Research Article

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Lipid characterization of 14 macroalgal species from Madeira Archipelago: implications for animal and human nutrition

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Abstract: The lipid and fatty acid profiles of 14 marine macroalgal species from the Madeira Archipelago, including two green (Ulvales and Dasycladales), three red (Corallinales, Bonnemaisoniales, and Ceramiales) and nine brown (Fucales, Dictyotales, and Sphacelariales) species were characterised in order to determine their potential use for animal and human nutrition. The total lipid content of species analysed was generally low, varying from 0.2 to 5.2% of dry weight. All species presented an omega 6/omega 3 (n-6/n-3) ratio lower than 10, as recommended by the World Health Organization for proper human health. Polyunsaturated fatty acids (PUFA), including linoleic acid and alpha-linolenic acid were exceptionally high in the green macroalga *Ulva* sp. Red macroalgae were rich in n-3 long-chain PUFA, particularly *Asparagopsis taxiformis*, which contained 6.6% of docosahexaenoic acid, and *Halopithys incurva* with 9.3% of eicosapentaenoic acid. Within Ochrophyta, *Dictyota dichotoma* is an interesting source of n-3 PUFA due to its high stearidonic acid proportion (8.0%). In addition, *H. incurva* contained a high proportion of both mono- and digalactosyldiacylglycerols. According to their lipid profiles, most macroalgae analysed might be considered of particular interest for their potential exploitation for human nutrition and livestock and aquaculture production.

Keywords: lipid profile; macroalgae; nutrition; polyunsaturated fatty acids.

1 Introduction

Marine macroalgae are fast-growing multicellular, photosynthetic organisms, classified into three major groups based on their pigmentation: green macroalgae (Chlorophyta), red macroalgae (Rhodophyta) and brown macroalgae (Ochrophyta). Seaweeds are traditionally consumed as food in Asia, mainly Japan, China and Korea (Roleda et al. 2018). However, their demand as food has also extended to occidental societies, mainly due to a change in consumer preferences, being increasingly recognized as a type of healthy "superfood" that

leads to the production of algal-derived innovative products (FAO 2018a). Marine macroalgae are rich in nutritional and bioactive compounds, including minerals such as iodine, fibre, vitamins, carbohydrates, proteins, lipids, chiefly polyunsaturated fatty acids (PUFA), phytosterols (PTS), and phenolic compounds. Seaweeds also possess high contents of pigments that exert antioxidant and anticancer activities (Chandini et al. 2008; Nunes et al. 2020; Stengel et al. 2011; Zárate et al. 2020). In addition, several other compounds of macroalgae are described to have potential medical applications, including antitumor, anticoagulant, antiviral, antiprotozoal, antifungal, and antibacterial properties (for details, see Barzkar et al. 2019).

Given the versatility of seaweeds for their application in industries, their global production is expected to increase in the coming decades. Seaweed aquaculture has been practiced for decades in Asian countries (Campbell et al. 2019), especially in China, the main world producer in both value and volume (FAO 2018a). Farmed macroalgae represent 96% of the total global supply (Harwood 2019), and this is almost exclusively used for direct human consumption (FAO 2018a, b). In Western countries, seaweeds are mainly exploited for the industrial production of phycocolloids such as alginate, agar-agar and carrageenan (Dellatorre et al. 2020), although its production by aquaculture has been recently established as a commercial activity (Campbell et al. 2019).

Seaweeds are a promising protein source, presenting a higher content of essential amino acids than vegetables (Fleurence 1999). In this regard, seaweed consumption is expected to increase due to the growing demand for protein sources that can overcome the anticipated challenges of a growing world population and food scarcity, and the demand for alternative proteins in Western countries. In recent years, lipid composition of macroalgae has also raised considerable interest due to their valuable content of omega-3 (n-3) PUFA and of a certain type of lipids. In general, marine macroalgae have low lipid levels (<5% of dry weight, DW) (Dellatorre et al. 2020; Schmid et al. 2018), and fluctuating fatty acid (FA) profiles, which vary greatly among taxa (Stengel et al. 2011). These variations have been attributed to several factors, including seasonal and geographical changes, environmental parameters, physiological status, and even molecular mechanisms in response to environmental factors (Verma et al. 2017).

Glycolipids and phospholipids are the major lipid classes in algae (Guihéneuf et al. 2015), together with triacylglycerols (TAG) (Harwood 2019). Glycolipids are mainly located in photosynthetic membranes playing a crucial role in maintaining optimal photosynthesis efficiency (Nakamura and Li-Beisson 2016), and are predominantly represented by monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG). Furthermore, major phospholipids are phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylglycerol (Guihéneuf et al. 2015), which are mostly localized in non-plastid membranes, except for the latter which is present in the chloroplast envelope (Nakamura and Li-Beisson 2016). MGDG, DGDG or phosphatidylglycerol have been described as anti-inflammatory and anti-thrombotic compounds, while PTS are known to lower total and low-density lipoprotein cholesterol levels in humans (Ibañez and Cifuentes 2013). Despite the low lipid level reported in macroalgae (Dellatorre et al. 2020; Schmid et al. 2018), their PUFA content is greater than that of terrestrial plants (Kendel et al. 2015). Within PUFA, the long-chain PUFA (LC-PUFA) are physiologically important molecules (Trushenski and Rombenso 2019) involved in cell membrane structure, transcription, regulation and cellular signalling (Lee et al. 2016; Zárate et al. 2017). Furthermore, a high dietary intake of n-3 LC-PUFA has been shown to prevent some human diseases, including colon and breast cancers, neurodegenerative or inflammatory illnesses, and even to reduce the prevalence of dementia (Harwood 2019; Lee et al. 2016; Zárate et al. 2017). Particularly, eicosapentaenoic acid (EPA; 20:5n-3) and docosahexaenoic acid (DHA; 22:6n-3) have been demonstrated to reduce cardiovascular disease and arthritis, and to improve brain function (Harwood 2019). Consequently, global demand for n-3 FA has significantly increased over the last decades. Fish and other marine products are almost the only natural source of n-3 LC-PUFA for humans (Zárate et al. 2017). However, the source of these FA is generally not fish itself but marine phytoplankton and macroalgae, which form their major dietary source (Colombo et al. 2019). Algae possess not just the capacity to synthesize *de novo* alpha-linolenic acid (ALA; 18:3n-3) and linoleic acid (LA; 18:2n-6), but also LC-PUFA, whose content differs among taxa (Bourgougnon et al. 2011). To date, the feeding of aquatic captive-reared species has relied heavily on fishmeal and fish oils obtained from wild pelagic fish populations, whose stocks are currently either fully exploited or overexploited (FAO 2018b). Recently, various plant-based sources have been tested to replace marine ingredients due to their higher availability, sustainability and reduced cost. Nonetheless, terrestrial alternatives present low digestibility, contain some antinutritional factors, and are deficient in certain essential amino acids and n-3 LC-PUFA, resulting in a significant reduction of the nutritional quality of the edible product (Welker et al. 2016). By contrast, the inclusion of small amounts of macroalgae in aquafeeds seems to positively affect fish growth performance and feed efficiency due to their high nutritional value and balanced composition (Norambuena et al. 2015).

For all these reasons, a wide variety of seaweeds can be potentially exploited as a main source of n-3 LC-PUFA, not just for direct human consumption but also for animal feed production, offering a continuous and sustainable supply of these essential compounds and contributing to satisfying the world population's needs. The main objective of the present study was to broadly characterize the lipid and FA profiles of the still understudied, but most representative 14 macroalgal species from the Madeira Archipelago, in order to evaluate their potential as sources for both n-3 LC-PUFA and other healthy lipid molecules with marked anti-hypercholesterolemic and anti-hypertriglycerolemic properties for human and animal nutrition.

2 Materials and methods

2.1 Specimens of seaweeds

Single samples were collected from representative species of the Madeira Archipelago, including two green macroalgal species (Chlorophyta, Ulvophyceae) *Dasycladus vermicularis* (Scopoli) Krasser (Order Dasycladales) and *Ulva* sp. (Order Ulvales), three species of red macroalgae (Rhodophyta, Florideophyceae) *Corallina officinalis* Linnaeus (Order Corallinales), *Asparagopsis taxiformis* (Delile) Trevisan (Order Bonnemaisoniales) and *Halopithys incurva* (Hudson) Batters (Order Ceramiales), and nine species of brown macroalgae (Ochrophyta, Phaeophyceae) *Cystoseira compressa* (Esper) Gerloff *et* Nizamuddin (Order Fucales), *Cystoseira usneoides* (Linnaeus) M. Roberts (Order Fucales), *Cystoseira humilis* Schousboe *ex* Kützing (Order Fucales), *Sargassum vulgare* C. Agardh (Order Fucales), *Dictyota dichotoma* (Hudson) J.V. Lamouroux (Order Dictyotales), *Lobophora* J. Agardh sp. (Order Dictyotales), *Padina pavonica* (Linnaeus) Thivy (Order Dictyotales), *Halopteris filicina* (Grateloup) Kützing (Order Sphacelariales), and *Halopteris scoparia* (Linnaeus) Sauvageau (Order Sphacelariales), and were analysed in triplicate.

