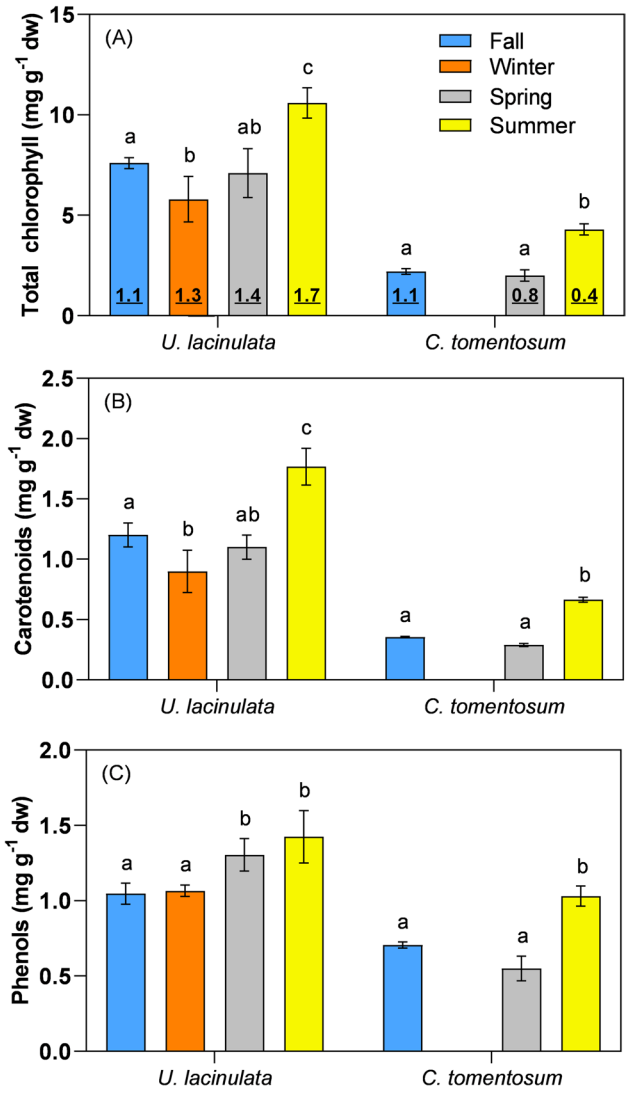


Fig. 1 Seasonal variation in the content of **A**) total chlorophyll and chlorophyll *a*/*b* ratio, **B**) carotenoids and **C**) phenolic compounds of *U. lacinulata* and *C. tomentosum*. Results are expressed as mean ± SD (n=3). The chlorophyll *a*/*b* ratio is represented by the numbers inside the bars. Phenols are expressed as gallic acid equivalents. Different letters indicate significant differences between sample means (seasons) within each species ($p < 0.05$). For detailed statistical output refer to the supplementary material (Table S1)

tomentosum (0.29 to 0.66 mg g^{-1} dw). Their levels increased significantly from winter to summer in *U. lacinulata* and from fall to summer in *C. tomentosum* (Fig. 1B).

The concentration of phenolic compounds in *U. lacinulata* increased significantly from fall (1.05 mg [gallic acid equivalents] g^{-1} dw algae) to summer (1.43 mg g^{-1} dw), with a mean value of 1.21 ± 0.18 mg g^{-1} dw determined year-round (Fig. 1C). The same pattern was observed in *C. tomentosum*, the highest levels of phenols were measured in summer (1.03 mg g^{-1} dw), with a mean value of 0.76 ± 0.25 mg g^{-1} dw year-round. Generally, *U. lacinulata* presented a higher concentration of phenols than *C. tomentosum*, except in summer, when the difference was not statistically significant.

Chemical composition

Tables 2 and 3 summarize the chemical composition of *U. lacinulata* and *C. tomentosum* collected throughout the year. The protein content of *U. lacinulata* was relatively stable across seasons, with the lowest values found in the spring (21.1%) and no significant difference among all other seasons (24.7–25.9%). The highest protein content in *C. tomentosum* was measured in the fall (21.0%) and decreased significantly towards the spring and summer (12.0–12.2%).

The lipid content of *U. lacinulata* ranged from 1.3–4.9%, with the minimum and maximum values measured in the fall and spring, respectively (Table 2). *Codium tomentosum* presented a higher fat content than *U. lacinulata*, which was relatively stable across seasons (5.8–6.7%), also peaking in the spring.

SFA represented between 54–64% of total fatty acids in *U. lacinulata*, MUFA varied between 20–32% and PUFA varied between 14–21% (Table 2). In *C. tomentosum*, the fatty acid profile consisted of 39–51% SFA, 4–29% MUFA and 29–45% PUFA. The content of PUFA in *C. tomentosum* increased significantly from fall (1.45% dw) to summer (2.62% dw). *Codium tomentosum* contained a lower proportion of SFA and was richer in PUFA compared with *U. lacinulata.*

Ulva lacinulata presented a higher content of dietary fiber than *C. tomentosum* (Table 2). In *U. lacinulata* the amount of dietary fiber was relatively stable throughout the year (37.0–42.7% dw), whereas in *C. tomentosum* its content varied from 18.7% dw in the spring to 31.1% dw in the fall.

The ash content in *U. lacinulata* varied significantly from 26.2% dw in the fall to 31.9% dw in the spring (Table 2). The ash content in *C. tomentosum* was significantly higher in all seasons compared to *U. lacinulata* ranging from a minimum of 35.3% dw in the fall to a maximum of 55.5% dw in the summer.

Both species presented high concentrations of minerals in their composition, including important micronutrients (Table 2). The levels of calcium ranged from 0.41–0.71% dw in *U. lacinulata* and 0.61–0.79% dw in *C. tomentosum*, with peak levels occurring in winter and spring, respectively. The content of magnesium in *U. lacinulata* increased significantly from 2.8% dw in winter to 4.8% dw in spring/summer, whereas in *C. tomentosum*, lower, and more stable values were measured throughout the year (1.3–1.5% dw).

The amount of iodine determined in *U. lacinulata* was relatively stable ranging between 28.5–43.1 mg kg^{-1} dw from summer to winter (Table 3). On the other hand, a very significant

Table 2 Macrochemical composition of *U. lacinulata* and *C. tomentosum* collected in different seasons. Except for moisture (expressed as % wet weight, ww) and fatty acid (FA) composition (relative abundance (%) of total), all other parameters are expressed as % dw

		Fall	Winter	Spring	Summer	S	Sp	SxSp
Moisture (% ww)	*U. lacinulata*	81.6 ± 2.0	86.7 ± 0.1	85.4 ± 0.8	86.1 ± 2.0	*	*	*
	C. tomentosum	93.1 ± 0.3	-	92.5 ± 0.1	92.1 ± 0.7			
Protein	*U. lacinulata*	25.9 ± 0.6	25.2 ± 0.8	21.1 ± 2.6	24.7 ± 1.5	*	*	*
	C. tomentosum	21.0 ± 2.3	-	12.2 ± 1.7	12.0 ± 1.8			
Lipid	*U. lacinulata*	1.3 ± 0.4	(1.5)[1]	4.9 ± 3.2	2.6 ± 0.8		*	
	C. tomentosum	5.8 ± 0.2	-	6.7 ± 0.1	6.4 ± 0.6			
Ash	*U. lacinulata*	26.2 ± 0.4	28.4 ± 2.4	31.9 ± 1.4	26.2 ± 2.0	*	*	*
	C. tomentosum	35.3 ± 0.9	-	47.7 ± 0.5	55.5 ± 13.6			
Fiber	*U. lacinulata*	40.1 ± 0.3	39.9 ± 9.9	37.0 ± 2.7	42.7 ± 2.8		*	
	C. tomentosum	31.1 ± 3.8	-	18.7 ± 4.1	19.3 ± 7.1			
Fatty acids	*U. lacinulata*	1.0 ± 0.4	0.6 ± 0.4	4.4 ± 3.0	2.4 ± 1.1	*	*	
	C. tomentosum	5.1 ± 0.0	-	6.5 ± 0.1	5.9 ± 0.9			
SFA (% FA)	*U. lacinulata*	58.0 ± 3.5	63.4 ± 3.7	54.3 ± 0.7	63.6 ± 1.7	*	*	
	C. tomentosum	45.7 ± 2.1	-	38.8 ± 2.9	51.1 ± 1.6			
MUFA (% FA)	*U. lacinulata*	28.2 ± 1.8	31.9 ± 9.5	25.1 ± 4.8	20.0 ± 1.1	*		*
	C. tomentosum	25.7 ± 1.0	-	28.5 ± 0.0	4.4 ± 0.3			
PUFA (% FA)	*U. lacinulata*	13.8 ± 1.9	(14.2)[1]	20.6 ± 5.5	16.4 ± 2.8		*	*
	C. tomentosum	28.6 ± 1.1	-	32.7 ± 2.9	44.5 ± 1.9			
Sodium	*U. lacinulata*	3.1 ± 0.4	4.8 ± 0.6	7.2 ± 1.0	3.6 ± 0.3	*	*	
	C. tomentosum	9.3 ± 0.3	-	13.6 ± 0.9	11.5 ± 1.6			
Magnesium	*U. lacinulata*	3.3 ± 0.3	2.8 ± 0.1	4.8 ± 0.1	4.8 ± 1.1	*	*	*
	C. tomentosum	1.5 ± 0.1	-	1.3 ± 0.1	1.3 ± 0.1			
Potassium	*U. lacinulata*	1.4 ± 0.4	1.7 ± 0.1	4.0 ± 0.4	1.8 ± 0.4	*	*	*
	C. tomentosum	3.0 ± 0.1	-	9.5 ± 0.9	6.1 ± 0.8			
Calcium	*U. lacinulata*	0.41 ± 0.01	0.71 ± 0.08	0.50 ± 0.04	0.52 ± 0.07	*	*	
	C. tomentosum	0.61 ± 0.02	-	0.79 ± 0.07	0.69 ± 0.07			

