Title:

Polar lipid profile of *Saccharina latissima,* a functional food from the sea

Felisa REY[a,b,*], Diana LOPES[a,b], Elisabete MACIEL[a,b], João MONTEIRO[a,b], Jorunn SKJERMO[c], Jon FUNDERUD[d], Diogo RAPOSO[d], Pedro DOMINGUES[b] Ricardo CALADO[a] & M Rosário DOMINGUES[b,e]

[a] Departamento de Biologia & CESAM & ECOMARE, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

[b] Centro de Espetrometria de Massa, Departamento de Química & QOPNA, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

[c] SINTEF Ocean, N-7465 Trondheim, Norway

[d] Seaweed Energy Solution AS, Bynesveien 48, 7018 Trondheim, Norway

[e] Departamento de Química & CESAM & ECOMARE, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal

*Corresponding author
E-mail address: felisa.rey@ua.pt (Felisa Rey)

Abstract

Increasing global demand for food has generated a need for new strategies to feed a fast-growing population. Oceans appear as a sustainable solution, providing alternative sources of food such as macroalgae. These sea vegetables have high nutritional value and provide functional and health benefits. The sugar kelp *Saccharina latissima* is an emerging edible seaweed used mainly for human consumption. Although much is known about its biochemical compositions its lipidome remains unexplored. The aim of the present study was to characterize the polar lipid profile of *S. latissima* using a lipidomic-mass spectrometry HILIC-LC-MS based analysis. This approach allowed the identification of 197 molecular species of polar lipids, including glycolipids, phospholipids and betaine lipids. Several molecular species identified are carriers of polyunsaturated fatty acids with nutritional value and have been reported with anti-inflammatory, anti-microbial and anti-proliferative activity. *Saccharina latissima* is an emerging candidate to promote blue biotechnology inspired by the ocean.

Key words

Functional Food; Glycolipids; HILIC–ESI–MS; Lipidomic; Phospholipids; Seaweeds

Highlights

- *Saccharina latissima* is a promising source of lipid for food and feed industries
- Lipidome of *S. latissima* reveals the presence of potential bioactive compounds
- High nutritional value of *S. latissima* as source of polyunsaturated fatty acids
- Lipids from *S. latissima* could be incorporated in new high-value products

1. Introduction

The increasing world population has driven a surge on the search for new nutritional sources and a growing concern for healthiness and fitness have led consumers to demand healthier foods. One way to match these demands is looking for alternative solution in marine environments [1]. Oceans occupy the largest area of the biosphere, although most of them remain yet unexplored. Marine environments harbor a vast array of organisms providing them with unique biological properties. This is the case of macroalgae, which are adapted to diverse environments, sometimes even extreme [2], and to natural fluctuations on biotic and abiotic conditions (e.g. temperature, light, availability of nutrients, interaction with other species) [3]. This plasticity is reflected in their chemical phenotype and in their valuable primary and secondary metabolites [4–6], namely polysaccharides, protein, mineral, phenolic components and lipids [6,7]. Although, lipid content in macroalgae is relatively low, it includes relevant lipids, such as glycolipids and *n*-3 fatty acids which have already been recognized by their bioactive and nutritional value [8,9]. The role of dietary lipids in health, wellbeing, to protect or as potential therapeutic targets for disease has been increasingly recognized in the last decades [10–12]. In this sense, several studies have identified bioactive compounds in polar lipids isolated from macroalgae [8,13], as well as relevant health benefits associated to their consumption [6,13]. Therefore, the characterization of macroalgae lipidome and the identification of potential bioactive compounds are essential for their bioprospection, for finding healthful resources, as well as to find alternative sources of lipids for food, feed or to be incorporated in new high-value products.

The sugar kelp *Saccharina latissima* is a native European Atlantic macroalgae used mainly for human consumption [14], but due to development of cultivation also with

emerging applications in animal feed and potential for production of renewable fuels [2,15]. This edible seaweed has been recognized by its high concentration of essential minerals (e.g. iodine, calcium), sugars (e.g. glucose and mannitol), protein and high value compounds, such as phenolics (e.g. phlorotannins), fucoidan, fucoxanthin and alginates [5,15]. However, in spite of its recognized potential, the lipidome of *S. latissima* is yet to be unraveled and it is still limited to the characterization of fatty acid profile [16]. The use of mass spectrometry based approaches applied to lipidomic analysis have brought new insights in the survey of macroalgae lipidome and the identification of compounds with potential bioactivity [8,12,17]. In this sense, polar lipids identified in the lipidome of macroalgae, namely glycolipids and phospholipids, have been recognized as a natural source of polyunsaturated fatty acids (PUFA) and bioactive compounds [18,19].

The main goal of the present study was to identify and to characterize the polar lipid profile of *S. latissima*, using hydrophilic interaction liquid chromatography-electrospray ionization-mass spectrometry (HILIC–ESI–MS and M/MS). This analysis aims to elucidate the nutritional valued of this seaweed, in order to promote its valorization and to prospect potential bioactive lipid compounds with health benefits, which can foster the valorization and consumption of this macroalga as a functional food and/or as a source of add value compounds for nutraceutics.

2. Materials and methods

2.1. Sampling

Samples of *S. latissima* were cultivated at Seaweed Energy Solutions´ farm Taraskjæra at Frøya, Norway (N 63° 42.279', E 8° 52.232') at a depth of approx. 2 m, from early February to end of May 2016. Biomass was harvested on 23rd – 24th of

May 2016. After collection, 2 kg biomass samples were washed in filtered seawater, frozen using individual quick freezing (IQF) (vacuum packed) and stored at – 20 ºC. Before analysis, samples were frozen to – 80 ºC overnight and freeze-dried by SINTEF Ocean (Norway) during 2-3 d at – 50 ºC. The dried biomass was milled in a Mikro-Feinmühle-Culatti (IKA-Labortechnik). Storage and shipping for further processing were performed at -18 °C.

2.2. Lipid extraction

The Bligh and Dyer [20] method was used to extract total lipids from *S. latissima* samples. Freeze-dried samples were homogenized in a mortar and pestle to obtain small-sized flakes. A total biomass of 250 mg of macroalga was mixed with 2.5 mL of methanol and 1.25 mL of chloroform in glass centrifuge tubes. Following vigorous homogenization, samples were incubated on ice for 2 h and 30 minutes on an orbital shaker. Samples were centrifuged at 2000 rpm for 10 minutes at room temperature and the organic phase contained lipids was collected. Lipid extraction was repeated twice in the macroalgal biomass. In order to resolve a two-phase system, 2.3 mL of ultrapure water was added to the organic phase and after that the samples were centrifuged at 2000 rpm for 10 minutes at room temperature. The lipids recovered from the organic lower phase were dried under a nitrogen stream and preserved at −20 °C for further analysis. The final weight of the crude lipid extract was determined by gravimetry.

2.3. Fractionation of lipid extract

In order to isolate polar lipids, total lipid extracts were fractionated using a modification of Pacetti's method according to Rey et al. method [21]. Fractionation was performed by solid-phase extraction using a glass column with 500 mg of silica

gel (40–60 µm, 60 Å, Åcros Organics). Column was activated with 6 mL of *n*-hexane and then 3 mg of total lipid extract was applied after to be dissolved on 300 µL of chloroform. Subsequently, the following sequential elution was performed to separate lipid fractions: 5 mL of chloroform, 8 mL of diethyl ether:acetic acid (98:2, *v*/*v*), 6 mL of acetone:methanol (9:1, *v*/*v*) and 6 mL of methanol. Total lipid extracts were fractionated in four lipid fractions: fraction 1 (rich in neutral lipids), fraction 2 (rich in pigments), fraction 3 (rich in glycolipids) and fraction 4 (rich in phospholipids and betaine lipids). Fractions 3 and 4 were recovered separated and dried under nitrogen stream and stored at –20 °C prior to analysis by HILIC−LC−MS.