The seaweeds were haphazardly harvested, taking the entire algal thallus (between 0.5 and 1 kg) at a maximum depth of 10 m by free diving, from different beaches of the Madeira Archipelago including Madeira and Porto Santo islands (Portugal; Figure 1). The sampling was carried out from March to June 2017, when water temperature ranged from 18.5 to 21 °C. After collection, samples were transported to the laboratory in seawater, where they were gently washed with filtered freshwater, frozen at −35 °C and freeze-dried under reduced pressure (4×10^{-4} mbar) with a cooling trap (Scanvac Coolsafe Model 55-4, Labogene, Lynge, Denmark) set at −56 °C for five days. Lyophilized samples were later milled to 200 µm particle size in an electric mill (IKA Werke Model M20, Staufen, Germany), packed under vacuum with a vacuum sealer (AudionVac Model VMS 153, Derby, UK) and stored at −35 °C until biochemical analysis.

2.2 Lipid extraction

Total lipid (TL) was extracted using 10 ml of chloroform/methanol (2:1, v/v) per 100 mg sample, according to the method described by Folch et al. (1957) with small modifications (Christie and Han 2010). The lipid content was gravimetrically determined after evaporation of the organic solvent under a stream of nitrogen. TL extracts were stored at 10 mg ml^{-1} in chloroform/methanol (2:1, v/v) containing 0.01% (w/v) of butylated hydroxytoluene (Sigma-Aldrich Co., St. Louis, Missouri, USA) as an antioxidant, under an inert atmosphere of nitrogen at −20 °C.

2.3 Lipid classes and fatty acid composition

Lipid classes were separated by one-dimensional double-development high-performance thin-layer chromatography (HPTLC), using 1-propanol/chloroform/methyl acetate/methanol/0.25% potassium chloride (5:5:5:2:1.8, v/v) for polar lipids, and hexane/diethyl ether/acetic acid (20:5:0.5, v/v) for neutral lipids. Lipid classes were then quantified by calibrated densitometry using a dual-wavelength flying spot scanner CAMAG TLC Visualizer (Camag, Muttenz, Switzerland), as described by Reis et al. (2019). Lipid class identification was performed by comparison to external lipid standards (cod roe lipid extract; DGDG and SQDG (Avanti Polar Lipids, Inc., Alabaster, Alabama, USA)) placed on the same HPTLC plate (Supplementary Figure S1).

Fatty acid methyl esters (FAME) were obtained by acid-catalyzed transmethylation of 1 mg of lipid extracts using 1% sulphuric acid in methanol (v/v) for 16 h at 50 °C (Christie and Han 2010). FAME were purified by thin-layer chromatography (TLC) with hexane/diethyl ether/acetic acid (90:10:1, v/v) as solvents (Christie and Han 2010), and subsequently separated and quantified using a TRACE-GC Ultra gas chromatograph (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) equipped with an on-column injector, a flame ionization detector and a fused silica capillary column, Supelcowax TM 10 (30 m × 0.32 mm I.D. × 0.25 µm; Sigma-Aldrich Co., St. Louis, Missouri, USA). Helium was used as carrier gas and temperature programming was 50–150 °C at 40 °C min^{-1} slope, then from 150 to 200 °C at 2 °C min^{-1}, to 214 °C at 1 °C min^{-1} and, finally, up to 230 °C at 40 °C min^{-1}. Individual FAME were identified by reference to a mixture of authentic standards (Mix C4-C24 and PUFA No. Three from menhaden oil (Supelco Inc., Bellefonte, Pennsylvania, USA) and a well characterized cod roe oil (for details, see Supplementary Figure S2), and the identity of FAME confirmed, when necessary, by GC–MS (DSQ II, Thermo Scientific).

2.4 Nutritional indices

Nutritional quality of macroalgal FA composition was assessed by calculating atherogenicity and thrombogenicity indices following Cardoso et al. (2017) and the ratio between hypocholesterolemic and hypercholesteloremic FA as described by Santos-Silva et al. (2002):

$$\text{Atherogenicity index (AI)} = [(4 \times 14{:}\,0) + 16{:}\,0 + 18{:}\,0]/(\sum\text{MUFA} + \sum n-6\,\text{PUFA} + \sum n-3\,\text{PUFA})$$

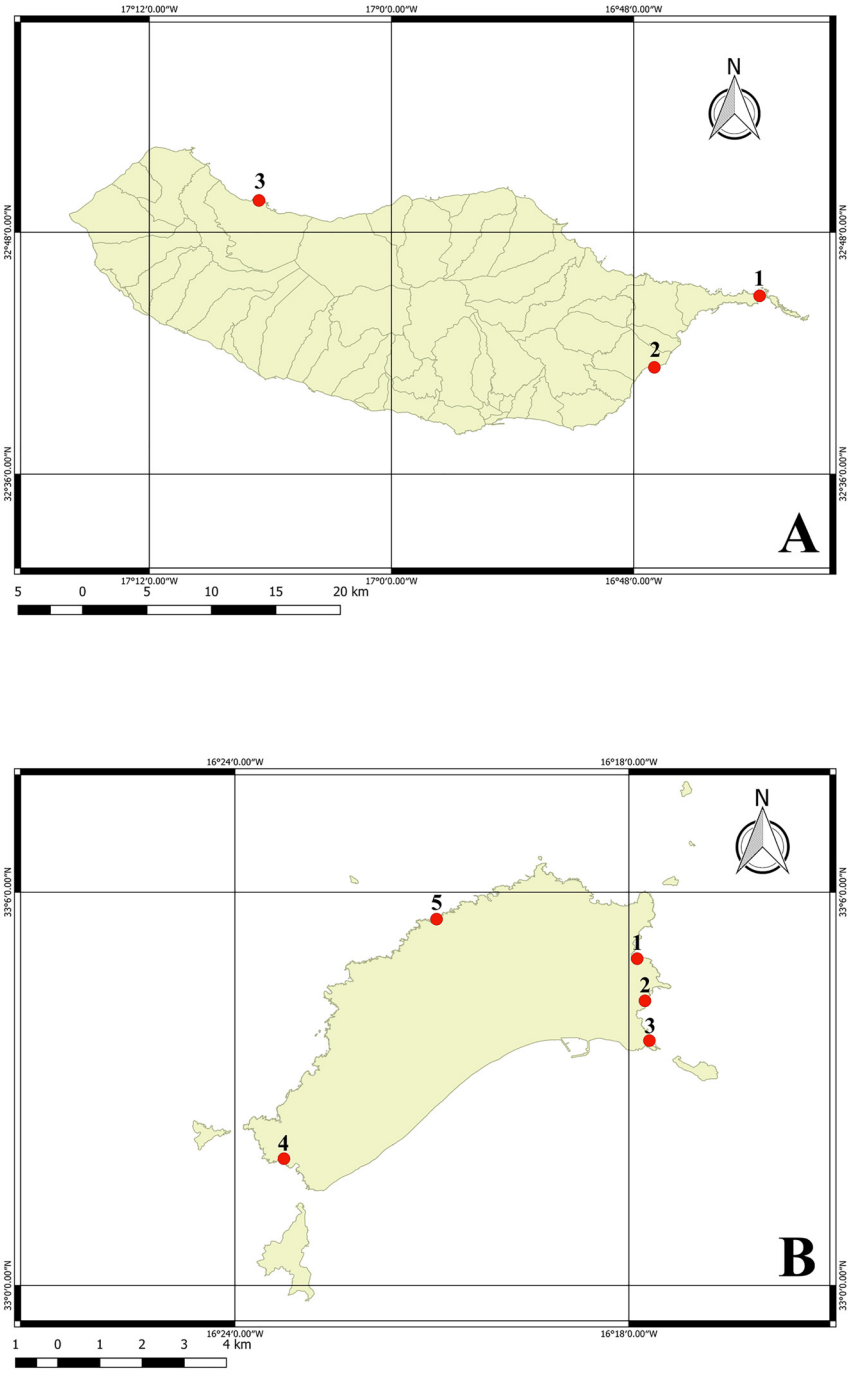

Figure 1: (A) Location of macroalgal sampling collection on Madeira island. Caniçal (1), *Halopteris scoparia*; Santa Cruz (2), *Sargassum vulgare*; Seixal (3), *Padina pavonica* and *Cystoseira humilis*. (B) Location of macroalgal sampling collection on Porto Santo island. Calhau Serra de Dentro (1), *Dasycladus vermicularis* and *Halopithys incurva*; Abas do Rio (2), *Dictyota dichotoma* and *Halopteris filicina;* Calhau da Baleia (3), *Lobophora* sp.; Praia do Zimbralinho (4), *Corallina officinalis* and *Asparagopsis taxiformis*; Porto das Salemas (5), *Ulva* sp., *Cystoseira compressa* and *Cystoseira usneoides*.

$$\text{Thrombogenicity index (TI)} = (14{:}\,0 + 16{:}\,0 + 18{:}\,0)/(0.5 \times \sum\text{MUFA} + 0.5 \times \sum n - 6\,\text{PUFA} + 3 \times \sum n - 3\,\text{PUFA} + n - 3/n - 6\,\text{ratio})$$

$$\text{Hypocholesterolemic (h)/hypercholesterolemic (H) ratio (hH)} = (18{:}\,1n - 9 + 18{:}\,2n - 6 + 20{:}\,4n - 6 + 8{:}\,3n - 3 + 20{:}\,5n - 3 + 22{:}\,5n - 3 + 22{:}\,6n - 3)/(14{:}\,0 + 16{:}\,0)$$

2.5 Statistical analysis

Before analysis, normality and homogeneity of data were confirmed within groups and, where necessary, appropriate variance stabilizing transformations were performed. When transformations did not succeed, Welch test followed by the Dunnett T3 test were used. Significant differences in lipid classes and FA

composition of red and brown macroalgae were assessed by one-way ANOVA followed by the Tukey HSD post-hoc test. Differences between green macroalgae were determined by Student's *t*-test or Mann-Whitney tests for normal or non-normal distribution of data, respectively. In addition, comparisons of TL, AI, TI, and hH indices between all species studied were also determined by one-way ANOVA.