Results are expressed as mean ± SD (n=3). SFA, Saturated fatty acids; MUFA, Monounsaturated fatty acids; PUFA, Polyunsaturated fatty acids. [1] Quantified in one single replicate, test result below the limit of quantification for other replicates

*Significant differences ($p < 0.05$) for season (S), species (Sp), or their interaction (SxSp). For detailed statistical output refer to the supplementary material (Table S2)

seasonal variation in iodine content was observed in *C. tomentosum,* reaching 105.1 mg kg^{-1} dw in the fall, which was almost 10 times the concentration determined in the spring/summer.

The levels of total arsenic found in *U. lacinulata* ranged from 3.2–7.2 mg kg^{-1} dw, being significantly lower than the levels determined in *C. tomentosum* (13.6–29.6 mg kg^{-1} dw; Table 3). The highest concentrations of arsenic in *C. tomentosum* and *U. lacinulata* occur in the fall and winter, respectively.

The levels of inorganic arsenic ranged from 0.05–0.81 mg kg^{-1} dw in *U. lacinulata* and 0.10–0.66 mg kg^{-1} dw in *C. tomentosum* (Table 3). These represent 1.1 to 17.6% and 0.5 to 2.2% of the total arsenic found in *U. lacinulata* and *C. tomentosum*, respectively. The lowest levels of inorganic arsenic (relative and absolute values) were observed during spring and summer in both species.

Concerning other hazardous elements, the mean levels of lead ranged from 0.17–0.71 mg kg^{-1} dw in *U. lacinulata* and 0.21–2.47 mg kg^{-1} dw in *C. tomentosum* (the content in one of the replicates reached 4.86 mg kg^{-1} dw; Table 3). The levels of cadmium ranged from 0.05–0.22 mg kg^{-1} dw in *U. lacinulata* and were below the limit of quantification of the method in *C. tomentosum* (<0.005 mg kg^{-1} dw), except for one of the replicates (0.11 mg kg^{-1} dw). The levels of mercury determined in the two species were also below the limit of quantification (<0.005 mg kg^{-1} dw), except for the *U. lacinulata* collected in the winter (0.011 mg kg^{-1} dw).

Antioxidant potential

The extracts of *U. lacinulata* and *C. tomentosum* displayed some antioxidant activity against the four radicals tested (Figure S1 in Supplementary material) allowing the calculation of IC_{25} or IC_{50} (Table 4). The antioxidant potential of *U. lacinulata* extracts against both $ABTS^{+\bullet}$ and DPPH increased significantly from fall to summer. On the other hand, the antioxidant potential against $O_2^{\bullet-}$ was higher in the winter (although not significantly different from the fall), while against $^{\bullet}NO$ it was higher from fall to spring decreasing in summer (Table 4). The

Table 3 Microchemical composition of *U. lacinulata* and *C. tomentosum* collected in different seasons. Results are expressed as mg kg^{-1} dw, unless otherwise stated

		Fall	Winter	Spring	Summer	S	Sp	SxSp
Iron	*U. lacinulata*	771.7 ± 588.7	931.7 ± 72.2	329.0 ± 297.3	139.0 ± 14.5	*		
	C. tomentosum	671.0 ± 104.7	-	128.0 ± 70.2	118.0 ± 8.2			
Iodine	*U. lacinulata*	40.8 ± 8.4	43.1 ± 3.6	34.5 ± 7.7	28.5 ± 5.0	*		*
	C. tomentosum	105.1 ± 32.6	-	11.0 ± 2.1	15.2 ± 1.6			
Arsenic (total)	*U. lacinulata*	3.7 ± 0.9	7.2 ± 0.5	3.2 ± 0.2	4.8 ± 0.1	*	*	*
	C. tomentosum	29.6 ± 5.5	-	13.6 ± 2.5	18.6 ± 1.8			
Inorganic Arsenic[1]	*U. lacinulata*	0.65	0.81	0.10	0.05	NA	NA	NA
	C. tomentosum	0.66	-	0.12	0.10			
Inorganic Arsenic (% of total)[2]	*U. lacinulata*	18.0 ± 3.9	11.2 ± 0.7	3.1 ± 0.2	1.1 ± 0.0	*	*	*
	C. tomentosum	2.3 ± 0.4	-	0.9 ± 0.2	0.6 ± 0.1			
Cadmium	*U. lacinulata*	0.05 ± 0.01	0.06 ± 0.01	0.12 ± 0.02	0.22 ± 0.03	*	NA	NA
	C. tomentosum	NQ	-	NQ	(0.11)[3]			
Lead	*U. lacinulata*	0.51 ± 0.49	0.71 ± 0.05	0.23 ± 0.23	0.17 ± 0.15			
	C. tomentosum	0.63 ± 0.08	-	0.21 ± 0.04	(0.08–4.86)[4]			
Mercury	*U. lacinulata*	NQ	0.011±0.001	NQ	NQ	NA	NA	NA
	C. tomentosum	NQ	-	NQ	NQ			

Results are expressed as mean ± SD (n=3). [1] Lyophilized samples (pooled sample composed of 3 experimental replicates); [2] Inorganic arsenic expressed as % of total arsenic; [3] Quantified in one single replicate, test result below the limit of quantification for other replicates; [4] Range values provided due to the result discrepancies between replicates; NQ, not quantified (below the limit of quantification < 0.005 mg kg^{-1} for both cadmium and mercury)

* Significant differences ($p < 0.05$) for season (S), species (Sp), or their interaction (SxSp). For detailed statistical output refer to the supplementary material (Table S3)

NA, not applicable

extracts of *C. tomentosum* showed a higher antioxidant capacity against $ABTS^{+\bullet}$ and $^{\bullet}NO$ in the summer (although the latter was not significantly different from the spring). On the other hand, the antioxidant potential against $O_2^{\bullet-}$ decreased significantly from fall to spring/summer, whereas against DPPH, the highest antioxidant potential was found in the fall, but not significantly different from spring and summer. Comparing the IC_{25}, *U. lacinulata* always demonstrated a better antioxidant potential against $ABTS^{+\bullet}$ and $O_2^{\bullet-}$ than *C. tomentosum*, but for $^{\bullet}NO$ and DPPH the results were season-dependent (Table 4).

Correlations

In general, correlation analysis revealed no correlation or a weak correlation between the fiber content of the two macroalgae and the antioxidant potential of their extracts, except for the activity against $O_2^{\bullet-}$ (r= −0.742) observed in *C. tomentosum* (Fig. 2A, B). The inverse correlation between IC_{25} and the fiber content indicates an increased antioxidant potential with increased fiber levels. In *U. lacinulata*, total chlorophyll, carotenoids and TPC presented an inverse correlation with IC_{25} for DPPH, r= −0.663, −0.717 and −0.798 respectively and to a lower extent also with IC_{25} for $ABTS^{+\bullet}$ (Fig. 2A). On the other hand, the correlation between the content of total chlorophyll, carotenoids and phenols of *C. tomentosum*, and the IC_{25} for the tested radicals was more evident for the radical $ABTS^{+\bullet}$ and $^{\bullet}NO$, and to a lower extent for DPPH (Fig. 2B).