2.4. Hydrophilic interaction liquid chromatography–mass spectrometry (HILIC-LC-MS)

Total lipid extracts and fractions were analyzed by hydrophilic interaction liquid chromatography on a high-performance liquid chromatography (HPLC) Thermo Scientific Accela^{TM} with an autosampler coupled online to a Q-Exactive hybrid quadrupole mass spectrometer (Thermo Fisher, Scientific, Bremen, Germany). Analyses were performed using a solvent system with two mobile phases. A mobile phase A consisted of water, acetonitrile and methanol (25%, 50%, 25%), with 1 mM ammonium acetate, and a mobile phase B consisted of acetonitrile and methanol (60%, 40%), with 1 mM ammonium acetate. Solvent system started with 0% of mobile phase A, which was held isocratically for 8 minutes, a linear increase to 60% of mobile phase A was applied within 7 minutes and maintained for 15 minutes, returning to the initial conditions in 10 minutes. In order to analysis each sample by HILIC-LC-MS 10 µg of total lipid extract, 4 µL of phospholipid standards mix (dMPC - 0.02 µg, dMPE - 0.02 µg, , LPC - 0.02 µg, , dPPI - 0.08 µg, dMPG - 0.012 µg, dMPS - 0.04 µg) and 86 µL of eluent B were introduced into the Ascentis Si

column HPLC Pore column (15 cm × 1 mm, 3 µm, Sigma-Aldrich) with a flow rate of 40 µL minutes^{-1} at 30 ºC. Acquisition in the e Orbitrap® mass spectrometer was performed in positive (electrospray voltage 3.0 kV) and negative (electrospray voltage −2.7 kV) modes with high resolution with 70 000 and AGC target of 1e6, the capillary temperature was 250 ºC and the sheath gas flow was 15 U. In MS/MS experiments, a resolution of 17 500 and AGC target of 1e5 was used and the cycles consisted in one full scan mass spectrum and ten data-dependent MS/MS scans were repeated continuously throughout the experiments with the dynamic exclusion of 60 seconds and intensity threshold of 1e4. Normalized collision energy™ (CE) ranged between 25, 30 and 35 eV. Data acquisition was performed using the Xcalibur data system (V3.3, Thermo Fisher Scientific, USA) [21,22].

In lipidomic studies normal phase (NP) or HILIC columns and reversed-phase C18 columns have been used. The separation of lipids in these methods are based on different properties: NP or HILIC columns allow the separation based on their hydrophilicity, which is mainly dependent on the polar head properties, allowing the separation of different lipid classes in a single run. In reversed-phase C18 columns, lipids elute based on their hydrophobic properties, and thus separate the lipid species based on the number of carbons and the degree of unsaturation of the fatty acyl substituents, co-eluting ions of different classes [23].

Polar lipid molecular species were identified by recognition of ions observed in LC-MS spectra (Supplementary Figure S12) and using the bioinformatic software MZmine 2.30. Molecular species were confirmed by interpretation of HILIC-ESI-MS/MS fragmentation (see Supplementary Material for fragmentation interpretation) and mass accuracy (Qual Browser) of ≤ 5ppm (Supplementary Table S1).

2.5. Fatty acid analysis by gas chromatography – mass spectrometry (GC-MS)

Fatty acids of total lipid extract were transmethylated to be analyzed by GC-MS. In order to obtain fatty acid methyl esters (FAMEs) 30 μg of lipid were transferred to a Pyrex glass tube and dried under nitrogen. Dry lipids were mixed with 1 mL of n-hexane containing a $C_{19:0}$ internal standard (0.746 μg mL^{-1}, CAS number 1731-94-8, Merck, Darmstadt, Germany) and 200 μL of a methanolic solution of potassium hydroxide (2 mol L^{-1}), followed by 2 minutes of homogenization. After this step, 2 mL of a saturated solution of sodium chloride was added and the sample was centrifuged for 5 minutes at 2000 rpm to separate the phases. The organic (upper) phase containing the FAMEs was transferred to a microtube and completely dried under nitrogen [24]. FAMEs were then dissolved in 50 μL *n*-hexane and 2 μL of this solution was used for GC-MS analysis on an Agilent Technologies 6890 N Network chromatograph (Santa Clara, CA, USA) equipped with a DB-FFAP column (123-3232, J & W Scientific, Folsom, CA, USA) with 30 m length, an internal diameter of 0.32 mm and a film thickness of 0.25 μm. The GC was connected to an Agilent 5973 Network Mass Selective Detector operating with an electron impact mode at 70 eV and scanning the mass range *m/z* 50 – 550 in a 1 s cycle in a full scan mode acquisition. The oven temperature was programmed from an initial temperature of 80 °C, standing at this temperature for 3 minutes, followed by three consecutive linear increments to 160 °C at 25 °C $minute^{-1}$, to 210 °C at 2 °C $minute^{-1}$, and to 250 °C at 30 °C $minute^{-1}$. Temperature was maintained at 250 ºC for 10 minutes. The injector and detector temperatures were 220 and 280 °C, respectively. Helium was used as carrier gas at a flow rate of 1.3 mL $minute^{-1}$ [22]. Fatty acid identification was performed by comparing their retention time and mass spectrum with MS spectra of the commercial FAME standards Supelco 37 and confirmed by interpretation of MS spectra [25]. Fatty acid amounts were calculated using calibration curves of FAME

standards Supelco 37 Component FAME Mix (ref. 47885-U, Sigma-Aldrich, Darmstadt, Germany) analyzed by GC-MS under the same conditions of FAMEs samples and using $C_{19:0}$ (0.746 μg mL^{-1}) as internal standard. Fatty acids were expressed both as total amount of fatty acid by biomass dry weight (DW) (μg mg DW^{-1}) and as percentage (%).

3. Results and Discussion

The lipid extract of *S. latissima* represented 1.9% ± 0.4 (± SD) of total dry biomass. Analyses of lipid extracts by HILIC−LC−MS and MS/MS allowed the identification of polar lipids, such as glycolipids, phospholipids, arsenolipids and betaine lipids. Overall, 197 molecular species were identified, being grouped as follows: glycolipids (57), phospholipids (120), arsenolipids (12) and betaine lipids (8) (Supplementary Table S1).

3.1. Glycolipid profile

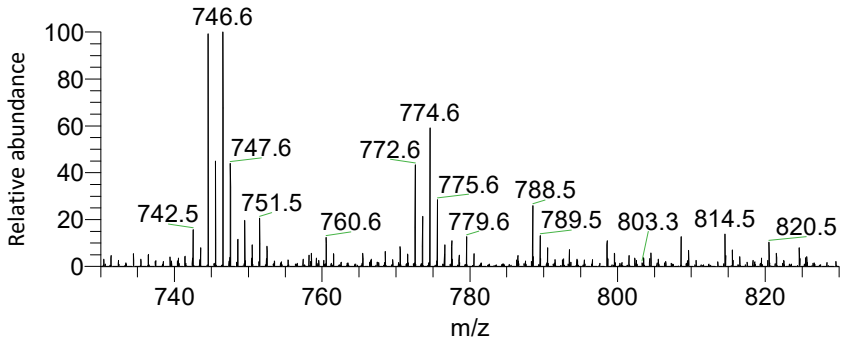
Glycolipids were distributed between galactolipids (monogalactosyl diacylglycerol, MGDG and digalactosyl diacylglycerol, DGDG) and sulfolipids (sulfoquinovosyl monoacylglycerol, SQMG and sulfoquinovosyl diacylglycerol, SQDG) classes.

3.1.1 Galactolipids

The neutral glycolipids identified corresponded to two classes of galactolipids, MGDG (Fig. 1) and DGDG (Fig. 2). Galactolipids were observed in HILIC-LC-MS spectra as positive $[M + NH_4]^+$ ions and their composition were confirmed by the interpretation of the MS/MS spectra (see Supplementary Figure S1 and S2, respectively). A total of 14 molecular species of MGDG were identified, being the most abundant species observed as $[M + NH_4]^+$ ions at *m/z* 744.6 and 746. 6 and assigned as MGDG (18:2/14:0) and MGDG (18:1/14:0), respectively (Fig. 1).