Two principal component analyses (PCA), one for the lipid classes and the other for the main FA, of all macroalgae were carried out. Two hierarchical cluster analyses subsequently used factor scores to identify macroalgae with similar lipid classes and FA profiles. The hierarchical cluster analysis were performed with the Ward linkage method and the squared Euclidean distances.

Results are presented as mean ± standard deviation (SD, $n = 3$) and the statistical significance was set at $p < 0.05$. All statistical analyses were performed using IBM® SPSS Statistics 25.0 software package (IBM Corp., New York, USA) for Windows.

3 Results

3.1 Total lipid content

The lipid content of analysed seaweeds strongly varied among species, from 0.2% DW (*Dasycladus vermicularis*) to 5.2% DW (*Dictyota dichotoma*) ($p < 0.05$; Table 1).

3.2 Lipid class profiles

All species studied presented higher proportions of total neutral lipids (TNL; from 31 to 62% of TL) than of total polar lipids (TPL; from 14 to 37% of TL) (Tables 2–4), except for *Dasycladus vermicularis* (Chlorophyta) and *Padina*

Table 1: Total lipid content (% of dry weight) of macroalgae.

Group/Phylum	Species	TL content
Chlorophyta	*Dasycladus vermicularis*	0.2 ± 0.1^{a}
	Ulva sp.	0.9 ± 0.1^{ab}
Rhodophyta	*Corallina officinalis*	1.3 ± 0.1^{bcd}
	Asparagopsis taxiformis	2.1 ± 0.9^{de}
	Halopithys incurva	1.2 ± 0.1^{acd}
Ochrophyta	*Cystoseira compressa*	1.8 ± 0.0^{bcd}
	C. usneoides	0.8 ± 0.0^{ab}
	C. humilis	2.9 ± 0.4^{e}
	Dictyota dichotoma	5.2 ± 0.2^{f}
	Halopteris filicina	1.0 ± 0.2^{ac}
	H. scoparia	1.2 ± 0.4^{acd}
	Lobophora sp.	1.2 ± 0.1^{acd}
	Padina pavonica	0.8 ± 0.0^{ab}
	Sargassum vulgare	2.0 ± 0.7^{ce}

Results are presented as mean ± SD ($n = 3$). Different superscript letters indicate significant differences among all macroalgal species ($p < 0.05$). TL, total lipid.

Table 2: Main lipid class composition of green macroalgae (% of total lipid).

	Dasycladus vermicularis	*Ulva* sp.
PC	2.3 ± 0.6	1.9 ± 0.3
PS + PI	1.1 ± 0.4	1.1 ± 0.2
SQDG + PE	12.8 ± 2.4	16.0 ± 0.8
DGDG	10.5 ± 1.4	9.7 ± 1.4
MGDG	2.3 ± 0.7	1.3 ± 0.5*
UkPL	2.7 ± 0.7	2.2 ± 0.3
TPL	31.7 ± 4.7	32.2 ± 2.3
P	37.1 ± 1.4	15.6 ± 0.8*
DAG	6.4 ± 0.8	6.1 ± 0.9
PTS	11.7 ± 3.5	9.0 ± 0.4
FFA	5.8 ± 1.4	20.2 ± 1.5*
TAG	4.5 ± 2.0	11.3 ± 0.8*
SE	2.9 ± 1.0	4.9 ± 1.1
UkNL	nd	0.6 ± 0.7
TNL	31.2 ± 6.1	52.2 ± 1.6*

Results are presented as mean ± SD ($n = 3$). *Indicates significant difference between the species ($p < 0.05$). PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol; PE, phosphatidylethanolamine; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; UkPL, unknown polar lipids; TPL, total polar lipids;
P, pigments; DAG, diacylglycerols; PTS, phytosterols; FFA, free fatty acids; TAG, triacylglycerols; SE, sterol esters; UkNL, unknown neutral lipids; TNL, total neutral lipids; nd, not detected.

Table 3: Main lipid class composition of red macroalgae (% of total lipid).

	Asparagopsis taxiformis	*Corallina officinalis*	*Halopithys incurva*
PC	3.5 ± 0.1	3.3 ± 0.4	6.2 ± 1.3
PS + PI	1.0 ± 0.6	1.2 ± 0.2	1.3 ± 0.6
SQDG + PE	3.7 ± 1.7^{a}	3.3 ± 0.5^{a}	7.7 ± 1.8^{b}
DGDG	1.8 ± 0.4^{b}	1.1 ± 0.1^{a}	6.7 ± 0.3^{c}
MGDG	1.6 ± 0.8^{a}	4.7 ± 0.6^{b}	5.6 ± 0.4^{b}
UkPL	2.3 ± 0.8	2.6 ± 1.1	1.9 ± 0.6
TPL	13.9 ± 2.5^{a}	16.2 ± 1.4^{a}	29.3 ± 4.6^{b}
P	23.8 ± 3.7	29.2 ± 0.8	29.6 ± 1.1
DAG	5.3 ± 1.3	4.4 ± 1.7	4.6 ± 1.0
PTS	6.2 ± 0.6^{a}	14.4 ± 0.4^{b}	14.6 ± 1.1^{b}
FFA	18.7 ± 6.3^{ab}	25.0 ± 2.3^{b}	11.1 ± 1.1^{a}
TAG	30.0 ± 4.0^{b}	4.1 ± 1.8^{a}	5.2 ± 2.1^{a}
SE	2.1 ± 1.2^{a}	6.8 ± 0.8^{b}	5.6 ± 2.4^{ab}
TNL	62.3 ± 3.9^{b}	54.6 ± 1.6^{b}	41.1 ± 5.4^{a}

Results are presented as mean ± SD ($n = 3$). Different superscript letters within each row indicate significant differences between species ($p < 0.05$). PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol; PE, phosphatidylethanolamine; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; UkPL, unknown polar lipids; TPL, total polar lipids; P, pigments; DAG, diacylglycerols; PTS, phytosterols; FFA, free fatty acids; TAG, triacylglycerols; SE, sterol esters; TNL, total neutral lipids.

Table 4: Main lipid class composition of brown macroalgae (% of total lipid).

	Cystoseira compressa	*C. usneoides*	*C. humilis*	*Dictyota dichotoma*	*Halopteris filicina*	*H. scoparia*	*Lobophora* sp.	*Padina pavonica*	*Sargassum vulgare*
PC	1.8 ± 0.4^{abc}	1.3 ± 0.3^{abc}	0.9 ± 0.4^{a}	1.2 ± 0.4^{ab}	2.9 ± 1.1^{bc}	3.0 ± 0.3^{bc}	3.1 ± 0.9^{c}	1.4 ± 1.1^{abc}	2.1 ± 0.4^{abc}
PS + PI	4.5 ± 0.3^{bc}	9.6 ± 1.9^{d}	2.4 ± 0.5^{ab}	4.1 ± 0.5^{bc}	1.1 ± 0.4^{a}	1.2 ± 0.4^{a}	6.0 ± 1.6^{c}	4.8 ± 1.4^{c}	5.0 ± 0.4^{c}
SQDG + PE	8.0 ± 1.4^{abc}	15.8 ± 3.0^{e}	10.6 ± 1.2^{cd}	5.3 ± 0.3^{a}	6.5 ± 1.2^{ab}	8.7 ± 2.1^{abcd}	9.1 ± 1.3^{bcd}	13.1 ± 1.3^{de}	8.7 ± 0.3^{abcd}
DGDG	3.8 ± 0.7	5.8 ± 0.7	5.8 ± 0.6	3.7 ± 0.1	4.3 ± 0.3	5.1 ± 1.1	4.6 ± 0.8	6.3 ± 0.5	3.7 ± 0.2
MGDG	1.0 ± 0.4^{a}	2.2 ± 0.7^{abc}	1.4 ± 0.6^{ab}	1.4 ± 0.8^{ab}	1.7 ± 0.7^{abc}	2.1 ± 0.8^{abc}	3.1 ± 0.4^{bc}	3.2 ± 0.4^{c}	1.6 ± 0.5^{abc}
UkPL	0.6 ± 0.3	1.9 ± 0.7	2.6 ± 0.4	1.3 ± 0.2	3.5 ± 0.3	7.6 ± 2.8	3.5 ± 0.4	5.4 ± 1.4	2.6 ± 1.2
TPL	19.7 ± 1.8^{ab}	36.6 ± 6.3^{c}	23.7 ± 2.5^{ab}	17.0 ± 1.4^{a}	20.0 ± 1.8^{ab}	27.7 ± 4.1^{bc}	29.4 ± 3.8^{bc}	34.3 ± 5.2^{c}	23.7 ± 1.9^{ab}
P	23.7 ± 0.8^{d}	22.7 ± 0.4^{ac}	21.2 ± 0.5^{a}	29.9 ± 0.5^{e}	27.5 ± 3.4^{abcde}	25.8 ± 3.1^{abcde}	27.2 ± 0.9^{bde}	31.2 ± 1.8^{cde}	21.2 ± 1.4^{ab}
DAG	9.4 ± 0.2^{ce}	2.2 ± 0.6^{a}	13.0 ± 1.2^{de}	14.8 ± 0.9^{e}	8.2 ± 1.6^{abcde}	4.2 ± 1.5^{abc}	6.7 ± 0.2^{bd}	3.5 ± 0.3^{a}	2.4 ± 0.4^{a}
PTS	10.9 ± 0.7^{a}	24.7 ± 3.6^{d}	17.0 ± 0.6^{bc}	18.9 ± 1.6^{bc}	19.3 ± 1.9^{bc}	15.5 ± 1.3^{b}	21.9 ± 1.9^{cd}	16.3 ± 1.5^{b}	19.6 ± 1.4^{bcd}
FFA	16.4 ± 1.0^{cd}	3.6 ± 1.3^{a}	14.4 ± 1.2^{c}	7.0 ± 0.8^{b}	15.2 ± 1.8^{cd}	15.5 ± 1.2^{cd}	9.2 ± 1.0^{b}	7.1 ± 1.3^{b}	19.8 ± 1.5^{d}
TAG	18.9 ± 0.5^{d}	4.6 ± 0.7^{bc}	3.8 ± 1.0^{abcd}	5.2 ± 1.2^{bcd}	6.3 ± 6.1^{abcd}	6.8 ± 1.0^{c}	1.4 ± 0.3^{a}	2.9 ± 0.2^{ab}	7.4 ± 1.4^{bcd}
SE	1.1 ± 0.4^{a}	1.6 ± 0.4^{a}	4.1 ± 1.1^{ab}	1.1 ± 1.1^{ab}	2.5 ± 1.1^{ab}	4.5 ± 0.6^{b}	2.6 ± 0.0^{ab}	4.7 ± 0.9^{ab}	4.9 ± 1.6^{ab}
UkNL	nd	3.8 ± 1.2	2.9 ± 1.1	6.2 ± 1.5	0.9 ± 1.1	nd	1.6 ± 0.5	nd	0.9 ± 0.3
TNL	56.7 ± 2.0^{d}	40.7 ± 6.4^{abcd}	55.1 ± 2.9^{bd}	53.1 ± 1.3^{cd}	52.5 ± 1.7^{bcd}	46.5 ± 1.0^{ab}	43.4 ± 3.0^{abc}	34.5 ± 3.4^{a}	55.1 ± 3.3^{bcd}