Concerning the relationship between the culture conditions and the composition of the macroalgae, strong correlations were established between the abiotic parameters and the content of protein, lipid, ash, and fiber in *C. tomentosum*, whereas no correlation or weak correlations were observed for *U. lacinulata* (Fig. 3A, B). The levels of protein and fiber in *C. tomentosum* were negatively correlated with the abiotic parameters, while for lipid and ash that correlation was positive (Fig. 3B). A strong correlation was observed between the photosynthetically available radiation (PAR) during cultivation and the levels of total chlorophyll (r= 0.790 – 0.852), carotenoids (r= 0.823 – 0.850), and phenols (r= 0.739 – 0.784) in both species. A strong positive correlation was established between the chlorophyll a/b ratio in *U. lacinulata* and the culture parameters (r= 0.578 – 0.754), whereas an inverse correlation was evident for *C. tomentosum* (r= −0.477 – −0.643).

Discussion

Algae experience fluctuations in temperature, light regime (intensity, wavelength, and photoperiod), nutrient availability, and other environmental factors that affect primary

Table 4 IC_{50} and/or IC_{25} (mg mL^{-1}) of hydro-ethanolic extracts of *U. lacinulata* and ethanolic extracts of *C. tomentosum* against $ABTS^{+\bullet}$, DPPH, $O_2^{\bullet-}$ and $^{\bullet}NO$

ABTS						S	Sp	SxSp
		Fall	Winter	Spring	Summer			
U. lacinulata	IC_{50}	x	x	1.23 ± 0.15	1.07 ± 0.01			
	IC_{25}	0.96 ± 0.12	0.63 ± 0.09	0.45 ± 0.13	0.41 ± 0.02			
C. tomentosum	IC_{50}	3.07 ± 0.02	-	x	2.47 ± 0.13	*	*	*
	IC_{25}	1.25 ± 0.03	-	1.62 ± 0.14	0.94 ± 0.05			
Trolox	IC_{50}	0.006 ± 0.0001 (24.0 ± 0.6 µM)						
	IC_{25}	0.003 ± 0.00005 (11.9 ± 0.2 µM)						
DPPH								
		Fall	Winter	Spring	Summer			
U. lacinulata	IC_{50}	x	x	x	3.9 ± 0.2			
	IC_{25}	3.3 ± 0.8	3.4 ± 0.5	2.8 ± 0.7	1.7 ± 0.2			
C. tomentosum	IC_{50}	6.9 ± 2.0	-	8.4 ± 0.2	5.8 ± 0.5	*		*
	IC_{25}	2.3 ± 0.4	-	2.9 ± 0.1	2.4 ± 0.1			
Trolox	IC_{50}	0.007 ± 0.001 (29.3 ± 3.9 µM)						
	IC_{25}	0.0035 ± 0.0002 (14.1 ± 0.7 µM)						
Superoxide								
		Fall	Winter	Spring	Summer			
U. lacinulata	IC_{50}	x	x	x	x			
	IC_{25}	2.9 ± 0.8	2.2 ± 0.3	4.2 ± 0.1	4.3 ± 0.4			
C. tomentosum	IC_{50}	(12.5)	-			*	*	*
	IC_{25}	5.8 ± 0.8	-	10.1 ± 0.6	9.0 ± 0.6			
Gallic acid	IC_{50}	0.013 ± 0.003 (76.9 ± 18.6 µM)						
	IC_{25}	0.005 ± 0.001 (26.9 ± 5.3 µM)						
Nitric Oxide								
		Fall	Winter	Spring	Summer			
U. lacinulata	IC_{50}	1.3 ± 0.1	1.4 ± 0.2	1.4 ± 0.5	1.3 ± 0.4			
	IC_{25}	0.3 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.6 ± 0.1			
C. tomentosum	IC_{50}	x	-	x	2.0 ± 0.5	*	*	*
	IC_{25}	1.0 ± 0.1	-	1.4 ± 0.7	0.4 ± 0.1			
Gallic acid	IC_{50}	x						
	IC_{25}	0.0013 ± 0.0003 (7.8 ±1.8 µM)						

Results are expressed as mean ± SD (n=3). X, Parameter could not be determined due to color interference of the extract in the wavelength used for determination

* Significant differences in IC_{25} ($p < 0.05$) for season (S), species (Sp), or their interaction (SxSp). For detailed statistical output refer to the supplementary material (Table S4)

NA, not applicable

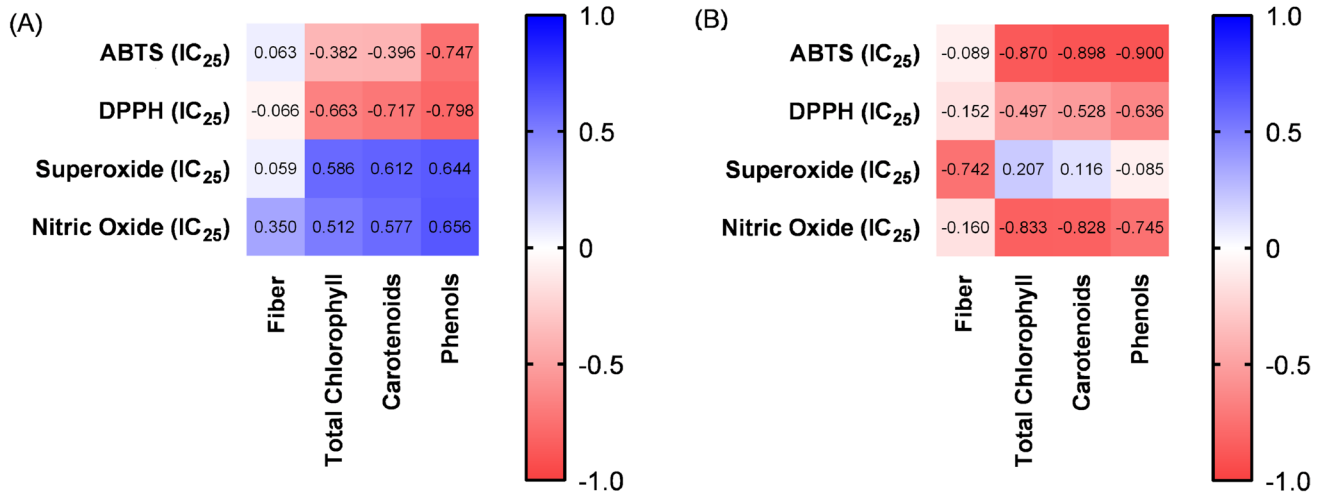


Fig. 2 Correlation analysis between the fiber and phytochemical content of *U. lacinulata* (**A**) and *C. tomentosum* (**B**), and their respective antioxidant potential (IC_{25} (mg extract/mL) against the radicals $ABTS^{+\bullet}$, DPPH, superoxide ($O_2^{\bullet-}$), and nitric oxide ($^{\bullet}NO$))