Regarding DGDG, a total of 15 molecular species were identified. The DGDG (20:5/18:4) correspond to $[M + NH_4]^+$ ion at *m/z* 976.6 and DGDG (20:5/18:3) correspond to *m/z* 978.6 represented the molecular species with higher relative abundance (Fig. 2).

a)

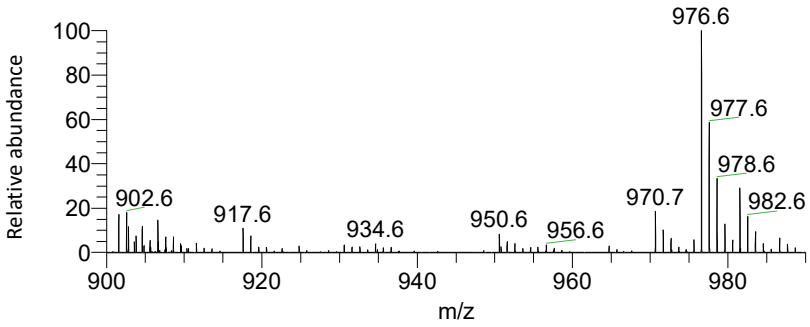


b)

$[M+NH_4]^+$ *m/z*	Lipid species (C:N)	Fatty acyl chains
740.5305	MGDG(32:4)	18:4/14:0; 18:3/14:1; 16:4/16:0
742.5461	MGDG(32:3)	18:3/14:0; 16:3/16:0
744.5610	**MGDG(32:2)**	**18:2/14:0; 18:1/14:1**
746.5780	**MGDG(32:1)**	**18:1/14:0; 16:1/16:0**
748.5941	MGDG(32:0)	18:0/14:0; 16:0/16:0
768.5624	MGDG(34:4)	20:4/14:0; 18:4/16:0; 18:3/16:1;18:2/16:2
770.5755	MGDG(34:3)	18:3/16:0; 18:2/16:1; 18:1/16:2; 18:0/16:3; 20:3/14:0
772.5932	MGDG(34:2)	18:2/16:0; 18:1/16:1
774.6090	MGDG(34:1)	18:1/16:0
776.6229	MGDG(34:0)	18:0/16:0; 20:0/14:0
788.5296	MGDG(36:8)	18:4/18:4; 20:5/16:3
790.5458	MGDG(36:7)	18:4/18:3
814.5462	MGDG(38:9)	20:5/18:4; 20:4/18:5; 20:6/18:3
828.6591	MGDG(38:2)	22:2/16:0

Figure 1 Lipidomic profile of monogalactosyl diacylglycerol (MGDG) identified in *Saccharina latissima* samples. a) HILIC-ESI-MS spectrum of MGDG showing the molecular species detected as $[M + NH_4]^+$ ions. b) Molecular species of MGDG (C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains; bold *m/z* values correspond to the most abundant molecular species detected in HILIC-ESI-MS spectrum).

a)



b)

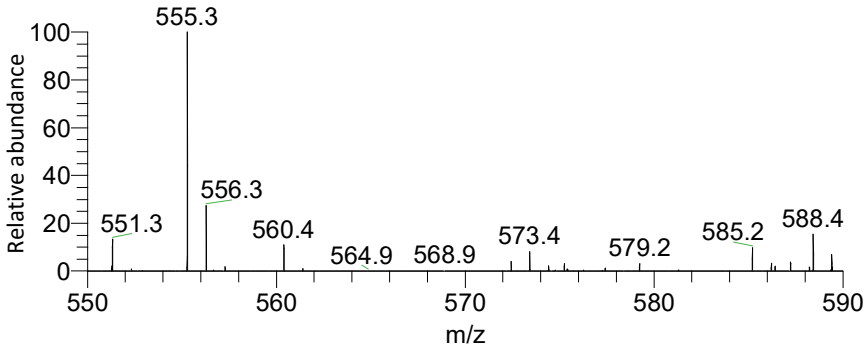
$[M+NH_4]^+$ *m/z*	Lipid species(C:N)	Fatty acyl chains
902.5854	DGDG(32:4)	18:4/14:0; 20:4/12:0; 16:2/16:2
904.5981	DGDG(32:3)	18:3/14:0
906.6140	DGDG(32:2)	18:2/14:0
908.6299	DGDG(32:1)	18:1/14:0; 16:1/16:0; 18:0/14:1
932.6304	DGDG(34:3)	18:3/16:0; 18:2/16:1; 20:3/14:0
934.6461	DGDG(34:2)	18:2/16:0; 18:1/16:1; 20:1/14:1; 20:2/14:0
936.6613	DGDG(34:1)	18:1/16:0; 18:0/16:1; 20:1/14:0
938.6765	DGDG(34:0)	18:0/16:0; 20:0/14:0
950.5841	DGDG(36:8)	18:4/18:4; 20:5/16:3
952.5982	DGDG(36:7)	18:4/18:3; 20:5/16:2; 20:4/16:3; 20:6/16:1
956.6315	DGDG(36:5)	20:5/16:0; 18:3/18:2; 22:5/14:0; 20:4/16:1; 18:4/18:1
976.5979	**DGDG(38:9)**	**20:5/18:4**
978.6127	DGDG(38:8)	20:5/18:3; 20:4/18:4
980.6276	DGDG(38:7)	20:4/18:3; 20:5/18:2; 20:3/18:4; 20:6/18:1; 20:2/18:5
984.6610	DGDG(38:5)	20:4/18:1; 20:5/18:0; 20:1/18:4

Figure 2 Lipidomic profile of digalactosyl diacylglycerol (DGDG) in *Saccharina latissima* samples. a) HILIC-ESI-MS spectrum of DGDG showing the molecular species detected as $[M + NH_4]^+$ ions; b) Molecular species of DGDG (C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains; bold *m/z* value corresponds to the most abundant molecular species detected in HILIC-ESI-MS spectrum).

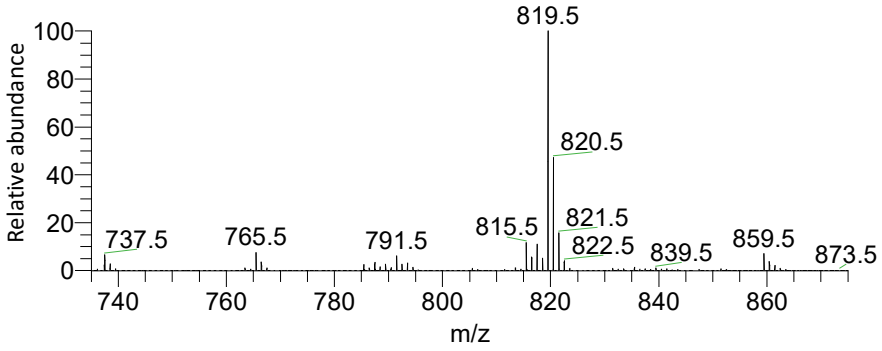
3.1.2 Sulfolipids

Two classes of anionic glycolipids were identified by HILIC-LC-MS and MS/MS in negative mode. These classes were the SQDG and its lyso form SQMG (Fig. 3), both identified in the LC-MS spectra as $[M - H]^-$ ions (see Supplementary Figure S3). Three molecular species of SQMG were identified, and the most abundant was detected at *m/z* 555.3, corresponding to the SQMG (16:0) (Fig. 3). Concerning SQDG class, 25 molecular species were identified and the two most abundant were identified at *m/z* 819.5 and 821.5, corresponding to the$[M - H]^-$ ions of the SQDG (18:1/16:0) and SQDG (18:0/16:0), respectively (Fig. 3).

a)



b)



c)

[M-H]⁻ *m/z*	Lipid species	Fatty acyl chain
555.2838	**SQMG (16:0)**	**16:0**
575.2532	SQMG (18:4)	18:4
581.3002	SQMG (18:1)	18:1

d)

[M-H]⁻ *m/z*	Lipid species (C:N)	Fatty acyl chains
737.4512	SQDG (28:0)	14:0/14:0
763.4662	SQDG (30:1)	16:1/14:0; 16:0/14:1
765.4815	SQDG (30:0)	16:0/14:0
779.4947	SQDG (31:0)	16:0/15:0; 17:0/14:0
785.4514	SQDG (32:4)	18:4/14:0
787.4663	SQDG (32:3)	18:3/14:0
791.4974	SQDG (32:1)	16:1/16:0; 18:1/14:0
793.5121	SQDG (32:0)	16:0/16:0; 18:0/14:0
805.5127	SQDG (33:1)	17:1/16:0; 18:1/15:0; 19:1/14:0
807.5264	SQDG (33:0)	17:0/16:0
813.4834	SQDG (34:4)	18:4/16:0; 20:4/14:0; 18:3/16:1; 18:2/16:2
815.4967	SQDG (34:3)	18:3/16:0
817.5134	SQDG (34:2)	18:2/16:0; 20:2/14:0; 18:1/16:1
819.5299	**SQDG (34:1)**	**18:1/16:0**
821.5437	SQDG (34:0)	18:0/16:0; 20:0/14:0
833.5430	SQDG (35:1)	19:1/16:0; 18:1/17:0
835.5604	SQDG (35:0)	19:0/16:0
837.4837	SQDG (36:6)	18:3/18:3
839.4970	SQDG (36:5)	20:5/16:0; 18:3/18:2; 20:4/16:1; 18:4/18:1
841.5135	SQDG (36:4)	20:4/16:0; 18:2/18:2; 18:3/18:1; 18:4/18:0
843.5277	SQDG (36:3)	20:3/16:0; 18:2/18:1; 18:3/18:0
845.5448	SQDG (36:2)	20:2/16:0; 18:1/18:1; 18:2/18:0
849.5760	SQDG (36:0)	20:0/16:0; 18:0/18:0; 22:0/14:0
859.4665	SQDG (38:9)	20:5/18:4
871.5601	SQDG (38:3)	22:3/16:0; 18:2/18:1; 18:3/18:0

Figure 3 Lipidomic profile of sulfolipids identified in *Saccharina latissima* samples. a) HILIC-ESI-MS spectrum of sulfoquinovosyl monoacylglycerol (SQMG) showing the molecular species detected as $[M -H]^-$ ions. b) HILIC-ESI-MS spectrum of sulfoquinovosyl diacylglycerol (SQDG) showing the molecular species detected as $[M -H]^-$ ions. c) Molecular species of SQMG. d) Molecular species of SQDG (C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains; bold *m/z* values correspond to the most abundant molecular species detected in HILIC-ESI-MS spectrum).