Results are presented as mean ± SD ($n = 3$). Different superscript letters within each row indicate significant differences between species ($p < 0.05$). PC, phosphatidylcholine; PS, phosphatidylserine; PI, phosphatidylinositol; SQDG, sulfoquinovosyldiacylglycerol; PE, phosphatidylethanolamine; DGDG, digalactosyldiacylglycerol; MGDG, monogalactosyldiacylglycerol; UkPL, unknown polar lipids; TPL, total polar lipids; P, pigments; DAG, diacylglycerol; PTS, phytosterols; FFA, free fatty acids; TAG, triacylglycerols; SE, sterol esters; UkNL, unknown neutral lipids; TNL, total neutral lipids; nd, not detected.

pavonica (Ochrophyta) which contained similar levels of both lipid fractions (31–34% of TL).

Ulva sp. contained higher levels of free fatty acids (FFA) and TAG (20.2 and 11.3%, respectively) than *Dasycladus vermicularis* (5.8 and 4.5%) ($p < 0.05$). Among polar lipids, only MGDG varied significantly among species (Table 2).

Within red macroalgae, TAG was highest in *Asparagopsis taxiformis* (30.0%) whereas TAG was only 4.1 and 5.2% of TL in *Corallina officinalis* and *Halopithys incurva*, respectively. On the other hand, *H. incurva* ($p < 0.05$) contained the highest SQDG + PE and DGDG (Table 3).

PTS was particularly abundant in brown macroalgae (10.9–24.7% TL; Table 4). *Cystoseira compressa* had the highest TAG levels (18.9%), while in the other Ochrophyta analysed, values ranged between 1.4 and 6.8% of TL. Phosphatidylserine (PS) + phosphatidylinositol (PI) (9.6%), and SQDG + PE (15.8%) were remarkably high in *C. usneoides* ($p < 0.05$; Table 4).

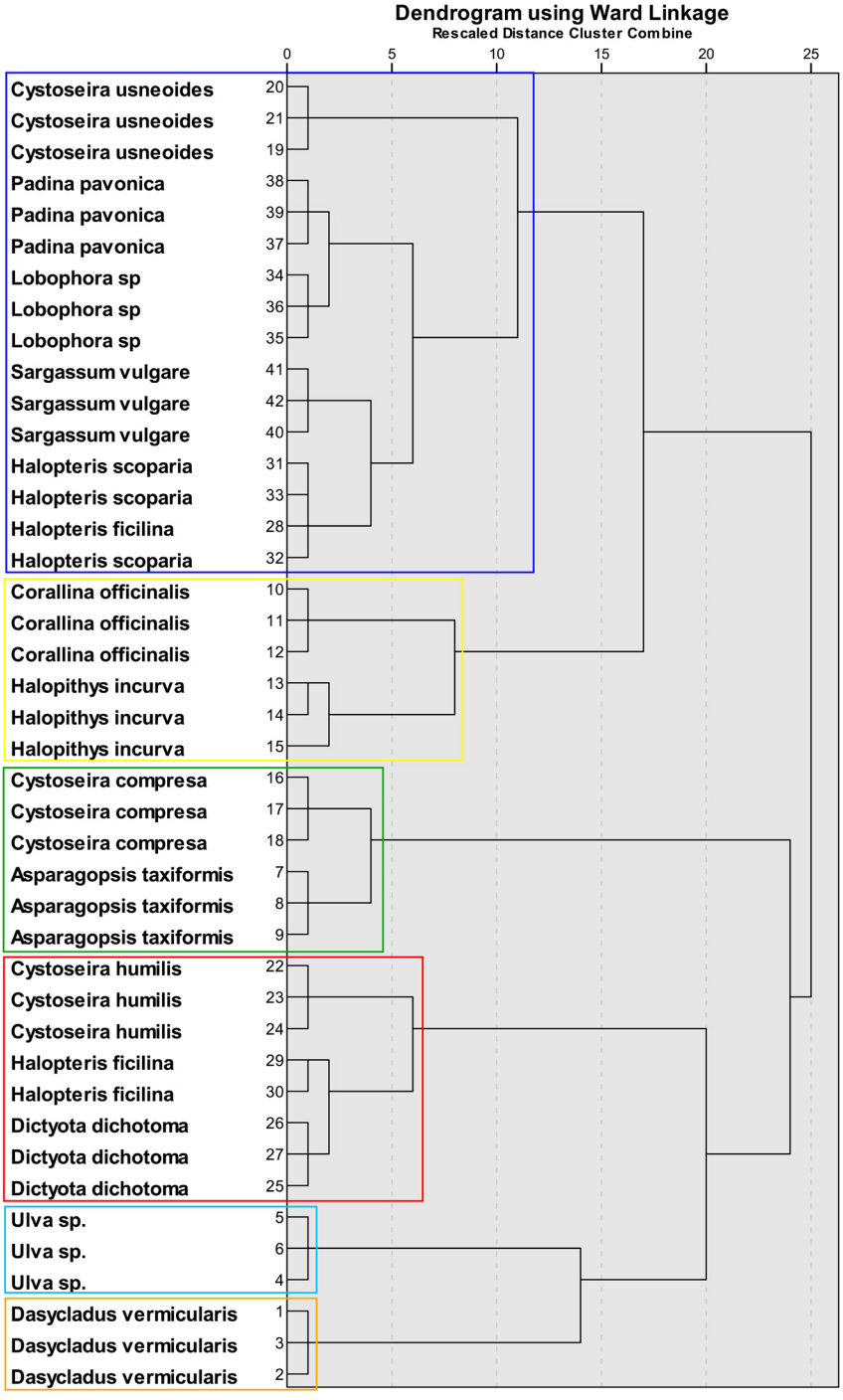

Figure 2: Hierarchical cluster analysis in a dendrogram format showing six clusters of macroalgae according to the lipid class composition.

The PCA of macroalgal lipid classes showed five components with eigenvalues >1, which accounted for more than 88% of the total variance. Factor loadings and communalities are shown in Supplementary Table S1. According to the dendrogram obtained, the macroalgae were classified into six clusters (Figure 2). Mean factor scores for each cluster of the dendrogram are given in Supplementary Table S2. Thus, Cluster one grouped most Ochrophyta species including *Cystoseira usneoides*, *Halopteris scoparia*, *Lobophora* sp., *Padina pavonica*, *Sargassum vulgare*, and one replicate of *H. ficilina*, all of which were mainly characterized by a high average content of PTS. Two of the three red macroalgae studied, *Corallina officinalis* and *Halopithys incurva*, formed Cluster 2, with high average proportions of FFA, sterol esters, PC and MGDG. Cluster three consisted of the third Rhodophyta species, *Asparagopsis taxiformis*, and *C. compressa* (Ochrophyta), which contained high average TAG and PS + PI. Cluster 4, which included the rest of the Ochrophyta species, *C. humilis*, *Dictyota dichotoma* and two replicates of *H. ficilina*, showed the highest average percentage of diacylglycerol. Finally, the green algae *Ulva* sp. and *Dasycladus vermicularis* were the only components of Clusters 5 and 6, which were characterized by high average SQDG + PE, DGDG and FFA content, and high SQDG + PE, DGDG and P, respectively.