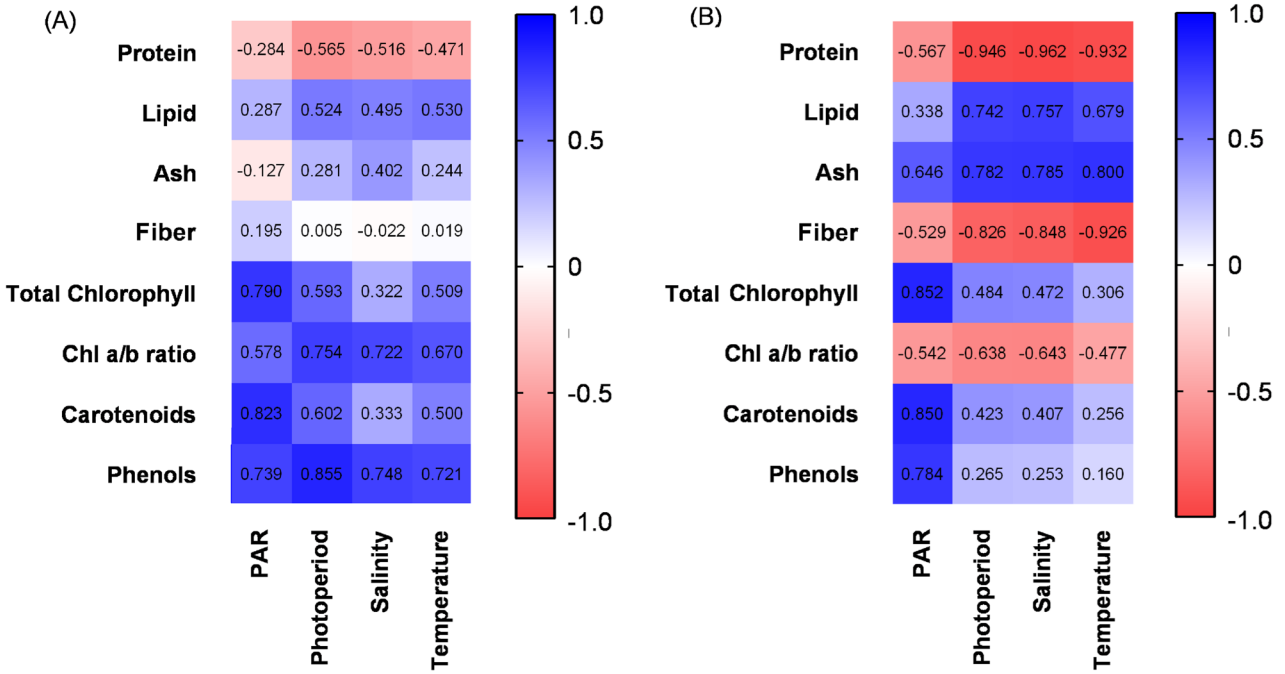


Fig. 3 Correlation analysis between the cultivation conditions (abiotic factors) and the composition of *U. lacinulata* (**A**) and *C. tomentosum* (**B**)

productivity and hence growth. These factors in addition to age or stage of development also affect algal composition (Strain et al. 1971; Eismann et al. 2020). In this study the composition and antioxidant potential of *U. lacinulata* and *C. tomentosum* were evaluated throughout the year.

Phytochemical composition

Macroalgae can be considered one of the most productive biological systems with a photosynthetic efficiency significantly higher than land plants (Margareta et al. 2020). Chlorophylls, namely chlorophyll *a* and *b*, are the photosynthetic pigments used by green macroalgae in photosynthesis to synthesize the organic compounds they need to grow. The concentration of total chlorophyll in *U. lacinulata* ranging from 5.8 to 10.6 mg g^{-1} dw was lower in the winter, intermediate in fall and spring and higher in the summer. A value of 5–6 mg chlorophyll g^{-1} dw in *Ulva* spp. has been reported (Martins et al. 2021). The levels of total chlorophyll determined in *C. tomentosum*

followed the same seasonal trend as *U. lacinulata*, but the actual figures were much lower (2.0–4.3 mg g^{-1} dw), in accordance with published values for the species (around 4.5 mg g^{-1} dw) (Marques et al. 2021). The increase in chlorophylls observed in summer in both species (33% in *U. lacinulata* and 57% in *C. tomentosum*) may result from long-term acclimation response to better light exposure. In fact, the correlation analysis revealed a strong positive correlation between the photosynthetically available radiation (PAR) during cultivation and the total chlorophyll content of the two species. The lower chlorophyll content in *C. tomentosum* may imply a lower photosynthetic performance and lower growth rates compared with *U. lacinulata*. The chlorophyll *a*/*b* ratio also differs in these species. In *U. lacinulata*, the ratio increased from fall to summer, while in *C. tomentosum*, the ratio was higher in fall (1.1) than in spring (0.8) and further decreased in summer (0.4), which encompasses the value of 0.7 previously reported for *Codium* species (Anderson 1983). A significant positive correlation was observed between the chlorophyll *a*/*b* ratio in *U. lacinulata* and the PAR during cultivation, whereas an inverse correlation was evident in *C. tomentosum*. The lower chlorophyll *a*/*b* ratio determined in *C. tomentosum* (in combination with the type of auxiliary carotenoids present in the *Codium* genus) has been related to enhanced light harvesting in deeper water habitats or turbid coastal waters with higher proportions of green and blue-green light available (Anderson 1983; Marques et al. 2021).

Like chlorophylls, the levels of carotenoids were much higher in *U. lacinulata* than in *C. tomentosum*, generally increasing from winter to summer. The values of carotenoids found in literature also point to higher values in *Ulva* than in *Codium*. Total carotenoids below 1 mg g^{-1} dw in air-dried *U. rigida* after 6 months of storage (Pinheiro et al. 2019) and around 0.8 mg g^{-1} dw (sum of all identified carotenoids) in *C. tomentosum* after a 5-week culture period (Marques et al. 2021) have been reported. The higher levels of carotenoids in *U. lacinulata* are expected, as species with high growth rates tend to have increased levels of carotenoids (Eismann et al. 2020). A direct correlation between carotenoid content and growth can also be deduced from the higher levels of carotenoids in summer observed in both species, matching a period of fast growth between spring and summer.

Ulva spp. and *Codium* spp. differ not only in the levels of carotenoids but also in their carotenoid profile, which should be considered if these species are intended as a source of these compounds. *Ulva* spp. contain violaxanthin, lutein, and neoxanthin, as well as small amounts of α and β-carotene (Bhat et al. 2021; Pappou et al. 2022), while *C. tomentosum* is characterized by the presence of siphonaxanthin and siphonaxanthin dodecenoate, and it also contains neoxanthin and β,ε-carotene (Marques et al. 2021). Some of these carotenoids are known to have light-harvesting functions, while others serve in photoprotection. In this study, a strong positive correlation was observed between PAR and the carotenoid content of the two species, which may suggest an adaptation to photoprotection. Because different carotenoids have different properties, further analyses may be necessary to assess the value of these macroalgae as a carotenoid source.

Among the phytochemicals found in *U. lacinulata* and *C. tomentosum*, phenolic compounds perform important ecological roles and were evaluated throughout the year. The concentration of phenolic compounds increased significantly from fall to summer in both species, with a mean value of 1.2 mg phenols g^{-1} dw found in *U. lacinulata* and 0.8 mg g^{-1} dw in *C. tomentosum*. Considerable variation in the levels of phenols in *Ulva* spp. was attributed to seasonal variation, with high concentrations in late summer, probably affording protection against extensive exposition to air and sunlight during summer (Flodin et al. 1999; Whitfield et al. 1999). Notably, a strong positive correlation was found between PAR during cultivation and the phenolic content determined in both species. Higher amounts of phenolic compounds in *U. lacinulata* in comparison to *C. tomentosum* were also expected since *Ulva* spp. are known to produce higher amounts of bromophenols than *Codium* species (Whitfield et al. 1999). Values of total phenolic compounds, measured in gallic acid equivalents, reported for *U. rigida* (12 mg g^{-1} dw (Trigui et al. 2013)) are also higher than those reported for *C. tomentosum* (e.g., 0.3 mg g^{-1} dw (Celikler et al. 2009) or 0.6 mg g^{-1} dw (Rodrigues et al. 2015)).

Simple bromophenols are common metabolites in green algae and *Ulva* and *Codium* species are characterized by the presence of mono, di and tribromophenols being usually 2,4,6-tribromophenol the most abundant compound (Flodin et al. 1999; Whitfield et al. 1999; Trigui et al. 2013). These metabolites are usually produced in limited quantities, serving for chemical defense and deterrence (Cotas et al. 2020). Bromophenols may exert some bioactivities, including antioxidant activity, but this is generally attributed to compounds containing a catechol group and not the simple bromophenols identified so far in the algal species under study (Jesus et al. 2019). Furthermore, it has been proven to be difficult to identify selective, safe, and effective new drugs derived from marine bromophenols, and the health benefits of these compounds are not clear (Dong et al. 2020).

Chemical composition

The use of macroalgae as a food source is well-established in many Asian countries and it is growing in other parts of the world (Echave et al. 2021). Animal feed is another potential field that requires consolidated knowledge of the nutritional composition of macroalgae.

Protein

The amount of protein found in *U. lacinulata* (21%–26% dw) agrees with previously reported values: *Ulva* spp. are known to have a high crude protein content, up to 26% dw (Fleurence et al. 1999; Astorga-España et al. 2016; Bikker et al. 2016), and a small variation in the protein content of *Ulva rigida* along seasons was also previously reported (Shuuluka et al. 2013). *Codium tomentosum* has a longer production cycle and the levels of crude protein decrease from fall to summer (21% to 12% dw) encompassing the reported value of 19% crude protein in this species (Rodrigues et al. 2015), which could be explained by the use of reserve nitrogen for active growth during spring and summer.