Glycolipids are abundant in photosynthetic organisms, being present in chloroplast and thylakoid membranes, where they play an essential role in physiological processes, as signal and regulatory molecules, and lipid trafficking between subcellular compartments [21,26]. This exclusive lipid class has sparked phytochemical researchers' interest because it is a natural source of PUFA and bioactive compounds, with recognized healthful benefits [13,18,19]. Several functional bioactivities have been identified in glycolipids isolated from marine organisms including macroalga, namely anti-inflammatory, antioxidant, antimicrobial and antitumoral properties [8,17,27]. The presence of biological activity is strongly linked with the chemical features of molecular species [13]. In glycolipids, bioactivity has been associated with the structure of the sugar moiety, its anomeric configuration, the position of the glycerol linkage to the sugar, and specially with the length, number and position of double bonds in the acyl chains (Bruno et al., 2005; Zhang et al., 2014). Several studies have identified biological activity in some molecular species of glycolipids, and reported that the presence of PUFA in their composition is a key feature in the structure-activity relationship, corroborating that fatty acyl composition is closely related with glycolipid bioactivity [13,28]. Previous studies have recognized bioactive effects in several molecular species of glycolipids that have been identified in the lipidome of *S. latissima*. For instance, the sulfolipids SQDG (20:5/16:0) was mentioned to have antitumoral activity as inhibitor of eukaryotic DNA polymerases [31], SQDG (16:0/16:0) and SQDG (18:0/14:0) with antiviral activity against herpes simplex virus (HSV-1 and HSV-2) and Coxsackie virus B3 (Cox B3) [32]; SQMG (16:0) and SQDG (18:2/16:0) have been referred to display high antitumoral activity against human breast carcinoma (MCF-7 cells) and human hepato carcinoma (HepG2), as well as antimicrobial activity, with a high growth inhibition of *Bacillus*

subtilis and *Escherichia coli* [33]. Moreover, galactolipids isolated from macroalgae have been reported to display bioactive proprieties. The MGDG (18:4/|16:0) and DGDG (20:5/16:0) identified in richness extract of polar lipids showed inhibition of nitric oxide production revealing their anti-inflammatory potential [18,34]. These glycolipids have been identified in the present study, as molecular species of *S. latissima* lipidome. Bioactive polar lipids molecules present in their structure C_{16} and C_{18} saturated and unsaturated fatty acids, and C_{20} PUFA [31,34]. These fatty acids are well documented for their health benefits to humans, specially the long chain *n*-3 fatty acids, such as α-linolenic (ALA, $C_{18:3n-3}$), eicosapentaenoic (EPA, $C_{20:5n-3}$) and docosahexaenoic acids (DHA, $C_{22:6n-3}$) [35,36].

3.2 Phospholipid profile

Analysis of HILIC-LC-MS allowed the identification of eight phospholipid classes and 120 molecular species. Additionally, a total of 12 molecular species of arsenolipids, concretely arsenic-containing phospholipids (AsPL), were also identified.

PGs and LysoPGs (LPGs) were identified by HILIC-LC-MS in negative mode as $[M - H]^-$ ions (see Supplementary Figure S4). A total of 8 and 22 molecular species of LPGs and PG (Fig. 4) have been identified, respectively. The most abundant molecular species of LPG were the LPG (16:1) and LPG (16:0), correspond to $[M - H]^-$ ions at *m/z* 481.3 and *m/z* 483.3, respectively (Fig. 4). Furthermore, the most abundant molecular species of PG were PG (16:0/18:2) and PG (16:0/18:1), correspond to $[M - H]^-$ ions at *m/z* 745.5 and *m/z* 747.5, respectively (Fig. 4). Phosphatidylcholines (PC) and LysoPC (LPC) were observed as $[M + H]^+$ ions in HILIC-LC-MS spectra and acyl chains were confirmed by HILIC-LC-MS/MS as $[M + CH_3COO]^-$ ions (see Supplementary Figure S5). The analysis of HILIC-LC-MS

spectra allowed the identification of 6 and 30 molecular species of LPC (Supplementary Table S2) and PC (Fig. 5), respectively. The most abundant molecular species of PC were PC (14:0/20:4) and PC (16:0/20:4), correspond to $[M + H]^+$ ions at *m/z* 754.5 and *m/z* 782.6, respectively (Fig. 5).

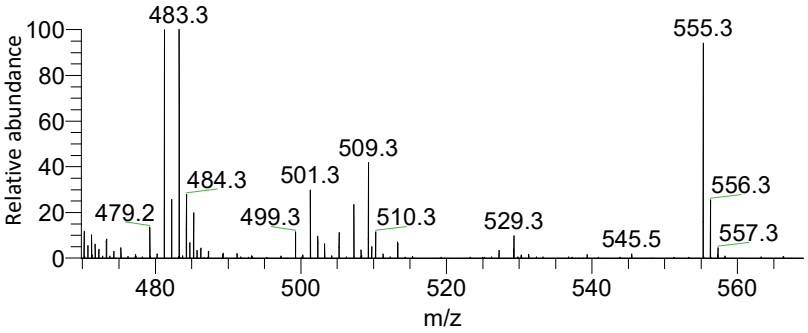
A total of 12 molecular species of phosphatidylethanolamines (PE) have been identified as $[M + H]^+$ ions (Supplementary Figure S6). The most abundant molecular species of PE were PE (30:3) and PE (36:1), correspond to $[M + H]^+$ ions at *m/z* 658.4 and *m/z* 746.6, respectively (Supplementary Figure S6).

Phosphatidylinositols (PI) and LysoPI (LPI) were analyzed in negative ion mode with the formation of $[M - H]^-$ ions. A total of 4 and 22 molecular species of LPI (Supplementary Figure S7) and PIs (Supplementary Figure S8) have been identified, respectively. The most abundant molecular species of PI were PI (14:0/18:3) and PI (16:0/18:1), correspond to $[M - H]^-$ ions at *m/z* 803.5 and 835.5, respectively (Supplementary Figure S7).

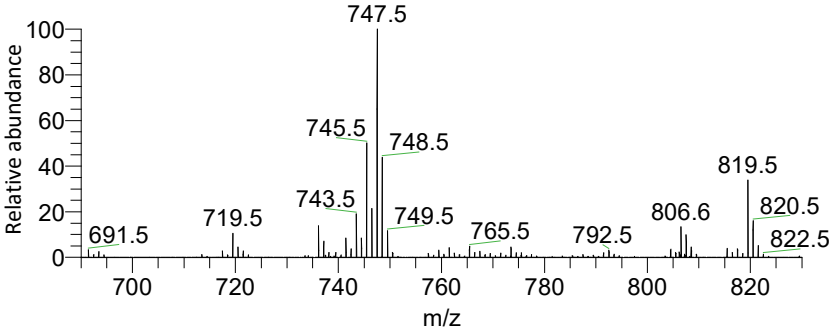
Phosphatidic acid (PA) were analyzed by HILIC-LC-MS in negative-ion mode with the formation of $[M - H]^-$ ions. Overall, 16 molecular species have been identified, being the PA (14:0/16:0) the most abundant molecular species, corresponds to $[M - H]^-$ ion at *m/z* 619.4 (Supplementary Figure S9).