3.3 Fatty acid profile

The FA profiles of green seaweeds strongly differed among species. *Ulva* sp. had higher contents of saturated fatty acids (SFA), mainly palmitic acid (16:0), and n-3 PUFA ($p < 0.05$; Table 5), while *Dasycladus vermicularis* was richer in monounsaturated fatty acids (MUFA). The n-3 LC-PUFA content was low in both species, where DHA represented only 0.5 and 0.8% of total FA in *D. vermicularis* and *Ulva* sp., respectively.

Similarly, the FA profiles varied considerably within the Rhodophyta. Only total SFA and 16:0 were not significantly different among the species, with the latter being the most abundant FA in all three species (Table 6). *Asparagopsis taxiformis* had the highest amount of DHA (22:6n-3; 6.6% of total FA), while *Halopithys incurva* was richer in arachidonic acid (ARA, 20:4n-6), ALA and EPA, leading to higher total n-6 and total n-3 PUFA contents. The n-6/n-3 ratio was highest in *Corallina officinalis* (1.6) and lowest in *A. taxiformis* (0.4; Table 6).

Brown macroalgae contained the highest levels of SFA of all species studied (from 34.1 to 52.3% of total FA in *Dictyota dichotoma* and *Halopteris scoparia*, respectively), followed by MUFA (26.2% in *Cystoseira usneoides* and 34.2% in *Padina pavonica*) (Table 7). Within Ochrophyta, n-3 PUFA was remarkably high in *D. dichotoma*, mainly due to the higher levels of EPA (5.0% of total FA) and stearidonic acid (SDA, 18:4n-3; 8.0%), while DHA was only 0.5% of total FA. On the other hand, *C. compressa* showed the highest value of DHA (3.9%). Total n-6 PUFA was more abundant in *C. usneoides* and *Sargassum vulgare* due to the high proportions of both LA and ARA.

PCA for seaweed FA revealed that five components had eigenvalues >1 and together accounted for more than 86% of the total variance. Factor loadings and communalities after applying varimax rotation to enhance the interpretability of the results are displayed in Supplementary Table S3. The dendrogram obtained from the hierarchical cluster analysis, which used the factor scores as input variables, revealed that macroalgae could be classified into six clusters (Figure 3). Supplementary Table S4 shows factor

Table 5: Main fatty acid composition (% of total FA) of green macroalgae.

	Dasycladus vermicularis	*Ulva* sp.
14:0	3.6 ± 0.5	1.7 ± 0.1*
15:0	1.1 ± 0.2	0.7 ± 0.0
16:0	18.7 ± 2.6	38.4 ± 0.1*
17:0	1.0 ± 0.2	0.5 ± 0.1*
18:0	4.3 ± 1.6	2.8 ± 0.2
Σ SFA[1]	28.7 ± 5.0	45.0 ± 0.4*
16:1[#]	15.5 ± 0.8	4.8 ± 0.1*
18:1[##]	26.7 ± 2.4	21.2 ± 0.7*
Σ MUFA[2]	44.3 ± 1.5	27.2 ± 0.6*
18:2n-6	14.3 ± 2.2	8.5 ± 0.2
20:3n-6	nd	0.7 ± 0.0
20:4n-6	0.6 ± 0.3	1.5 ± 0.1*
Σ n-6 PUFA[3]	14.9 ± 2.5	12.5 ± 0.2
16:3n-3	nd	1.7 ± 0.0
18:3n-3	0.7 ± 0.1	6.8 ± 0.1*
18:4n-3	0.8 ± 0.2	1.9 ± 0.1*
20:5n-3	1.2 ± 0.3	1.2 ± 0.1
22:6n-3	0.5 ± 0.1	0.8 ± 0.1*
Σ n-3 PUFA[4]	3.2 ± 0.7	13.5 ± 0.4*
Σ n-3 LC-PUFA[4]	1.7 ± 0.4	3.2 ± 0.3*
Σ PUFA[3,4,5]	22.9 ± 3.9	26.3 ± 1.0*
n-6/n-3	4.7 ± 0.3	0.9 ± 0.0*
DHA/EPA	0.4 ± 0.1	0.7 ± 0.0*
ARA/EPA	0.5 ± 0.1	1.2 ± 0.1*

Results are presented as mean ± SD ($n = 3$). *Indicates significant difference between the species ($p < 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long chain polyunsaturated fatty acids (≥C20 and ≥ 2 double bonds); DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ARA, arachidonic acid; nd, not detected. [1]Also includes 22:0; [2]also includes 15:1, 17:1, 20:1n-9 and 22:1; [3]also includes 20:2n-6 and 22:2n-6; [4]also includes 20:4n-3; [5] also includes 16:2n-4 and 16:3n-4. [#]Mainly n-7 isomer; [##]mainly n-9 and n-7 isomers.

Table 6: Main fatty acid composition (% of total FA) of red macroalgae.

	Asparagopsis taxiformis	*Corallina officinalis*	*Halopithys incurva*
14:0	10.1 ± 1.1[b]	4.7 ± 1.6[a]	6.8 ± 0.2[a]
15:0	0.7 ± 0.0[a]	1.6 ± 0.3[b]	0.4 ± 0.1[a]
16:0	32.0 ± 3.2	35.7 ± 1.8	32.0 ± 0.6
17:0	1.6 ± 0.0[a]	1.1 ± 0.2[b]	0.7 ± 0.1[a]
18:0	3.1 ± 0.1[b]	5.0 ± 0.4[c]	2.1 ± 0.3[a]
Σ SFA[1]	46.8 ± 4.3	48.7 ± 1.3	42.3 ± 1.0
16:1[#]	8.5 ± 0.7[b]	7.9 ± 2.0[ab]	6.1 ± 0.1[a]
18:1[##]	20.9 ± 1.3[b]	19.8 ± 0.4[b]	12.9 ± 0.3[a]
Σ MUFA[2]	32.5 ± 0.9[b]	31.0 ± 1.3[b]	20.0 ± 0.3[a]
18:2n-6	5.0 ± 0.6[b]	4.9 ± 0.9[b]	2.1 ± 0.1[a]
20:3n-6	nd	nd	0.3 ± 0.0
20:4n-6	0.4 ± 0.0[a]	5.1 ± 0.8[b]	11.7 ± 0.8[c]
Σ n-6 PUFA	5.5 ± 0.6[a]	9.9 ± 0.9[b]	14.2 ± 0.8[c]
18:3n-3	1.0 ± 0.1[a]	1.0 ± 0.3[a]	10.9 ± 0.6[b]
18:4n-3	0.8 ± 0.1[b]	0.5 ± 0.0[ab]	0.3 ± 0.1[a]
20:5n-3	2.2 ± 0.4[a]	3.4 ± 0.6[a]	9.3 ± 0.6[b]
22:6n-3	6.6 ± 1.4[c]	1.4 ± 0.2[b]	0.5 ± 0.1[a]
Σ n-3 PUFA[3]	13.6 ± 2.6[b]	6.2 ± 0.9[a]	21.6 ± 0.7[c]
Σ n-3 LC-PUFA[3]	11.8 ± 2.3[b]	4.8 ± 0.8[a]	10.4 ± 0.4[b]
Σ PUFA[3,4]	20.5 ± 3.4[a]	17.7 ± 1.8[a]	36.0 ± 1.2[b]
n-6/n-3	0.4 ± 0.0[a]	1.6 ± 0.3[c]	0.7 ± 0.0[b]
DHA/EPA	3.0 ± 0.1[c]	0.4 ± 0.0[a]	0.1 ± 0.0[b]
ARA/EPA	0.2 ± 0.0[a]	1.5 ± 0.1[c]	1.3 ± 0.1[b]

Results are presented as mean ± SD (n = 3). Different superscript letters within each row indicate significant differences between species ($p < 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long chain polyunsaturated fatty acids (≥C20 and ≥ 2 double bonds); DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ARA, arachidonic acid; nd, not detected. [1]Also includes 20:0 and 22:0; [2]also includes 14:1, 15:1, 17:1, 20:1n-9 and 22:1; [3]also includes 20:3n-3, 20:4n-3 and 22:5n-3; [4]also includes 16:2, 16:3n-4, 16:4n-1 and 20:2n-9. [#]Mainly n-7 isomer and n-9 isomers; [##]mainly n-9 isomer.

scores for each cluster given as mean ± SD Hence, Cluster 1, grouping together *Ulva* sp. (Chlorophyta), and three Ochrophyta species (*Cystoseira usneoides*, *C. humilis* and *Sargassum vulgare*), was characterized by the highest average percentage of 16:0 and medium-high average content of LA and 20:3n-6. Clusters 2–4 comprised only one species each: *Halopithys incurva* (Cluster 2) had the highest average proportion of ALA, ARA and EPA; *Dictyota dichotoma* (Cluster 3) was characterized by the lowest average percentage of DHA, and high 14:0 and 20:3n-6; *Dasycladus vermicularis* (Cluster 4) had the highest average proportions of 15:0, 17:0, 18:0 and LA, and low ARA and EPA contents. Cluster five contained the red macroalga *Asparagopsis taxiformis* and the brown *Cystoseira compressa*, which had the highest proportion of DHA and low percentages of ALA, ARA and LA. Finally, the red macroalga *Corallina officinalis*, and the four brown macroalgae (*Lobophora* sp., *Halopteris ficilina*, *H. scoparia* and *Padina pavonica*) were grouped in Cluster 6, and were characterized by medium-high average content of all SFA.