Because of their substantial amount of protein, *Ulva* spp. have been studied for human and animal nutrition, either directly or as protein concentrates (Wong and Cheung 2001; Angell et al. 2016). A significant portion (30 to 65%) of the protein found in the photosynthetic tissues of primary producers, including macroalgae, is RuBisCO. Like storage proteins, RuBisCO is considered a complete protein with a balanced proportion of all essential amino acids. However, its digestibility in whole macroalgae may be limited and the potential use of protein concentrates in food products has been limited by difficulties of efficiently purifying them on a large scale in a cost-effective form that is safe for human consumption (Wong and Cheung 2001; Grácio et al. 2023). Even for monogastric livestock, using whole macroalgae as a protein source negatively affects animal growth, compared with traditional feedstuffs (Angell et al. 2016). Among other factors macroalgae proteins are too diluted by fiber and ash, the former being considered an antinutritional factor (Fleurence et al. 1999; Angell et al. 2016). Notably, Bikker and colleagues (2016) have determined a 20% increase in protein digestibility if a protein-rich extract is efficiently separated from the algae polysaccharides. Nevertheless, up to 10% *Ulva* meal was tested in Nile tilapia diets without compromising growth performance, protein utilization, and protein retention in juveniles (Marinho et al. 2013).

Total lipid

The lipid content of macroalgae is generally low, although some variation occurs, driven by environmental and developmental conditions (Holdt and Kraan 2011; Bikker et al. 2016; Rey et al. 2020; Queirós et al. 2021). In *U. lacinulata* the lipid content ranged from 1.3–4.9% dw, peaking in the spring (Table 2). In *C. tomentosum* the lipid content was relatively stable and reached higher levels (5.8–6.7% dw), also peaking in spring. Results agree with reported values for *Ulva* (3.1% fat; Khotimchenko et al. 2002) but are slightly higher than the ones reported for *Codium* (4.7–4.8% fat; Rey et al. 2020). SFA represent between 54–64% of total fatty acids in *U. lacinulata*, MUFA levels varied between 20–32%, while PUFA varied between 14–21%, a profile like the one described for *U. lactuca* (Bikker et al. 2016). In *C. tomentosum* 39–51% of total fatty acids were SFA, 4–29% were MUFA and 29–45 % were PUFA, with the highest PUFA levels found in summer. Comparing the two green macroalgae, *C. tomentosum* contained a lower proportion of SFA and was richer in PUFA than *U. lacinulata*. The levels and profile of fatty acids indicate that *C. tomentosum* is probably a better source of fatty acids for food and feed than *U. lacinulata.*

Fiber

Macroalgae have a high content of structural polysaccharides in their cell walls with an extensive intercellular matrix. Most of these polysaccharides are not digested by human endogenous enzymes forming a large pool of dietary fiber. In *U. lacinulata*, the amount of fiber was relatively constant, around 40% throughout the year, which is in accordance with the literature. *Ulva* spp. are reported to contain 30–40% total dietary fiber, of which 55% is soluble and 45% is insoluble (Lahaye 1991; Bobin-Dubigeon et al. 1997). Fiber in *C. tomentosum* varied from 19% in the spring/winter to 31% in the fall, which is lower than reported in the literature (around 38% (Echave et al. 2021)).

Fiber in *Ulva* spp. is expected to be poorly fermented by human colonic bacteria and to be resistant to colonic bacterial fermentation (Bobin-Dubigeon et al. 1997; Holdt and Kraan 2011). However, it has been postulated that in regular consumers of raw or lightly processed macroalgae human gut symbionts have acquired enzymes active in sulphated polysaccharides (most probably by horizontal gene transfer from marine bacteria) which may increase the dietary value of this species (Hehemann et al. 2010; Li et al. 2017).

Sulphated polysaccharides from green macroalgae are partially reminiscent of mucopolysaccharides present in every mammalian tissue and are regarded as safer and non-immunogenic raw materials with potential applications in drug delivery or tissue engineering (Tziveleka et al. 2019). For example, partial enzymatic depolymerization of ulvans originates sulphated oligosaccharides that mimic human endogenous sugars (Coste et al. 2015).

Minerals

Macroalgae uptake and accumulate minerals from their habitat, showing high contents of sodium, magnesium, potassium, calcium, iron, and specifically iodine compared with land plants (Mabeau and Fleurence 1993; Meinita et al.

2022). They can also accumulate hazardous elements in concentrations potentially toxic to consumers. The amounts accumulated depend, among other factors, on the surrounding environment, and local variations should always be assessed. Among hazardous elements accumulated by green macroalgae arsenic, cadmium, lead, and mercury are of concern and must be closely monitored if the macroalgae are intended for feed or food (ANSES 2017; Commission Regulation (EU) 2023/915; Directive 2002/32/EC; Superior Health Council No. 9149 (2015)).

The richness in minerals of the species under study can be inferred from ash contents that ranged from 26–32% in *U. lacinulata* and 35–56% in *C. tomentosum* (Table 2). The ash content in *U. lacinulata* peaked in spring, which agrees with the tendency described for other macroalgae (Holdt and Kraan 2011), while in *C. tomentosum* it peaked in summer. The results agree with reported values (20–26% for *Ulva* spp. (Bobin-Dubigeon et al. 1997) and 36% for *C. tomentosum* (Rodrigues et al. 2015)).

Concerning individual compounds, the two macroalgae can be considered a good source of important micronutrients such as calcium and magnesium. The levels of calcium were higher in *C. tomentosum* (6.1–7.9 g kg^{-1} dw) than in *U. lacinulata* (4.1–7.1 g kg^{-1} dw) and are consistent with values previously reported (Echave et al. 2021; Queirós et al. 2021). On the other hand, magnesium was higher in *U. lacinulata* (28–48 g kg^{-1} dw) than in *C. tomentosum* (13–15 g kg^{-1} dw) agreeing with reported values (Echave et al. 2021; Queirós et al. 2021). It has already been acknowledged that *Ulva* spp are particularly rich in magnesium used by the macroalgae in catalytic functions (2–5.2% of dry matter; Mabeau and Fleurence 1993).

Macroalgae also accumulate high levels of iodine that can lead to dangerously excessive iodine intakes and may cause hyperthyroidism (Nitschke and Stengel 2015; Commission Recommendation (EU) 2018/464). Iodine concentration varies seasonally, peaking in the winter (Nitschke and Stengel 2015). The amounts determined in *U. lacinulata* from summer to winter (29 to 43 mg kg^{-1} dw) are slightly lower than the mean value previously reported by other authors (around 66 mg kg^{-1} dw; Romarís–Hortas et al. 2011). A very significant seasonal variation in iodine content was observed in *C. tomentosum,* reaching 105 mg kg^{-1} dw in the fall, almost 10 times the concentration determined in the spring/summer. Intermediate values of iodine (42 mg kg^{-1}) were reported for this species (Soares et al. 2021). As iodine can pose health problems if ingested in high levels, daily consumption should not exceed 1–14 g dw (14–188 g fresh weight) of *C. tomentosum* and 3–5 g dw (20–33 g fresh weight) of *U. lacinulata*, depending on season, considering the recommended 150 µg daily intake of iodine for an adult (Bouga & Combet 2015). Noteworthy, a simple 30-second water blanching at 45ºC was reported to effectively reduce the iodine content of iodine-rich macroalga sugar kelp (*Saccharina latissima*) by 88%, together with its overall ash content (Nielsen et al. 2020).

Arsenic is another mineral of concern in macroalgae intended for food and feed (Directive 2002/32/EC of the European Parliament and of the Council; Superior Health Council No. 9149 (2015)). Arsenic can be concentrated to several mg kg^{-1} dw from seawater where it is ubiquitously present at low concentrations (1 to 5 µg L^{-1}) (Morita and Shibata 1990; Lide 2005; Taylor and Jackson 2016). European regulations impose a limit of 40 mg kg^{-1} arsenic on animal feed derived from macroalgae (feed with a moisture content of 12%) from which inorganic arsenic should not exceed 2 mg kg^{-1}. A maximum level of 3 mg kg^{-1} dw of inorganic arsenic is recommended for edible macroalgae sold in France (ANSES 2017).