AsPL were analyzed by HILIC-LC-MS in positive-ion mode with the formation of $[M + H]^+$ ions, and a total of 12 molecular species have been identified, being the ion AsPL (32:0) the most abundant, corresponding to $[M + H]^+$ at *m/z* 959.5 (Supplementary Figure S10).

a)



b)



c)

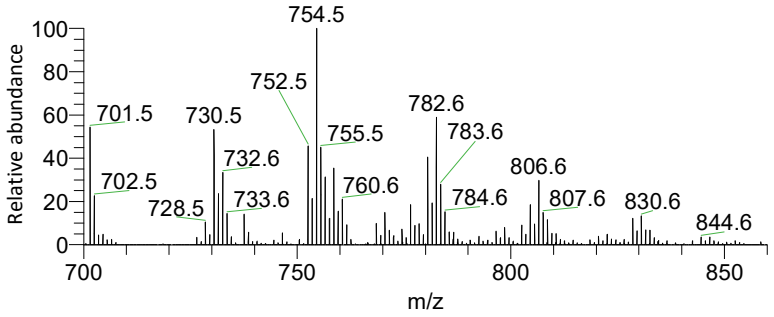
[M-H]⁻ *m/z*	Lipid species (C:N)	Fatty acyl chain
481.2570	**LPG(16:1)**	**16:1**
483.2730	**LPG(16:0)**	**16:0**
505.2569	LPG(18:3)	18:3
507.2732	LPG(18:2)	18:2
509.2880	LPG(18:1)	18:1
529.2578	LPG(20:5)	20:5
535.3038	LPG(20:2)	20:2
557.2894	LPG(22:5)	22:5

d)

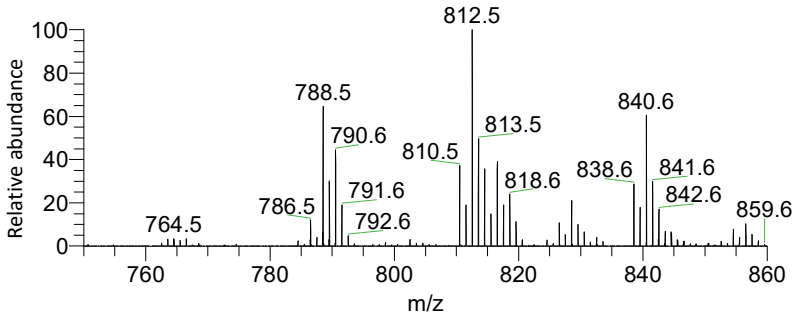
[M-H]⁻ *m/z*	Lipid species (C:N)	Fatty acyl chains
691.4550	PG(30:1)	14:0/16:1; 14:1/16:0
693.4710	PG(30:0)	14:0/16:0
717.4714	PG(32:2)	14:0/18:2; 16:1/16:1; 16:0/16:2
719.4861	PG(32:1)	16:0/16:1; 14:0/18:1
721.5025	PG(32:0)	16:0/16:0
739.4547	PG(34:5)	14:0/20:5; 16:3/18:2; 16:1/18:4; 14:1/20:4
741.4700	PG(34:4)	16:1/18:3; 14:0/20:4; 16:0/18:4
743.4863	PG(34:3)	16:0/18:3; 16:1/18:2
745.5026	PG(34:2)	16:0/18:2; 16:1/18:1; 14:0/20:2
747.5187	**PG(34:1)**	**16:0/18:1; 14:0/20:1**
749.5336	PG(34:0)	16:0/18:0; 14:0/20:0
759.5147	PG(35:2)	16:1/19:1; 17:0/18:2
761.5335	PG(35:1)	16:0/19:1
763.5499	PG(35:0)	16:0/19:0
765.4717	PG(36:6)	18:3/18:3; 18:2/18:4; 16:1/20:5; 16:0/20:6
767.4866	PG(36:5)	18:2/18:3; 16:0/20:5; 16:1/20:4; 14:0/22:5
769.5027	PG(36:4)	18:2/18:2; 18:1/18:3; 16:0/20:4
771.5176	PG(36:3)	18:1/18:2; 16:0/20:3; 16:1/20:2
773.5332	PG(36:2)	18:1/18:1; 18:0/18:2; 16:0/20:2
775.5475	PG(36:1)	18:1/18:0; 16:0/20:1
795.5174	PG(38:5)	16:0/22:5; 18:0/20:5; 18:1/20:4; 18:2/20:3; 18:3/20:2
821.5329	PG(40:6)	18:1/22:5; 18:0/22:6; 20:2/20:4

Figure 4 Lipidomic profile of phosphatidylglycerol (PG) and lysoPG (LPG) identified in *Saccharina latissima* samples. a) HILIC-ESI-MS spectrum of LPG showing the molecular species detected as $[M -H]^-$ ions. b) HILIC-ESI-MS spectrum of PG showing the molecular species detected as $[M -H]^-$ ions. c) Molecular species of LPG. d) Molecular species of PG (C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains; bold *m/z* values correspond to the most abundant molecular species detected in HILIC-ESI-MS spectrum).

a)



b)



c)

[M+H]$^+$ *m/z*	[M+CH_3COO]$^-$ *m/z*	Lipid species (C:N)	Fatty acyl chains
704.5249	762.5285	PC(30:1)	**
726.5065	784.5128	PC(32:4)	**
728.5233	786.5288	PC(32:3)	14:2/18:1; 14:1/18:2
730.5370	788.5425	PC(32:2)	14:0/18:2
732.5538	790.5593	PC(32:1)	14:0/18:1; 16:0/16:1
746.5667	804.5722	PC(33:1)	16:0/17:1; 14:0/19:1
752.5230	810.5285	PC(34:5)	14:0/20:5
754.5388	**812.5443**	**PC(34:4)**	**14:0/20:4**
756.5537	814.5592	PC(34:3)	14:0/20:3; 16:0/18:3
758.5707	816.5762	PC(34:2)	16:0/18:2; 16:1/18:1
760.5838	818.5893	PC(34:1)	16:1/18:0
768.5533	826.5588	PC(35:4)	**
774.5065	832.5120	PC(36:8)	18:4/18:4
776.5200	834.5255	PC(36:7)	18:2/18:5; 16:1/20:6
778.5359	836.5414	PC(36:6)	18:1/18:5; 16:0/20:6; 16:2/20:4
780.5525	838.5580	PC(36:5)	16:0/20:5
782.5679	840.5734	PC(36:4)	16:0/20:4
784.5849	842.5904	PC(36:3)	16:0/20:3; 18:1/18:2; 18:0/18:3
786.6004	844.6059	PC(36:2)	18:1/18:1; 18:0/18:2; 16:0/20:2; 14:0/22:2
802.5370	860.5425	PC(38:8)	18:2/20:6
804.5516	862.5571	PC(38:7)	18:1/20:6; 18:3/20:4; 18:4/20:3; 18:5/20:2
806.5685	864.5740	PC(38:6)	18:2/20:4
808.5829	866.5884	PC(38:5)	18:1/20:4
810.5993	868.6048	PC(38:4)	18:0/20:4; 20:0/18:4
826.5384	884.5439	PC(40:10)	20:4/20:6
828.5539	886.5594	PC(40:9)	20:4/20:5
830.5698	888.5753	PC(40:8)	20:4/20:4
832.5840	890.5895	PC(40:7)	20:3/20:4; 20:2/20:5
834.5987	892.6042	PC(40:6)	18:2/22:4
852.5538	910.5593	PC(42:11)	20:5/22:6

Figure 5 Lipidomic profile of phosphatidylcholine (PC) identified in *Saccharina latissima* samples. a) HILIC-ESI-MS spectrum of PC showing the molecular species detected as $[M+H]^+$ ions and b) $[M+CH_3COO]^-$ ions. c) Molecular species of PC (C represents the total number of carbon atoms and N the total number of double bonds on the fatty acyl chains; bold *m/z* value corresponds to the most abundant molecular species detected in HILIC-ESI-MS spectrum).

** Molecular species identified only by mass accuracy.

Phospholipids are recognized by their structural role in cell membranes, whose properties, such as fluidity, depend on their fatty acid composition. However, recent studies have identified phospholipids as a source of PUFA (e.g. *n*-3 fatty acids) by delivering their fatty acids, and even endorsed their therapeutic and beneficial health effects when they are ingested in the diet [37]. The use of phospholipids as source of PUFA was studied by Tang et al. [38], who have compared the efficiency of *n*-3 fatty acids absorption in the forms of free fatty acids, ethyl esters, esterified into triglycerides or phospholipids, in mice. Phospholipid bound *n*-3 fatty acids presented a superior bioavailability, increasing the concentration of DHA in the tissues analyzed (e.g. brain and liver), likely due to the amphiphilic character of these compounds and their interaction with cellular membranes [38,39]. Furthermore, phospholipid bound *n*-3 fatty acids was identified by decreasing hepatic and serum total cholesterol and triglycerides levels [38]. Therefore, phospholipids represent an available source of PUFA, providing beneficial nutritional effects through the transport of *n*-3 fatty acids, which for instance are essential to a healthy human brain or even protect against different diseases such as cancer, cardiovascular risks or neurological disorders [19,37].