Overall, the grouping of macroalgae based on their FA profile did not follow a similar pattern to that described for their lipid class composition (Figure 2). Only the pairs *Halopteris scoparia* and *Padina pavonica*; *Cystoseira usneoides* and *Sargassum vulgare*; and *Asparagopsis taxiformis* and *C. compressa* were similar in their lipid class and FA profiles. Of all species analysed, *Dasycladus vermicularis* (Chlorophyta) had a particular and unique lipid profile.

3.4 Nutritional indices

Dasycladus vermicularis had the lowest AI (0.6) and *Dictyota dichotoma* the lowest TI (0.4), whereas *Halopteris scoparia* had the highest AI (1.6) and TI (1.3) values (Table 8). *Ulva* sp., *H. scoparia* and *Padina pavonica* showed the lowest hH ratios (0.7), while *D. dichotoma* and *Sargassum vulgare* had the highest values.

4 Discussion

The seaweeds analysed differed greatly in their lipid content, lipid classes and FA profiles providing evidence of strong interspecific variations. Several factors have been suggested to affect the biochemical composition of algae, including the part of the macroalgal thallus used for the analysis (Alsufyani et al. 2014; Pereira et al. 2012), which was strictly controlled in the present study. However, our one-off collection methodology did not allow us to evaluate seasonal, environmental or even geographical factors, or the impact of species-dependent fatty acid transformation and decomposition that should be considered in future research to provide a complete lipid description of the selected macroalgae.

The TL content of the macroalgae studied were broadly similar to the low levels described in earlier literature (Bourgougnon et al. 2011; Kendel et al. 2015; Mæhre et al. 2014; Nunes et al. 2020; Verma et al. 2017). Nevertheless, some differences, probably related to both geographical and seasonal factors, were detected. Thus, the two Chlorophyta species had lower lipid contents than other species of *Ulva*, such as *Ulva lactuca* and *U. reticulata* from India, *U. lactuca* from Hong Kong, and *U. rigida* from South Africa (Foster and Hodgson 1998; Verma et al. 2017; Wong and Cheung 2000). In contrast, the lipid level of *Ulva* sp. was

Table 7: Main fatty acid composition (% of total FA) of brown macroalgae.

	Cystoseira compressa	*C. usneoides*	*C. humilis*	*Dictyota dichotoma*	*Halopteris ficilina*	*H. scoparia*	*Lobophora sp.*	*Padina pavonica*	*Sargassum vulgare*
14:0	7.8 ± 0.9^{bcd}	3.2 ± 1.2^{abcd}	7.0 ± 0.2^{bc}	10.2 ± 0.4^{de}	5.9 ± 0.0^{b}	7.6 ± 0.2^{cd}	11.6 ± 0.5^{e}	8.0 ± 0.2^{d}	3.6 ± 0.1^{a}
15:0	0.7 ± 0.1^{ab}	0.6 ± 0.3^{ab}	0.5 ± 0.0^{a}	0.6 ± 0.1^{ab}	0.7 ± 0.0^{b}	0.8 ± 0.0^{b}	0.8 ± 0.1^{ab}	1.0 ± 0.1^{ab}	0.5 ± 0.0^{ab}
16:0	31.5 ± 1.1^{bde}	32.9 ± 0.7^{bcd}	33.7 ± 0.5^{cd}	20.7 ± 0.4^{a}	32.8 ± 1.3^{cd}	36.6 ± 1.4^{c}	29.2 ± 0.3^{b}	34.7 ± 0.7^{ce}	30.9 ± 0.4^{bd}
17:0	0.9 ± 0.5^{abc}	0.4 ± 0.2^{abc}	0.4 ± 0.0^{a}	nd	0.9 ± 0.1^{b}	1.0 ± 0.1^{b}	0.7 ± 0.1^{b}	0.6 ± 0.0^{b}	0.4 ± 0.0^{a}
18:0	3.1 ± 0.3^{b}	1.7 ± 0.2^{a}	1.8 ± 0.5^{ab}	1.8 ± 0.2^{a}	3.9 ± 1.4^{abc}	4.7 ± 1.1^{abc}	2.2 ± 0.1^{ab}	2.4 ± 0.2^{ab}	5.1 ± 0.3^{c}
Σ SFA[1]	44.7 ± 1.3^{bcd}	40.6 ± 0.7^{abcd}	44.2 ± 0.8^{bc}	34.1 ± 0.9^{a}	44.9 ± 0.9^{bcd}	52.3 ± 1.1^{cd}	45.8 ± 0.8^{d}	47.6 ± 1.5^{bcd}	41.8 ± 0.3^{d}
16:1[#]	8.3 ± 0.2^{bcd}	5.4 ± 0.6^{a}	5.7 ± 0.0^{a}	9.6 ± 0.4^{cde}	8.0 ± 0.3^{b}	9.4 ± 0.2^{de}	10.2 ± 0.5^{ef}	10.2 ± 0.1^{f}	8.2 ± 0.3^{bcd}
18:1[##]	21.8 ± 0.7^{bcd}	20.6 ± 0.2^{b}	20.5 ± 0.8^{bcd}	21.0 ± 0.3^{b}	18.7 ± 2.0^{abcd}	18.6 ± 0.4^{ac}	22.7 ± 0.3^{d}	24.0 ± 0.1^{d}	16.8 ± 0.4^{a}
Σ MUFA[2]	33.0 ± 0.8^{cd}	26.2 ± 0.3^{a}	26.6 ± 0.8^{ab}	31.4 ± 0.7^{bc}	27.8 ± 2.3^{abcd}	28.9 ± 0.3^{a}	33.3 ± 0.5^{d}	34.2 ± 0.1^{d}	27.5 ± 0.8^{ab}
18:2n-6	5.8 ± 0.6^{ade}	7.5 ± 0.5^{ade}	7.7 ± 0.6^{e}	2.7 ± 0.2^{a}	6.9 ± 0.1^{ce}	9.3 ± 1.2^{def}	4.2 ± 0.1^{bd}	3.8 ± 0.5^{abc}	11.7 ± 0.5^{f}
20:3n-6	nd	1.0 ± 0.1^{b}	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}	nd	nd	0.9 ± 0.0^{b}	0.6 ± 0.0^{a}	0.6 ± 0.0^{a}
20:4n-6	2.8 ± 0.7^{abc}	7.2 ± 0.3^{d}	7.5 ± 0.1^{d}	6.7 ± 0.1^{cd}	3.0 ± 0.3^{ab}	2.0 ± 0.2^{a}	4.4 ± 0.2^{b}	2.0 ± 0.2^{a}	6.5 ± 0.0^{cd}
Σ n-6 PUFA[3]	8.7 ± 1.4^{abc}	19.5 ± 0.4^{e}	15.7 ± 0.6^{c}	13.2 ± 0.1^{b}	13.3 ± 1.2^{abcd}	11.3 ± 1.0^{abc}	10.3 ± 0.5^{ab}	9.2 ± 0.5^{a}	18.8 ± 0.5^{de}
18:3n-3	2.5 ± 0.1^{b}	3.4 ± 0.1^{d}	6.0 ± 0.0^{f}	3.5 ± 0.0^{cd}	3.0 ± 0.4^{abcde}	2.2 ± 0.3^{abcd}	1.5 ± 0.1^{a}	2.4 ± 0.2^{abcd}	4.8 ± 0.2^{e}
18:4n-3	1.7 ± 0.3^{ab}	2.9 ± 0.1^{d}	3.2 ± 0.1^{d}	8.0 ± 0.2^{e}	2.2 ± 0.3^{bc}	1.2 ± 0.1^{a}	2.6 ± 0.1^{cd}	1.7 ± 0.2^{b}	1.8 ± 0.1^{b}
20:5n-3	2.2 ± 0.1^{bc}	3.2 ± 0.3^{d}	2.2 ± 0.1^{bc}	5.0 ± 0.2^{e}	3.6 ± 0.2^{d}	1.7 ± 0.4^{b}	3.4 ± 0.1^{d}	1.0 ± 0.1^{a}	2.4 ± 0.2^{c}
22:6n-3	3.9 ± 0.4^{c}	0.5 ± 0.1^{ab}	0.3 ± 0.1^{a}	0.5 ± 0.1^{ab}	1.1 ± 0.4^{b}	0.6 ± 0.3^{ab}	0.5 ± 0.1^{ab}	0.4 ± 0.2^{ab}	0.5 ± 0.1^{ab}
Σ n-3 PUFA[4]	12.0 ± 1.2^{c}	10.9 ± 0.4^{bc}	12.1 ± 0.2^{c}	18.8 ± 0.4^{d}	11.9 ± 0.8^{c}	5.7 ± 0.6^{a}	9.1 ± 0.5^{b}	6.8 ± 0.5^{a}	10.0 ± 0.5^{bc}
Σ n-3 LC-PUFA[4]	7.8 ± 0.9^{c}	4.6 ± 0.6^{b}	2.9 ± 0.0^{a}	7.2 ± 0.2^{c}	6.6 ± 0.4^{c}	2.4 ± 0.5^{a}	4.9 ± 0.4^{b}	3.1 ± 0.5^{a}	3.5 ± 0.2^{ab}
Σ PUFA[3,4,5]	21.5 ± 2.2^{b}	30.6 ± 0.6^{de}	28.0 ± 0.5^{cd}	33.0 ± 0.4^{e}	25.4 ± 2.3^{c}	17.0 ± 0.8^{a}	19.5 ± 0.8^{ab}	16.4 ± 1.1^{a}	28.8 ± 0.9^{cd}
n-6/n-3	0.7 ± 0.1^{a}	1.8 ± 0.1^{ef}	1.3 ± 0.1^{cd}	0.7 ± 0.0^{a}	1.1 ± 0.0^{bc}	2.0 ± 0.4^{abcdef}	1.1 ± 0.0^{bc}	1.3 ± 0.0^{de}	1.9 ± 0.1^{f}
DHA/EPA	1.8 ± 0.1^{b}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	0.1 ± 0.0^{a}	0.3 ± 0.1^{a}	0.4 ± 0.1^{a}	0.2 ± 0.0^{a}	0.4 ± 0.3^{a}	0.2 ± 0.0^{a}
ARA/EPA	1.3 ± 0.3^{abcdef}	2.2 ± 0.2^{de}	3.5 ± 0.1^{f}	1.3 ± 0.1^{b}	0.8 ± 0.1^{a}	1.2 ± 0.2^{abc}	1.3 ± 0.0^{abe}	2.0 ± 0.1^{cd}	2.7 ± 0.2^{df}