The levels of total arsenic found in *U. lacinulata* (3.2–7.2 mg kg^{-1} dw) agree with the values previously published (3.1 mg kg^{-1} dw; Díaz et al. 2012), the highest value being within the range of arsenic reported in other *Ulva* species (4.1 to 14.7 mg kg^{-1} dw; Taylor and Jackson 2016). The arsenic determined in *C. tomentosum* (14 to 30 mg kg^{-1} dw) is slightly lower than that measured in *C. tomentosum* extracts obtained by subcritical water extraction (47 mg kg^{-1}; Soares et al. 2021). As expected, the highest concentrations of arsenic occur in fall/winter, when macroalgae growth rates are slower increasing the arsenic/biomass ratio (Besada et al. 2009). This can also explain the lower levels of arsenic found in *U. lacinulata,* a species with faster growth rates and a shorter production cycle than *C. tomentosum*.

The toxicity of arsenic depends on its molecular nature, with inorganic arsenic considered the most toxic (Almela et al. 2006; Feldmann and Krupp 2011; Taylor and Jackson 2016). However, in algae and other marine organisms, inorganic arsenic usually represents a small proportion of total arsenic (Feldmann and Krupp 2011; Taylor and Jackson 2016). All samples analyzed herein contained inorganic arsenic below 1 mg kg^{-1} dw, always representing less than 20% of total arsenic: in *U. lacinulata* this proportion ranged between 1.1 and 17.6%, and in *C. tomentosum* between 0.5 and 2.2%. The lowest levels of inorganic arsenic (absolute value and proportion) were observed during spring/summer. Besides season, many other factors may contribute to fluctuation in the concentration of inorganic arsenic including an increase in inorganic arsenic with storage time and "age" of the macroalgae at harvesting (Taylor and Jackson 2016). The concentration of total and inorganic arsenic measured in both species at different seasons were below maximum values defined by EU regulations on animal feed, as well as existing recommendations for human food (Directive 2002/32/EC of the European Parliament and of the Council; ANSES 2017). Additionally, it should be noted that arsenic was determined in fresh material, but macroalgae sold for

food are generally dried, and soaking is advised before consumption. This procedure alone can significantly reduce the amount of organic and inorganic arsenic (3 to 12 times) prior to consumption (Rose et al. 2007). The molecular nature is an important factor for arsenic toxicity. In marine organisms, it ranges from highly toxic inorganic species to the non-toxic arsenobetaine found in fish, with several organo-arsenic compounds with variable degrees of toxicity in between. The described profile of arsenic in green macroalgae includes low levels of inorganic arsenic (Almela et al. 2006; Besada et al. 2009) and substantial amounts of arsenosugars which are potentially toxic forms of arsenic (Morita and Shibata 1990; Feldmann and Krupp 2011). Arsenosugars have low but significant bioavailability in humans and some of their metabolites are cytotoxic (Leffers et al. 2013; Taylor and Jackson 2016).

Concerning other hazardous elements, the levels of cadmium, lead, and mercury determined in the two green macroalgae were below maximum levels defined by EU regulations for food supplements (3.0 mg kg^{-1} dw for cadmium; 3.0 mg kg^{-1} dw for lead and 0.1 mg kg^{-1} dw for mercury; Commission Regulation (EU) 2023/915) and feed (1 mg kg^{-1} dw for cadmium; 15 mg kg^{-1} dw for lead and 0.1 mg kg^{-1} dw for mercury; Directive 2002/32/EC of the European Parliament and of the Council). However, these levels may pose some toxicity risks in the case of long-term daily consumption of macroalgae-based meals (Superior Health Council No. 9149, 2015).

Antioxidant potential

The ability of organisms to cope with oxidative stress can be measured in the laboratory using prototype radicals, to simulate reactive species that cells must deal with. Among them, the 'stable' radicals DPPH and $ABTS^{+\bullet}$ as well as superoxide anion ($O_2^{\bullet-}$) and nitric oxide ($^{\bullet}NO$), the precursors of various reactive species, are commonly used to screen the antioxidant potential of natural extracts (Baliga et al. 2003; Müller et al. 2011; Morone et al. 2022). The extracts of *U. lacinulata* and *C. tomentosum* displayed some antioxidant activity against the four radicals tested allowing the calculation of IC_{25} and/or IC_{50}. The antioxidant potential of *U. lacinulata* against $ABTS^{+\bullet}$ and DPPH, and *C. tomentosum* against $ABTS^{+\bullet}$ and $^{\bullet}NO$ was generally better in the summer than in the other seasons. Comparing the IC_{25}, *U. lacinulata* always demonstrated a better antioxidant potential against $ABTS^{+\bullet}$ and $O_2^{\bullet-}$ than *C. tomentosum*, but for $^{\bullet}NO$ and DPPH the results were season-dependent. These results can be partly explained by the chemical composition of the macroalgal extracts. The increase in antioxidant potential correlates with the increase in the phytochemicals quantified, namely carotenoids, which are efficient $ABTS^{\bullet+}$ scavengers (Müller et al. 2011). Comparing the two macroalgae, *U. lacinulata* contains more carotenoids than *C. tomentosum* and has a better antioxidant potential against $ABTS^{\bullet+}$. The better antioxidant potential of *U. lacinulata* against $O_2^{\bullet-}$ can be explained by the presence of ulvan, a sulphated polysaccharide that is reported to have good superoxide scavenging activities (Qi et al. 2005), although, in this study, no correlation could be established between the amount of fiber found in *U. lacinulata* and the $O_2^{\bullet-}$ scavenging activity.

Conclusions

Both species of macroalgae contain high levels of protein, fiber and minerals. The protein, fiber and ash content of *U. lacinulata* was relatively stable throughout the year compared to *C. tomentosum*. The fatty acid composition indicates that *C. tomentosum* is a better source of fatty acids, while *U. lacinulata* seems to have a higher antioxidant potential. The two species can also be considered a good source of important micronutrients such as calcium and magnesium. The levels of hazardous compounds across all seasons were below the limits set by existing recommendations and regulations, but should be closely monitored. New regulations specific to macroalgae are expected as a result of the increasing consumption of macroalgae products in the EU. According to current knowledge, and existing recommendations and regulations, both species seem to have good potential as sea vegetables.

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Data availability Data is provided within the manuscript or supplementary information files.

Declarations

Competing interest Inês Oliveira is employed by ALGAplus SA, a producer and supplier of seaweed and derived products.

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References

Acker JG, Leptoukh G (2007) Online analysis enhances use of NASA Earth science data. Eos Trans AGU 88:14–17

Almela C, Clemente MJ, Vélez D, Montoro R (2006) Total arsenic, inorganic arsenic, lead and cadmium contents in edible seaweed sold in Spain. Food Chem Toxicol 44:1901–1908

Anderson JM (1983) Chlorophyll-protein complexes of a *Codium* species, including a light-harvesting siphonaxanthin-Chlorophylla ab-protein complex, an evolutionary relic of some Chlorophyta. Biochim Biophys Acta 724:370–380

Anderson M, Gorley R, Clarke K (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth, p 214

Angell AR, Angell SF, de Nys R, Paul NA (2016) Seaweed as a protein source for mono-gastric livestock. Trends Food Sci Technol 54:74–84

ANSES (2017) Opinion request No 2017-SA-0086 de l'Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (France) relatif au risque d'excès d'apport en iode lié à la consommation d'algues dans les denrées alimentaires. https://www.anses.fr/fr/system/files/NUT2017SA0086EN.pdf. Accessed 08/02/2024

Astorga-España MS, Rodríguez-Galdón B, Rodríguez-Rodríguez EM, Díaz-Romero C (2016) Amino acid content in seaweeds from the Magellan Straits (Chile). J Food Compos Anal 53:77–84

Badmu UO, Taggart MA, Elbourne P, Sterk HP, Boyd KG (2022) Effect of long-term storage and harvest site on the fatty acid profiles, mineral and antioxidant properties of selected edible Scottish seaweeds. Food Chem 377:131955

Baliga MS, Jagetia GC, Rao SK, Babu K (2003) Evaluation of nitric oxide scavenging activity of certain spices in vitro: a preliminary study. Nahrung 47:261–264

Barbier M, Charrier B, Araujo R, Holdt SL, Jacquemin B, Rebours C (2019) PEGASUS - PHYCOMORPH European Guidelines for a sustainable aquaculture of seaweeds. COST Action FA1406, Roscoff, France. Bot Mar 63:5–16