Very few studies have addressed phospholipids bioactivity, although some molecular species of PG have been reported with anti-inflammatory proprieties. Banskota et al. [18] identified two PG molecular species with strong inhibitory activity against lipopolysaccharide induced nitric oxide production. One of these PG molecular species identified in this study [18] was the PG (16:0/20:5), which was also present in *S. latissima* lipidome identified in the present study.

Arsenolipids were identified for the first time in the brown alga *Undaria pinnatifida* (wakame) [40]. This class of lipids occurs naturally in marine foods, such as fish and

macroalgae [41–43]. Their presence in *S. latissima* was already reported by Raab et al. [44], who suggested that they can related with the protection against peroxidation by intensive UV radiation and/or salinity changes, typical damages of macroalgae inhabiting intertidal environments. Although the presence of inorganic arsenic in water is considered an environmental health problem, few studies have addressed the toxicity of organic arsenic. Nevertheless, several studies have classified certain molecular species of arsenolipids (i.e. arsenic-containing hydrocarbons and arsenic-containing fatty acids) as potentially cytotoxic for human cells, by their ability to cross the blood brain barrier (studies performed in the fruit fly *Drosophila melanogaster*) [45,46]. More toxicological studies are needed to evaluate the risk of these compounds.

3.3 Betaine lipids

Two classes of betaine lipids have been identified as $[M + H]^+$ ions, monoacylglyceryl-N,N,N-trimethyl homoserine (MGTS) and diacylglyceryl-N,N,N-trimethyl homoserine (DGTS). One molecular species of MGTS was identified MGTS (16:2), corresponds to the *m/z* $[M + H]^+$ ion at 470.4. Furthermore, 7 molecular species of DGTS were identified, being DGTS (18:1/16:1) the most abundant and corresponding to the *m/z* $[M + H]^+$ ion at 736.6 (Supplementary Figure S11). Betaine lipids are widely distributed in algae, where they display different functions as donors of diacylglycerols and fatty acids to be used in the biosynthesis of other lipid classes [47].

3.4 Fatty acid profile

Analysis by GC-MS showed that total fatty acid content represented 2.8 ± 0.3 µg mg DW $^{-1}$, corresponding with 15.2% ± 2.5 of total lipid extract and 0.3% ± 0.0 of total biomass. The fatty acid profile of *S. latissima* displayed $C_{16:0}$ and $C_{18:0}$ (0.8 ± 0.2 and

0.4 ± 0.2 µg mg DW $^{-1}$, respectively) as the most abundant fatty acids, representing 29.4 % ± 3.0 and 15.5 % ± 4.6 of total fatty acid content, respectively (Fig. 6). Additionally, relevant PUFA have been identified, such as $C_{18:2n\text{-}6}$ (0.2 ± 0.0 µg mg DW $^{-1}$, 6.5% ± 1.3), $C_{18:3n\text{-}3}$ (0.1 ± 0.0 µg mg DW $^{-1}$, 3.5% ± 0.5), $C_{18:4n\text{-}3}$ (0.1 ± 0.0 µg mg DW $^{-1}$, 4.6% ± 0.7), $C_{20:4n\text{-}6}$ (0.2 ± 0.0 µg mg DW $^{-1}$, 5.6% ± 1.0) and $C_{20:5n\text{-}3}$ (0.2 ± 0.0 µg mg DW $^{-1}$, 5.5% ± 0.8). *Saccharina latissima* samples showed a *n*-6/*n*-3 ratio of 0.9 ± 0.1.

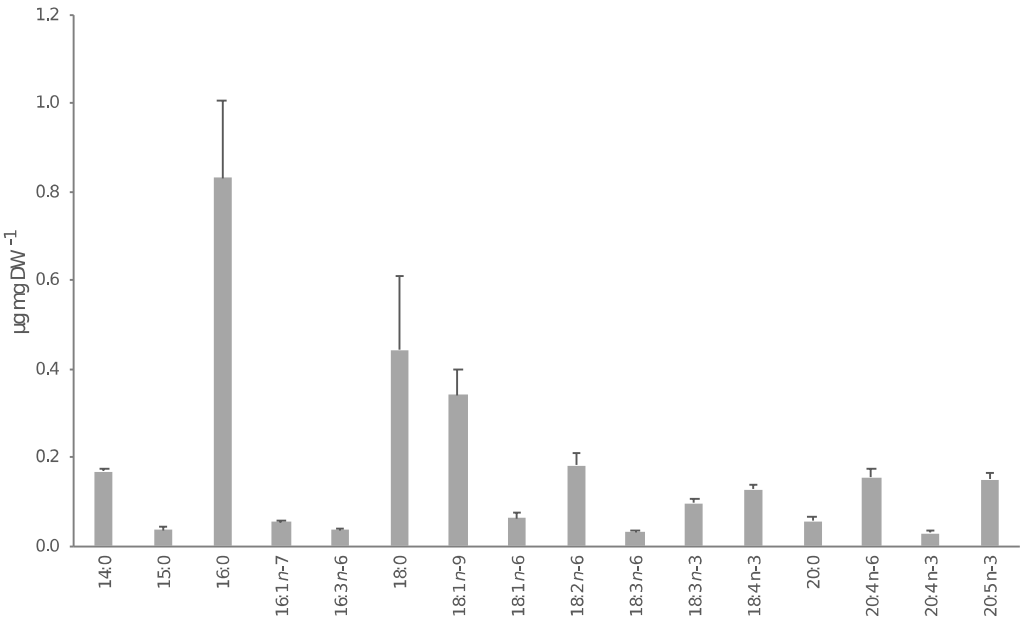


Figure 6 Fatty acid profile of *Saccharina latissima* samples as µg mg dry weight $^{-1}$ (DW), determined by GC-MS analysis of five samples (n = 5).

This macroalga revealed to be a rich source of PUFA and, as referred above, these are mainly esterified in polar lipid molecular species (e.g. glycolipids and phospholipids). Essential PUFA are critical lipids that must be obtained from dietary sources to sustain homeostasis, being marine macroalgae one of their natural sources [48]. Humans, as most animals, lack the ability to synthesize linoleic (LnA, $C_{18:2n-6}$) and ALA. The latter is the precursor of two physiologically essential fatty acids, EPA and DHA. Nevertheless, humans have a limited ability to desaturate and elongate the precursors of these fatty acids to fulfil their own demand. Therefore, certain PUFA such as EPA and DHA must be supplied through the diet, being seaweed a rich source of these PUFA. Nowadays, western diets present high levels of *n*-6 PUFA, with a nutritional ratio *n*-6/*n*-3 greater than 2, which has been associated with increasing risks of mortality due to cancer, cardiovascular, inflammatory and autoimmune diseases [49]. However, marine macroalgae, such as *S. latissima*, present a much higher prevalence of *n*-3 PUFA than land vegetables. A diet rich in *n*-3 PUFA can reduce *n*-6/*n*-3 ratio, being nutritionally more healthful and contributing to the prevention of chronic diseases [36,50]. Several studies have demonstrated the nutritional value of *n*-3 PUFA and the health benefits associated with their consumption [12,36,38,50]. Additionally, *n*-3 PUFA play a relevant role in the normal development and functioning of brain and central nervous system, being involved in memory formation and neuronal signaling [36,51]. The protective role of *n*-3 PUFA has proved to be crucial during early brain development stages, suggesting that a low consumption of these PUFA during childhood may be associated with neurocognitive disorders [52].

4. Conclusions

Lipidome characterization of *S. latissima* was performed by the identification of 197 molecular species of polar lipids, including glycolipids, phospholipids and betaine lipids, some of which have been recognized by their bioactivity. This study endorses *S. latissima* as a promising source of natural bioactive lipid compounds with potential health and therapeutic benefits. Furthermore, this macroalgae is featured by its high nutritional value as a source of PUFA and low *n*-6/*n*-3 ratio. *Saccharina latissima* is an emerging candidate to promote blue biotechnology inspired by the ocean, with applications in food, feed and nutraceutical industries.

Acknowledgments

This study was financed by the Project GENIALG, funding from the European Union's Horizon 2020 Framework Programme under grant agreement No 727892. The authors are thankful to the Marine Lipidomics Laboratory, University of Aveiro, Fundação para a Ciência e a Tecnologia (FCT, Portugal), European Union, QREN, Programa Operacional Factores de Competitividade (COMPETE) and FEDER. Thanks are also due to FCT/MEC, for the financial support to QOPNA research Unit (FCT UID/QUI/00062/2019), to CESAM (UID/AMB/50017/2019), to the Portuguese Mass Spectrometry Network (LISBOA-01-0145-FEDER-402-022125), to FCT/MEC through national funds, and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020.