Results are presented as mean ± SD ($n = 3$). Different superscript letters within each row indicate significant differences between species ($p < 0.05$). SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids; LC-PUFA, long chain polyunsaturated fatty acids (≥C20 and ≥ 2 double bonds); DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ARA, arachidonic acid; nd, not detected. [1]Also includes 20:0, 22:0 and 24:0; [2]also includes 15:1, 20:1n-11, 20:1n-9 and 22:1; [3]also includes 20:2n-6, 22:2n-6 and 22:4n-6; [4]also includes 20:3n-3, 20:4n-3 and 22:5n-3; [5]also includes 16:2, 16:3n-4 and 16:4n-1. [#]Mainly n-7 and n-9 isomers; [##]mainly n-9 isomer.

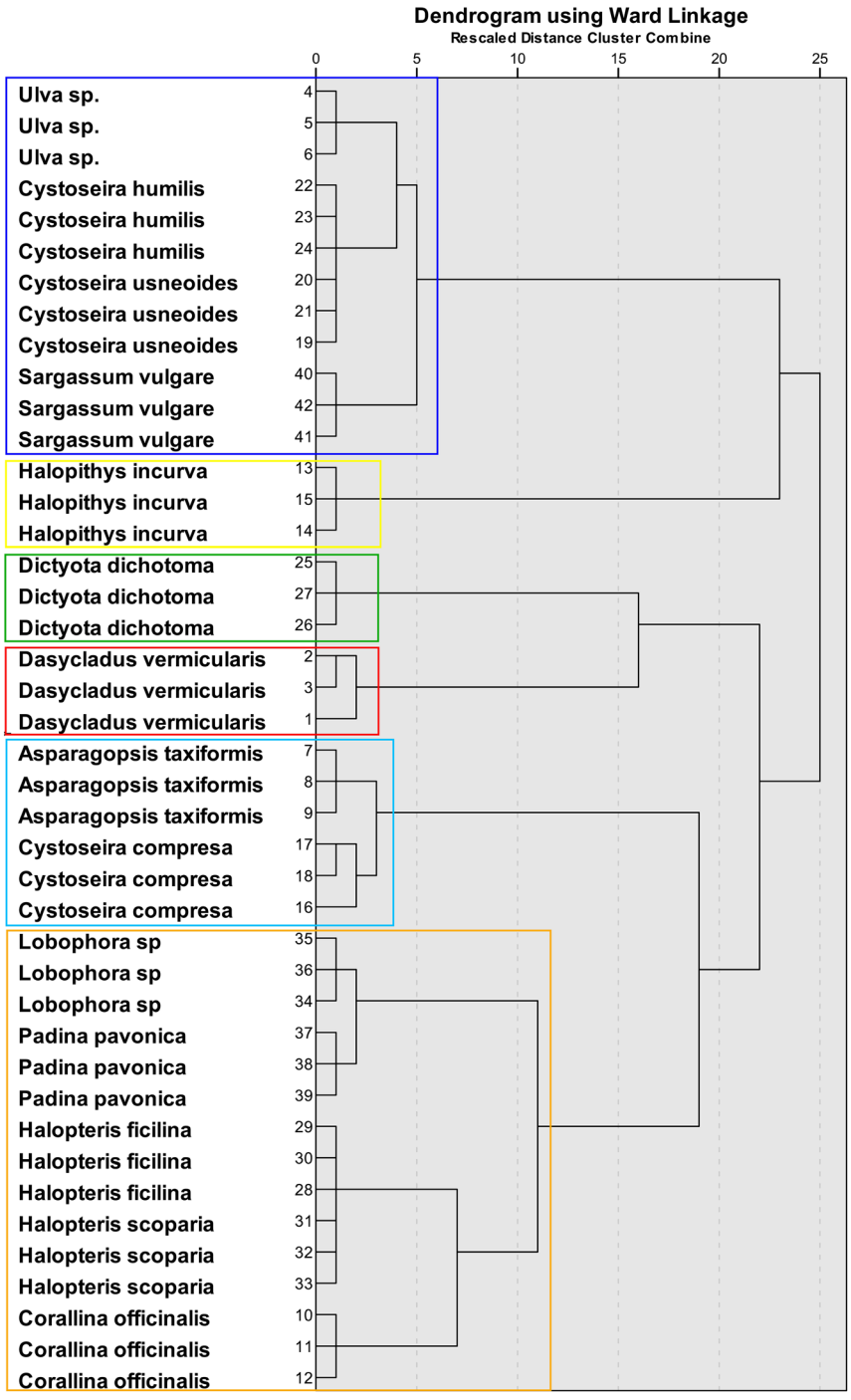

Figure 3: Hierarchical cluster analysis in a dendrogram format showing six clusters of macroalgae according to the fatty acid composition.

slightly higher than that of *U. lactuca* collected in North Yorkshire, UK (Marsham et al. 2007).

Among Rhodophyta, *Asparagopsis taxiformis* stood out from the rest in its high lipid content (~2% of TL in DW) and *Corallina officinalis* had a higher lipid content than that reported by Marsham et al. (2007) for the same species. Gosch et al. (2012) described that Ochrophyta, chiefly species from the Dictyotales, such as *Dictyota bartayresii*, *Dictyota dichotoma* and *Spatoglossum macrodontum*, had large lipid contents of 10–12%. In our present work, *D. dichotoma* had the highest lipid content (5.2%) of all species studied, higher than that cited by Verma et al. (2017), which is probably related to the recognized higher lipid levels of *Dictyota* species in temperate climates (McDermid and Stuercke 2003).

Table 8: Atherogenicity index (AI), thrombogenicity index (TI) and hypocholesterolemic/hypercholesteloremic fatty acids ratio (hH) of macroalgae.

Group/Phylum	Species	AI	TI	hH
Chlorophyta	*Dasycladus vermicularis*	0.6 ± 0.1^{a}	0.7 ± 0.2^{bc}	1.7 ± 0.4^{e}
	Ulva sp.	0.9 ± 0.0^{abc}	0.7 ± 0.0^{bc}	0.7 ± 0.0^{a}
Rhodophyta	*Asparagopsis taxiformis*	1.5 ± 0.3^{fg}	0.7 ± 0.2^{bc}	0.8 ± 0.2^{ac}
	Corallina officinalis	1.3 ± 0.1^{deg}	1.1 ± 0.1^{ef}	0.8 ± 0.1^{ab}
	Halopithys incurva	1.1 ± 0.1^{be}	0.5 ± 0.0^{ab}	1.1 ± 0.1^{abcd}
Ochrophyta	*Cystoseira compressa*	1.2 ± 0.1^{def}	0.7 ± 0.1^{bc}	0.9 ± 0.1^{ad}
	C. usneoides	0.8 ± 0.1^{ab}	0.7 ± 0.0^{bc}	1.2 ± 0.0^{cd}
	C. humilis	1.2 ± 0.0^{ce}	0.7 ± 0.0^{bc}	1.1 ± 0.0^{bcd}
	Dictyota dichotoma	1.0 ± 0.0^{bcd}	0.4 ± 0.0^{a}	1.3 ± 0.1^{d}
	Halopteris filicina	1.1 ± 0.0^{be}	0.7 ± 0.0^{c}	0.8 ± 0.1^{ac}
	H. scoparia	1.6 ± 0.1^{g}	1.3 ± 0.1^{f}	0.7 ± 0.0^{a}
	Lobophora sp.	1.5 ± 0.1^{fg}	0.9 ± 0.0^{cd}	0.8 ± 0.0^{ac}
	Padina pavonica	1.4 ± 0.1^{eg}	1.1 ± 0.1^{de}	0.7 ± 0.0^{a}
	Sargassum vulgare	0.9 ± 0.0^{abc}	0.7 ± 0.0^{c}	1.2 ± 0.0^{d}

Results are presented as mean ± SD ($n = 3$). Different superscript letters in the same column indicate significant differences among all macroalgal species ($p < 0.05$).