Beaumont M, Tran R, Vera G, Niedrist D, Rousset A, Pierre R, Shastri VP, Forget A (2021) Hydrogel-forming algae polysaccharides: from seaweed to biomedical applications. Biomacromolecules 22:1027–1052

Besada V, Andrade JM, Schultze F, González JJ (2009) Heavy metals in edible seaweeds commercialised for human consumption. J Mar Syst 75:305–313

Bhat I, Haripriya G, Jogi N, Mamatha BS (2021) Carotenoid composition of locally found seaweeds of Dakshina Kannada district in India. Algal Res 53:102154

Bikker P, van Krimpen MM, van Wikselaar P, Houweling-Tan B, Scaccia N, van Hal JW, Huijgen WJ, Cone JW, López-Contreras AM (2016) Biorefinery of the green seaweed *Ulva lactuca* to produce animal feed, chemicals and biofuels. J Appl Phycol 28:3511–3525

Bobin-Dubigeon C, Lahaye M, Barry J-L (1997) Human colonic bacterial degradability of dietary fibres from sea-lettuce (*Ulva* sp). J Sci Food Agric 73:149–159

Bouga M, Combet E (2015) Emergence of seaweed and seaweed-containing foods in the UK: Focus on labeling, iodine content, toxicity and nutrition. Foods 4:240–253

Braga AFM, Lens PNL (2023) Pretreatment optimisation of *Ulva* spp. for production of bio H_2 and lactic acid as added-value product. Int J Hydrog Energy 48:33466–33482

Celikler S, Vatan O, Yildiz G, Bilaloglu R (2009) Evaluation of anti-oxidative, genotoxic and antigenotoxic potency of *Codium tomentosum* Stackhouse ethanolic extract in human lymphocytes in vitro. Food Chem Toxicol 47:796–801

Coleman MA (2023) Algae in the anthropocene: Managing, conserving, and utilizing algae in an era of rapid environmental change. J Phycol 60:1–3

Collén J, Del Rio MJ, García-Reina G, Pedersén M (1995) Photosynthetic production of hydrogen peroxide by *Ulva rigida* C. Ag. (Chlorophyta). Planta 196:225–230

Commission Recommendation (EU) 2018/464 on the monitoring of metals and iodine in seaweed, halophytes and products based on seaweed. Off J Eur Union L 78/16. http://data.europa.eu/eli/reco/2018/464/oj. Accessed 08/02/2024

Commission Regulation (EU) 2023/915 on maximum levels for certain contaminants in food and repealing Regulation (EC) No 1881/2006. Off J Eur Union L 119/103. http://data.europa.eu/eli/reg/2023/915/oj

Connan S (2015) Spectrophotometric assays of major compounds extracted from algae. Meth Mol Biol 1308:75–101

Coste O, Malta E-J, López JC, Fernández-Díaz C (2015) Production of sulfated oligosaccharides from the seaweed *Ulva* sp. using a new ulvan-degrading enzymatic bacterial crude extract. Algal Res 39:101422

Cotas J, Leandro A, Monteiro P, Pacheco D, Figueirinha A, Gonçalves AMM, da Silva GJ, Pereira L (2020) Seaweed phenolics: from extraction to applications. Mar Drugs 18:384

Díaz O, Tapia Y, Muñoz O, Montoro R, Velez D, Almela C (2012) Total and inorganic arsenic concentrations in different species of economically important algae harvested from coastal zones of Chile. Food Chem Toxicol 50:744–749

Directive (2002) 2002/32/EC of the European Parliament and of the council on undesirable substances in animal feed - Council statement. http://data.europa.eu/eli/dir/2002/32/oj. Accessed 08/02/2024

Dong H, Dong S, Hansen EP, Stagos D, Lin X, Liu M (2020) Progress of bromophenols in marine algae from 2011 to 2020: Structure, bioactivities, and applications. Mar Drugs 18:411

Echave J, Lourenço-Lopes C, Carreira-Casais A, Rivo FN, Otero P, García-Pérez P, Baamonde S, Saa F, Cao H, Xiao J, Prieto M, Simal-Gandara J (2021) Nutritional composition of the Atlantic seaweeds *Ulva rigida, Codium tomentosum, Palmaria palmata* and *Porphyra purpurea*. Chem Proc 5:67

Eismann AI, Reis RP, da Silva AF, Cavalcanti DN (2020) *Ulva* spp. carotenoids: Responses to environmental conditions. Algal Res 48:101916

Feldmann J, Krupp EM (2011) Critical review or scientific opinion paper: Arsenosugars-a class of benign arsenic species or justification for developing partly speciated arsenic fractionation in foodstuffs? Anal Bioanal Chem 399:1735–1741

Fernández PV, Arata PX, Ciancia M (2014) Polysaccharides from *Codium* species: Chemical structure and biological activity. Their role as components of the cell wall. Adv Bot Res 71:253–278

Fleurence J, Chenard E, Luçcon M (1999) Determination of the nutritional value of proteins obtained from *Ulva armoricana*. J Appl Phycol 11:231–239

Flodin C, Helidoniotis F, Whitfield FB (1999) Seasonal variation in bromophenol content and bromoperoxidase activity in *Ulva lactuca*. Phytochemistry 51:135

Grácio M, Oliveira S, Lima A, Ferreira RB (2023) RuBisCO as a protein source for potential food applications: a review. Food Chem 419:135993

Hehemann JH, Correc G, Barbeyron T, Helbert W, Czjzek M, Michel G (2010) Transfer of carbohydrate-active enzymes from marine bacteria to Japanese gut microbiota. Nature 464:908–912

Holdt SL, Kraan S (2011) Bioactive compounds in seaweed: functional food applications and legislation. J Appl Phycol 23:54

Huang H, Ullah F, Zhou D-X, Yi M, Zhao Y (2019) Mechanisms of ROS regulation of plant development and stress responses. Front Plant Sci 10:800

Hurd CL, Gattuso J-P, Boyd PW (2023) Air-sea carbon dioxide equilibrium: Will it be possible to use seaweeds for carbon removal offsets? J Phycol 58:347–363

ISO (1999a) Animal feeding stuffs – Determination of moisture and other volatile matter content (ISO 6496:1999). International Organization for Standardization

ISO (1999b) Animal feeding stuffs – Determination of fat content (ISO 6492:1999). International Organization for Standardization

ISO (2002) Animal feeding stuffs – Determination of crude ash (ISO 5984:2002). International Organization for Standardization

ISO (2008) Food products – Determination of the total nitrogen content by combustion method – Part 1: Method for general analysis (ISO 16634-1:2008). International Organization for Standardization

Jesus A, Correia-da-Silva M, Afonso C, Pinto M, Cidade H (2019) Isolation and potential biological applications of haloaryl secondary metabolites from macroalgae. Mar Drugs 17:73

Khotimchenko SV, Vaskovsky VE, Titlyanova TV (2002) Fatty acids of marine algae from the Pacific Coast of North California. Bot Mar 45:17–22

Lahaye M (1991) Marine algae as sources of fibres: Determination of soluble and insoluble dietary fibre contents in some 'sea vegetables.' J Sci Food Agric 54:587–594

Lai YC, Tsai YC, Shin YN, Chou YC, Shen YR, Lin NC, Chang SH, Kao YM, Tseng SH, Wang DY (2022) Inorganic arsenic speciation analysis in food using HPLC/ICP-MS: Method development and validation. J Food Drug Anal 30:644–653

Leffers L, Ebert F, Taleshi MS, Francesconi KA, Schwerdtle T (2013) In vitro toxicological characterization of two arsenosugars and their metabolites. Mol Nutr Food Res 57:1270–1282

Li M, Shang Q, Li G, Wang X, Yu G (2017) Degradation of marine algae-derived carbohydrates by Bacteroidetes isolated from human gut microbiota. Mar Drugs 15:92

Lichtenthaler HK (1987) Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Meth Enzymol 148:350–382

Lide DR (2005) Geophysics, astronomy, and acoustics; abundance of elements in the earth's crust and in the sea. CRC Handbook of Chemistry and Physics, 85th edn. CRC Press, Boca Raton

Liu M, Hansen PE, Lin X (2011) Bromophenols in marine algae and their bioactivities. Mar Drugs 9:1273–1292

Mabeau S, Fleurence J (1993) Seaweed in food products: biochemical and nutritional aspects. Trends Food Sci Technol 4:103–107