Felisa Rey (BPD/UI51/7192/2017 from European Union's Horizon 2020 and SFRH/BPD/115347/2016) and Diana Lopes (SFRH/BD/119027/2016) were supported by FCT, Programa Operacional do C Framework Programme apital Humano (POCH) and European Union through European Social Fund (ESF).

Conflict of interest

The authors declare that they have no competing interests.

Authors contributions

- Conception and design of the study: Felisa Rey, Ricardo Calado, Rosário Domingues
- Analysis and interpretation of the data: Felisa Rey, Diana Lopes, Elisabete Maciel, João Monteiro, Rosário Domingues
- Drafting of the article: Felisa Rey, Rosário Domingues
- Critical revision of the article for important intellectual content: Felisa Rey, Diana Lopes, Elisabete Maciel, Jorunn Skjermo, Jon Funderud, Diogo Raposo, Pedro Domingues, Ricardo Calado, Rosário Domingues
- Final approval of the article: Felisa Rey, Rosário Domingues
- Provision of study materials: Jorunn Skjermo, Jon Funderud, Diogo Raposo
- Obtaining of funding: Jorunn Skjermo, Jon Funderud, Pedro Domingues, Ricardo Calado, Rosário Domingues
- Collection and assembly of data: Felisa Rey, Diana Lopes, Elisabete Maciel
- Administrative, technical, or logistic support: Felisa Rey, Rosário Domingues

References

[1] Science Advice for Policy by European Academies, Food from the oceans, Berlin, 2017. doi:10.26356/foodfromtheoceans.

[2] B. Charrier, M.H. Abreu, R. Araujo, A. Bruhn, J.C. Coates, O. De Clerck, C. Katsaros, R.R. Robaina, T. Wichard, Furthering knowledge of seaweed growth and development to facilitate sustainable aquaculture, New Phytol. (2017). doi:10.1111/nph.14728.

[3] M. Schmid, F. Guihéneuf, D.B. Stengel, Ecological and commercial implications of temporal and spatial variability in the composition of pigments and fatty acids in five Irish macroalgae, Mar. Biol. 164 (2017) 1–18. doi:10.1007/s00227-017-3188-8.

[4] M.C. Leal, M.H.G. Munro, J.W. Blunt, J. Puga, B. Jesus, R. Calado, R. Rosa, C. Madeira, Biogeography and biodiscovery hotspots of macroalgal marine natural products, Nat. Prod. Rep. 30 (2013) 1380–1390. doi:10.1039/c3np70057g.

[5] M.Y. Roleda, J. Skjermo, H. Marfaing, R. Jónsdóttir, C. Rebours, A. Gietl, D.B. Stengel, U. Nitschke, Iodine content in bulk biomass of wild-harvested and cultivated edible seaweeds: Inherent variations determine species-specific daily allowable consumption, Food Chem. 254 (2018) 333–339. doi:10.1016/j.foodchem.2018.02.024.

[6] M.L. Wells, P. Potin, J.S. Craigie, J.A. Raven, S.S. Merchant, K.E. Helliwell, A.G. Smith, M.E. Camire, S.H. Brawley, Algae as nutritional and functional food sources: revisiting our understanding, J. Appl. Phycol. 29 (2017) 949–982. doi:10.1007/s10811-016-0974-5.

[7] L.E. Rioux, L. Beaulieu, S.L. Turgeon, Seaweeds: A traditional ingredients for

new gastronomic sensation, Food Hydrocoll. 68 (2017) 255–265. doi:10.1016/j.foodhyd.2017.02.005.

[8] E. da Costa, T. Melo, A. Moreira, C. Bernardo, L. Helguero, I. Ferreira, M. Cruz, A. Rego, P. Domingues, R. Calado, M. Abreu, M. Domingues, Valorization of lipids from *Gracilaria* sp. through lipidomics and decoding of antiproliferative and anti-inflammatory activity, Mar. Drugs. 15 (2017) 62. doi:10.3390/md15030062.

[9] D. Rodrigues, A.C. Freitas, L. Pereira, T.A.P. Rocha-Santos, M.W. Vasconcelos, M. Roriz, L.M. Rodríguez-Alcalá, A.M.P. Gomes, A.C. Duarte, Chemical composition of red, brown and green macroalgae from Buarcos bay in Central West Coast of Portugal, Food Chem. 183 (2015) 197–207. doi:10.1016/j.foodchem.2015.03.057.

[10] A. Valdés, A. Cifuentes, C. León, Foodomics evaluation of bioactive compounds in foods, TrAC - Trends Anal. Chem. 96 (2017) 2–13. doi:10.1016/j.trac.2017.06.004.

[11] S.M. Cardoso, O.R. Pereira, A.M.L. Seca, D.C.G.A. Pinto, A.M.S. Silva, Seaweeds as preventive agents for cardiovascular diseases: From nutrients to functional foods, Mar. Drugs. 13 (2015) 6838–6865. doi:10.3390/md13116838.

[12] L. Méndez, G. Dasilva, N. Taltavull, M. Romeu, I. Medina, Marine lipids on cardiovascular diseases and other chronic diseases induced by diet: An insight provided by proteomics and lipidomics, Mar. Drugs. 15 (2017). doi:10.3390/md15080258.

[13] I.P.S. Fernando, J.W. Nah, Y.J. Jeon, Potential anti-inflammatory natural products from marine algae, Environ. Toxicol. Pharmacol. 48 (2016) 22–30. doi:10.1016/j.etap.2016.09.023.

[14] C. Peteiro, Ó. Freire, Biomass yield and morphological features of the seaweed *Saccharina latissima* cultivated at two different sites in a coastal bay in the Atlantic coast of Spain, J. Appl. Phycol. 25 (2013) 205–213. doi:10.1007/s10811-012-9854-9.

[15] G.S. Marinho, M. Alvarado-Morales, I. Angelidaki, Valorization of macroalga *Saccharina latissima* as novel feedstock for fermentation-based succinic acid production in a biorefinery approach and economic aspects, Algal Res. 16 (2016) 102–109. doi:10.1016/j.algal.2016.02.023.

[16] G.S. Marinho, S.L. Holdt, C. Jacobsen, I. Angelidaki, Lipids and composition of fatty acids of *Saccharina latissima* cultivated year-round in integrated multi-trophic aquaculture, Mar. Drugs. 13 (2015) 4357–4374. doi:10.3390/md13074357.

[17] E. da Costa, J. Silva, S.H. Mendonça, M.H. Abreu, M.R. Domingues, Lipidomic approaches towards deciphering glycolipids from microalgae as a reservoir of bioactive lipids, Mar. Drugs. 14 (2016). doi:10.3390/md14050101.

[18] A.H. Banskota, R. Stefanova, S. Sperker, S.P. Lall, J.S. Craigie, J.T. Hafting, A.T. Critchley, Polar lipids from the marine macroalga *Palmaria palmata* inhibit lipopolysaccharide-induced nitric oxide production in RAW264.7 macrophage cells, Phytochemistry. 101 (2014) 101–108. doi:10.1016/j.phytochem.2014.02.004.

[19] M.L. Cornish, A.T. Critchley, O.G. Mouritsen, Consumption of seaweeds and the human brain, J. Appl. Phycol. 29 (2017) 2377–2398. doi:10.1007/s10811-016-1049-3.

[20] E.G. Bligh, W.J. Dyer, A rapid method of total lipid extraction and purification, Can. J. Biochem. Physiol. 37 (1959) 911–917.

[21] F. Rey, E. da Costa, A.M. Campos, P. Cartaxana, E. Maciel, P. Domingues, M.R.M. Domingues, R. Calado, S. Cruz, Kleptoplasty does not promote major shifts in the lipidome of macroalgal chloroplasts sequestered by the sacoglossan sea slug *Elysia viridis*, Sci. Rep. 7 (2017) 11502. doi:10.1038/s41598-017-12008-z.

[22] E. da Costa, V. Azevedo, T. Melo, A.M. Rego, D. V. Evtuguin, P. Domingues, R. Calado, R. Pereira, M.H. Abreu, M.R. Domingues, High-Resolution Lipidomics of the Early Life Stages of the Red Seaweed *Porphyra dioica*, Molecules. 23 (2018). doi:10.3390/molecules23010187.

[23] E. Maciel, E. Alves, P. Domingues, R. Domingues, Lipidomics, in: P. Domingues, A. García, E. Skrzydlewska (Eds.), AACLifeSci Course Companion Man., 2018: pp. 120–134.