Despite their low lipid levels overall, the proportions of physiologically important PUFA in the macroalgae analysed were higher than those of terrestrial plants (Wielgosz-Collin et al. 2016). C18 PUFA such as LA and ALA are considered essential FA for vertebrates since they cannot be synthesized *de novo*, and therefore, their incorporation through diet becomes necessary. The human capacity to endogenously produce LC-PUFA from their C18 PUFA precursors through successive elongation and desaturation processes was reported to be much lower than presumed (Metherel and Bazinet 2019). Since nearly 70% of the world's population does not reach the minimum recommended daily intake of n-3, due either to unhealthy nutritional habits or to difficulties accessing them, it is mandatory to include sources of n-3 LC-PUFA in the human diet for general health and wellbeing (D'Angelo et al. 2020; Taha 2020).

The present work demonstrates that the lipid class and FA composition should not be considered to be useful biomarkers for taxonomic studies in seaweeds, due to the high interspecific variability detected (Figures 2 and 3). However, green macroalgal species are often described as being characterized by high amounts of C16 FA (including 16:3n-3 and 16:4n-3) and C18 PUFA (LA and ALA, similar to terrestrial plants), with LC-PUFA being usually absent (Kendel et al. 2015; Nakamura and Li-Beisson, 2016; Santos et al. 2019). Likewise, the green species analysed here showed high proportions of 16:0, oleic acid (18:1n-9; OA), and C18 PUFA, such as LA in *Dasycladus vermicularis* and ALA in *Ulva* sp., while LC-PUFA contents were low. DHA was found in trace amounts in both species, as previously reported by McCauley et al. (2016). In contrast, red macroalgae were characterised by high levels of 16:0, OA and ARA, which also constitute an important source of EPA (Sánchez-Machado et al. 2004; Schmid et al. 2018). In the present study, *Halopithys incurva* displayed the highest proportions of ARA (~12% of total FA) and EPA (~9% of total FA), while DHA was highest in *Asparagopsis taxiformis* (6.6% of total FA). Therefore, the red macroalgae studied might be considered attractive sources of n-3 LC-PUFA (Sánchez-Machado et al. 2004), potentially promoting animal and human health and wellbeing (Murata and Nakazoe 2001; Zárate et al. 2017). In particular, *H. incurva* and *A. taxiformis* might be promising candidates to partially substitute for marine ingredients in aquafeed formulation, as with other red macroalgal species (Morais et al. 2020; Younis et al. 2018). However, some safety factors, such as the production of the halogenated toxic compounds described in *A. taxiformis* (Machado et al. 2016), must be considered before recommending this seaweed as a food or feed ingredient.

In the present study, the FA profiles of Dictyotales and Fucales were similar to those previously described by Kumari et al. (2010) and Santos et al. (2019) where 14:0, 16:0, OA, and C18-C20 PUFA were reported as the major FA. Among Ochrophyta, *Dictyota dichotoma* presented a high n-3 PUFA, especially SDA (18:4n-3) with 8.0% of total FA. SDA is the first metabolic intermediate in the conversion of ALA into EPA and DHA (Whelan 2009). The consumption of vegetable oils containing high SDA, such as *Echium* oil, was reported to improve the incorporation of n-3 PUFA, and especially EPA, into human tissues compared with vegetable oils containing ALA (Guil-Guerrero 2007).

Nonetheless, the importance of SDA in animal and human health might go beyond its function as a precursor of EPA and DHA, and offer beneficial effects similar to those of EPA (Whelan 2009).

The n-3 and n-6 PUFA families often show opposite physiological functions, with their relative proportions having implications for animal physiological and pathological mechanisms (Liu et al. 2015; Simopoulos 2016; Tocher et al. 2019). High n-6/n-3 ratios hamper the biosynthesis of n-3 LC-PUFA (Smink et al. 2012) and impair eicosanoid and docosanoid production (Zárate et al. 2017). All the macroalgae analysed had an n-6/n-3 FA ratio well below 10, as recommended by WHO (Matanjun et al. 2009) for potential human and animal health and wellbeing applications.

It is widely accepted that a reduction of SFA consumption and an increase in PUFA ingestion impacts human health positively by decreasing blood cholesterol (Moussa et al. 2014). AI, TI and hH indices indicate the relationship between pro-thrombogenic (saturated) and anti-thrombogenic (unsaturated) FAs (Özden et al. 2020), and they have been proposed as both nutritional fat quality indicators and measures of dietary propensity to influence the incidence of coronary heart disease (Moussa et al. 2014; Pérez et al. 2014; Santos-Silva et al. 2002). Therefore, lower dietary AI, TI, and higher hH would prevent the risk of appearance of coronary diseases (Gerasimenko and Logvinov 2016). In this sense, *Dasycladus vermicularis* (green alga), *Cystoseira usneoides* and *Sargassum vulgare* (brown algae), displayed the most favourable values of all species analysed for these cardiovascular health indicators.

In animal nutrition, the type of lipid in which FA is provided seems to be particularly relevant (Lund et al. 2018; Reis et al. 2021). Algal lipids can be divided into neutral lipids as storage compounds and polar lipids, including glycolipids and phospholipids, with major structural functions. Betaine lipids, not isolated in our study, are also common lipids in non-plastid membranes of algae, especially in green and brown seaweeds, and are being considered as replacement compounds for phosphatidylcholine (PC) under phosphorus deficiency (Huang et al. 2019). In fact, a reciprocal relationship between certain betaine lipids and PC has been proposed (Künzler and Eichenberger 1997; Nakamura and Li-Beisson 2016).

Although several studies have shown that Dictyotales, Fucales (Ochrophyta) and Ulvales (Chlorophyta) do not have PC (Wielgosz-Collin et al. 2016), our study supports that of Jones and Harwood (1992), where small amounts of PC were detected in fucoids such as *Fucus vesiculosus* and *Ascophyllum nodosum*. On the other hand, PC is expected in Rhodophyta species, being reported to represent up to 55–75% of total phospholipids (Wielgosz-Collin et al. 2016). PC is an interesting source of LC-PUFA-rich marine lecithin, which is of increasing interest for cosmetic, food, and pharmaceutical sectors (Alhajj et al. 2020).

All the macroalgae studied here had a higher content of glycolipids than phospholipids. Thus, Chlorophyta species had remarkably high percentages of both SQDG and DGDG, in contrast to other studies where MGDG was higher than SQDG and DGDG in green macroalgae (Khotimchenko 2002). Furthermore, MGDG and DGDG have been described as the most characteristic glycolipids in red algae (Wielgosz-Collin et al. 2016), although Khotimchenko (2002) reported high variability in glycolipid content among species. In our study, *Halopithys incurva* had the highest contents of SQDG, DGDG and MGDG among red seaweeds, while brown macroalgae had low contents of MGDG. According to Wielgosz-Collin et al. (2016), glycolipids do not seem to be valuable as a taxonomic character since they are present in all brown species.

The glycolipids MGDG and DGDG from marine organisms have been reported to have antifungal, fibrinolytic and antitumor activities (Gerasimenko and Logvinov 2016; Kendel et al. 2015; Wielgosz-Collin et al. 2016), which make seaweeds potentially interesting dietary components for human and animal nutrition, in addition to their higher LC-PUFA content than in terrestrial plants (Sahaka et al. 2020).

Corallina officinalis, *Halopithys incurva*, *Cystoseira usneoides*, *Lobophora* sp. and *Sargassum vulgare*, had high contents of phytosterols (PTS). Macroalgal PTS include several molecules such as fucosterol, stigmasterol, sitosterol and saringosterol, together with variable amounts of cholesterol (Schepers et al. 2020). PTS present benefits for cardiovascular diseases and anti-inflammatory processes (Kendel et al. 2015), and also decrease intestinal cholesterol absorption, reducing low-density lipoprotein-cholesterol (LDL-C) and therefore, reduce cardiovascular disease risk factors (Patch et al. 2006). Humans cannot biosynthesize PTS *de novo* (Kendel et al. 2015), again suggesting that macroalgae might be a potential source of these beneficial compounds for human nutrition.

5 Conclusions

The present study provided evidence of a high variability in the lipid contents, lipid classes and FA profiles of macroalgae, making a definition of a characteristic pattern within each phylum highly complex. Seasonal, environmental and even geographical factors affect these lipid profiles and should be considered in future research to ensure

biochemical stability or even to identify algal species with a sufficiently high or diverse content of lipid molecules to be of commercial interest.

Overall, the species analysed contained lower lipid levels but higher PUFA proportions than terrestrial plants, and had low n-6/n-3 FA ratios as recommended by WHO. Red macroalgae are an attractive source of n-3 LC-PUFA for human consumption and might also be considered as a potential substitute for marine ingredients in aquafeed formulations and production. The high contents of DGDG, MGDG, and PTS, together with the high levels of EPA, and low n-6/n-3 ratios makes *Halopithys incurva* an interesting macroalga from a nutritional point of view. *Asparagopsis taxiformis* (Rhodophyta) also contained a high proportion of DHA, and *Dictyota dichotoma* (Ochrophyta) an unusually high content of the nutraceutical SDA.

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