Mallick N, Mohn FH (2000) Reactive oxygen species: response of algal cells. J Plant Physiol 157:183–193

Mantri VA, Kazi MA, Balar NB, Gupta V, Gajaria T (2020) Concise review of green algal genus *Ulva* Linnaeus. J Appl Phycol 32:2725–2741

Margareta W, Nagarajan D, Chang J-S, Lee D-J (2020) Dark fermentative hydrogen production using macroalgae (*Ulva* sp) as the renewable feedstock. Appl Energy 381:133404

Marinho G, Nunes C, Sousa-Pinto I, Pereira R, Rema P, Valente LMP (2013) The IMTA-cultivated Chlorophyta *Ulva* spp. as a sustainable ingredient in Nile tilapia (*Oreochromis niloticus*) diets. J Appl Phycol 25:1359–1367

Marques R, Cruz S, Calado R, Lillebø A, Abreu H, Pereira R, Pitarma B, da Silva JM, Cartaxana P (2021) Effects of photoperiod and light spectra on growth and pigment composition of the green macroalga *Codium tomentosum*. J Appl Phycol 33:471–480

Martins M, Fernandes APM, Torres-Acosta MA, Collén PN, Abreu MH, Ventura SPM (2021) Extraction of chlorophyll from wild and farmed *Ulva* spp. using aqueous solutions of ionic liquids. Sep Purif Technol 254:117589

Meinita MDN, Harwanto D, Choi JS (2022) A concise review of the bioactivity and pharmacological properties of the genus *Codium* (Bryopsidales, Chlorophyta). J. Appl. Phycol. 34:2827–2845

Molyneux P (2004) The use of the stable radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol 26:211–219

Morita M, Shibata Y (1990) Chemical form of arsenic in marine macroalgae. Appl Organometal Chem 4:181–190

Morone J, Lopes G, Morais J, Neves J, Vasconcelos V, Martins R (2022) Cosmetic application of cyanobacteria extracts with a sustainable vision to skincare: Role in the antioxidant and antiaging process. Mar Drugs 20:761

Müller L, Fröhlich K, Böhm V (2011) Comparative antioxidant activities of carotenoids measured by ferric reducing antioxidant power (FRAP), ABTS bleaching assay (αTEAC), DPPH assay and peroxyl radical scavenging assay. Food Chem 129:139–148

MODIS (2022) NASA Goddard Space Flight Center, Ocean Ecology Laboratory, Ocean Biology Processing Group. Moderate-resolution Imaging Spectroradiometer (MODIS) Aqua Photosynthetically Available Radiation Data, Version 2022; NASA OB.DAAC, Greenbelt, MD, USA. Accessed 10 Oct 2024

NEN-EN (2010) Foodstuffs – Determination of trace elements – Determination of arsenic, cadmium, mercury, and lead in foodstuffs by inductively coupled plasma mass spectrometry (ICP-MS) after pressure digestion (NEN-EN 15763:2010). Netherlands Standardization Institute (NEN)

Nielsen CW, Holdt SL, Sloth JJ, Marinho GS, Sæther M, Funderud J, Rustad T (2020) Reducing the high iodine content of *Saccharina latissima* and improving the profile of other valuable compounds by water blanching. Foods 9:569

Nitschke U, Stengel DB (2015) A new HPLC method for the detection of iodine applied to natural samples of edible seaweeds and commercial seaweed food products. Food Chem 172:326–334

Pappou S, Dardavila MM, Savvidou MG, Louli V, Magoulas K, Voutsas E (2022) Extraction of bioactive compounds from *Ulva lactuca*. Appl Sci 12:2117

Pinheiro VF, Marçal C, Abreu H, da Silva JAL, Silva AMS, Cardoso SM (2019) Physicochemical changes of air-dried and salt-processed *Ulva rigida* over storage time. Molecules 24:2955

Pizzolla PF (2007) *Codium tomentosum* Velvet horn. In: Tyler-Walters H, Hiscock K (eds) Marine Life Information Network: Biology and Sensitivity Key Information Reviews. Marine Biological Association of the United Kingdom, Plymouth. https://www.marlin.ac.uk/species/detail/1474. Accessed 26 Jan 2024

Qi H, Zhao T, Zhang Q, Li Z, Zhao Z, Xing R (2005) Antioxidant activity of different molecular weight sulfated polysaccharides from *Ulva pertusa* Kjellm (Chlorophyta). J Appl Phycol 17:527–534

Queirós AS, Circuncisão AR, Pereira E, Válega M, Abreu MH, Silva AMS, Cardoso SM (2021) Valuable nutrients from *Ulva rigida*: modulation by seasonal and cultivation factors. Appl Sci 11:6137

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999) Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med 26:1231–1237

Rey F, Cartaxana P, Melo T, Calado R, Pereira R, Abreu H, Domingues P, Cruz S, Domingues MR (2020) Domesticated populations of *Codium tomentosum* display lipid extracts with lower seasonal shifts than conspecifics from the wild—Relevance for biotechnological applications of this green seaweed. Mar Drugs 18:188

Ritchie RJ (2006) Consistent sets of spectrophotometric chlorophyll equations for acetone, methanol and ethanol solvents. Photosynth Res 89:27–41

Rodrigues D, Freitas AC, Pereira L, Rocha-Santos TA, Vasconcelos MW, Roriz M, Rodríguez-Alcalá LM, Gomes AM, Duarte AC (2015) Chemical composition of red, brown and green macroalgae from Buarcos bay in Central West Coast of Portugal. Food Chem 183:197–207

Romarís-Hortas V, García-Sartal C, Barciela-Alonso MDC, Domínguez-González R, Moreda-Piñeiro A, Bermejo-Barrera P (2011) Bioavailability study using an in-vitro method of iodine and bromine in edible seaweed. Food Chem 124:1747–1752

Rose M, Lewis J, Langford N, Baxter M, Origgi S, Barber M, MacBain H, Thomas K (2007) Arsenic in seaweed—Forms, concentration and dietary exposure. Food Chem Toxicol 45:1263–1267

Shuuluka D, Bolton JJ, Anderson RJ (2013) Protein content, amino acid composition and nitrogen-to-protein conversion factors of *Ulva rigida* and *Ulva capensis* from natural populations and *Ulva lactuca* from an aquaculture system, in South Africa. J Appl Phycol 28:511–524

Soares C, Paíga P, Marques M, Neto T, Carvalho AP, Paiva A, Simões P, Costa L, Bernardo A, Fernández N, Pinto E, Almeida A, Bronze MR, Delerue-Matos C, Grosso C (2021) Multi-step subcritical water extracts of *Fucus vesiculosus* L. and *Codium tomentosum* Stackhouse: Composition, health-benefits and safety. Processes 9:893

Stahl W, Sies H (2003) Antioxidant activity of carotenoids. Mol Aspects Med 24:345–351

Strain HH, Cope BT, Svec WA (1971) Analytical procedures for the isolation, identification, estimation, and investigation of the chlorophylls. Meth Enzymol 23:452–476

Superior Health Council No. 9149 (2015) on Arsenic and other elements in algae and dietary supplements based on algae. https://www.hgr-css.be/en/report/9149/arsenic. Accessed 08/02/2024

Taylor VF, Jackson BP (2016) Concentrations and speciation of arsenic in New England seaweed species harvested for food and agriculture. Chemosphere 163:6–13

Trigui M, Gasmi L, Zouari I, Tounsi S (2013) Seasonal variation in phenolic composition, antibacterial and antioxidant activities of *Ulva rigida* (Chlorophyta) and assessment of antiacetylcholinesterase potential. J Appl Phycol 25:319–328

Tziveleka LA, Ioannou E, Roussis V (2019) Ulvan, a bioactive marine sulphated polysaccharide as a key constituent of hybrid biomaterials: A review. Carbohydr Polym 218:355–370

Whitfield FB, Helidoniotis F, Shaw KJ, Svoronos D (1999) Distribution of bromophenols in species of marine algae from eastern Australia. J Agric Food Chem 47:2367–2373

Wong KH, Cheung PCK (2001) Nutritional evaluation of some subtropical red and green seaweeds Part II. In vitro protein digestibility and amino acid profiles of protein concentrates. Food Chem 72:11–17

Xie J, Schaich KM (2014) Re-evaluation of the 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) assay for antioxidant activity. J Agric Food Chem 62:4251–4260

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