[24] S. Aued-Pimentel, J.H.G. Lago, M.H. Chaves, E.E. Kumagai, Evaluation of a methylation procedure to determine cyclopropenoids fatty acids from *Sterculia striata* St. Hil. Et Nauds seed oil, J. Chromatogr. A. 1054 (2004) 235–239. doi:10.1016/j.chroma.2004.07.090.

[25] W.W. Christie, The Lipid Web, (2017). http://www.lipidhome.co.uk/ (accessed February 11, 2019).

[26] P. Dörmann, Lipid Synthesis, Metabolism and Transport, in: R.R. Wise, J.K. Hoober (Eds.), Struct. Funct. Plast., Springer, Dordrecht, The Netherlands, 2007: pp. 335–353.

[27] J. Zhang, C. Li, G. Yu, H. Guan, Total synthesis and structure-activity relationship of glycoglycerolipids from marine organisms, Mar. Drugs. 12 (2014) 3634–3659. doi:10.3390/md12063634.

[28] A. Bruno, C. Rossi, G. Marcolongo, A. Di Lena, A. Venzo, C.P. Berrie, D.

Corda, Selective in vivo anti-inflammatory action of the galactolipid monogalactosyldiacylglycerol, Eur. J. Pharmacol. 524 (2005) 159–168. doi:10.1016/j.ejphar.2005.09.023.

[29] E. Larsen, A. Kharazmi, L.P. Christensen, S.B. Christensen, An antiinflammatory galactolipid from rose hip (*Rosa canina*) that inhibits chemotaxis of human peripheral blood neutrophils in vitro, J. Nat. Prod. 66 (2003) 994–995. doi:10.1021/np0300636.

[30] D. Colombo, F. Compostella, F. Ronchetti, A. Scala, L. Toma, H. Tokuda, H. Nishino, Glycoglycerolipid analogues active as anti-tumor-promoters: The influence of the anomeric configuration, Eur. J. Med. Chem. 35 (2000) 1109–1113. doi:10.1016/S0223-5234(00)01193-4.

[31] K. Ohta, Y. Mizushina, N. Hirata, M. Takemura, F. Sugawara, A. Matsukage, S. Yoshida, K. Sakaguchi, Sulfoquinovosyldiacylglycerol, KM043, a new potent inhibitor of eukaryotic DNA polymerases and HIV-reverse transcriptase type 1 from a marine red alga, *Gigartina tenella*., Chem. Pharm. Bull. (Tokyo). 46 (1998) 684–686. doi:10.1248/cpb.37.3229.

[32] H. Wang, Y.L. Li, W.Z. Shen, W. Rui, X.J. Ma, Y.Z. Cen, Antiviral activity of a sulfoquinovosyldiacylglycerol (SQDG) compound isolated from the green alga *Caulerpa racemosa*, Bot. Mar. 50 (2007) 185–190. doi:10.1515/BOT.2007.022.

[33] B.F.K. El Baz, G.S. El Baroty, H.H.A. El Baky, O.I.A. El-Salam, E.A. Ibrahim, Structural characterization and biological activity of sulfolipids from selected marine algae, Grasas y Aceites. 64 (2013) 561–571. doi:10.3989/gya.050213.

[34] A.H. Banskota, R. Stefanova, S. Sperker, S. Lall, J.S. Craigie, J.T. Hafting, Lipids isolated from the cultivated red alga *Chondrus crispus* inhibit nitric

oxide production, J. Appl. Phycol. 26 (2014) 1565–1571. doi:10.1007/s10811-013-0174-5.

[35] S.C. Cottin, T.A. Sanders, W.L. Hall, The differential effects of EPA and DHA on cardiovascular risk factors, Proc. Nutr. Soc. 70 (2011) 215–231. doi:10.1017/S0029665111000061.

[36] N.G. Bazan, M.F. Molina, W.C. Gordon, Docosahexaenoic acid signalolipidomics in nutrition: significance in aging, neuroinflammation, macular degeneration, Alzheimer's, and other neurodegenerative diseases, Annu. Rev. Nutr. 31 (2011) 321–351. doi:10.1146/annurev.nutr.012809.104635.Docosahexaenoic.

[37] D. Küllenberg, L.A. Taylor, M. Schneider, U. Massing, Health effects of dietary phospholipids, Lipids Health Dis. 11 (2012) 3–18. doi:10.1186/1476-511X-11-3.

[38] X. Tang, Z.-J. Li, J. Xu, Y. Xue, J.-Z. Li, J.-F. Wang, T. Yanagita, C.-H. Xue, Y.-M. Wang, Short term effects of different omega-3 fatty acid formulation on lipid metabolism in mice fed high or low fat diet, Lipids Health Dis. 11 (2012) 70. doi:10.1186/1476-511X-11-70.

[39] S. Hiratsuka, K. Koizumi, T. Ooba, H. Yokogoshi, Effects of dietary docosahexaenoic acid connecting phospholipids on the learning ability and fatty acid composition of the brain., J. Nutr. Sci. Vitaminol. (Tokyo). 55 (2009) 374–380. doi:10.3177/jnsv.55.374.

[40] M. Morita, Y. Shibata, Isolation and identification of arseno-lipid from a brown alga, *Undaria pinnatifida* (Wakame), Chemosphere. 17 (1988) 1147–1152. doi:10.1016/0045-6535(88)90180-4.

[41] M.H. Al Amin, C. Xiong, R.A. Glabonjat, K.A. Francesconi, T. Oguri, J.

Yoshinaga, Estimation of daily intake of arsenolipids in Japan based on a market basket survey, Food Chem. Toxicol. 118 (2018) 245–251. doi:10.1016/j.fct.2018.05.019.

[42] M. Vyssotski, K. Lagutin, A. Mackenzie, K. Mitchell, D. Scott, Phospholipids of New Zealand Edible Brown Algae, (2017) 629–639. doi:10.1007/s11745-017-4266-x.

[43] X. Yu, C. Xiong, K.B. Jensen, R.A. Glabonjat, M. Stiboller, G. Raber, K.A. Francesconi, Mono-acyl arsenosugar phospholipids in the edible brown alga Kombu (*Saccharina japonica*), Food Chem. 240 (2018) 817–821. doi:10.1016/j.foodchem.2017.08.024.

[44] A. Raab, C. Newcombe, D. Pitton, R. Ebel, J. Feldmann, Comprehensive analysis of lipophilic arsenic species in a brown alga (*Saccharina latissima*), Anal. Chem. 85 (2013) 2817–2824. doi:10.1021/ac303340t.

[45] S. Meyer, M. Matissek, S.M. Müller, M.S. Taleshi, F. Ebert, K.A. Francesconi, T. Schwerdtle, In vitro toxicological characterisation of three arsenic-containing hydrocarbons, Metallomics. 6 (2014) 1023–1033. doi:10.1039/c4mt00061g.

[46] S.M. Müller, F. Ebert, G. Raber, S. Meyer, J. Bornhorst, S. Hüwel, H.J. Galla, K.A. Francesconi, T. Schwerdtle, Effects of arsenolipids on in vitro blood-brain barrier model, Arch. Toxicol. 92 (2018) 823–832. doi:10.1007/s00204-017-2085-8.

[47] V.M. Dembitsky, Betaine ether-linked glycerolipids: Chemistry and biology, Prog. Lipid Res. 35 (1996) 1–51. doi:10.1016/0163-7827(95)00009-7.

[48] C.C. Parrish, Essential Fatty Acids in Aquatic Food Webs, in: M.T. Arts, M.T. Brett, M.J. Kainz (Eds.), Lipids Aquat. Ecosyst., Springer, New York, USA,

2009: pp. 309–326.

[49] A.P. Simopoulos, The importance of the omega-6/omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases, Exp. Biol. Med. 233 (2008) 674–688. doi:10.3181/0711-MR-311.

[50] S.R. Shaikh, J.J. Kinnun, X. Leng, J.A. Williams, S.R. Wassall, How polyunsaturated fatty acids modify molecular organization in membranes: Insight from NMR studies of model systems, Biochim. Biophys. Acta - Biomembr. 1848 (2015) 211–219. doi:10.1016/j.bbamem.2014.04.020.

[51] S.C. Dyall, G.J. Michael, A.T. Michael-Titus, Omega-3 fatty acids reverse age-related decreases in nuclear receptors and increase neurogenesis in old rats, J. Neurosci. Res. 88 (2010) 2091–2102. doi:10.1002/jnr.22390.

[52] J.P. Schuchardt, M. Huss, M. Stauss-Grabo, A. Hahn, Significance of long-chain polyunsaturated fatty acids (PUFAs) for the development and behaviour of children, Eur. J. Pediatr. 169 (2010) 149–164. doi:10.1007/s00431-009-1035-8.