Major lipophilic pigments in Atlantic seaweeds as valuable food ingredients: analysis and assessment of quantification methods

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Abstract: Current trends towards the use of ingredients from natural origin in food, cosmetic and pharmaceutical industry, place macroalgae as a good reservoir of novel compounds. Among them, lipophilic major pigments such as chlorophylls and fucoxanthin, are of great interest because of their multiple applications as bioactive compounds and dyes. In this work, a mid-polarity medium was used to extract pigments from twenty-four species from North coast of Spain, including brown (Phaeophyceae) and red macroalgae (Rhodophyta). The fucoxanthin and chlorophyll a content was assessed by means of two different methods, spectrophotometric and high-performance liquid chromatography coupled to diode array detection (HPLC-DAD). The effect of dried processing on the pigment content of selected species was also evaluated. A linear relationship between the extractability of fucoxanthin and chlorophyll a was observed, being the highest content recorded among members belonging to the order Fucales and *Undaria pinnatifida*. This work provides good insights about the content on pigments in Spanish North Atlantic macroalgae with future commercial value in different industrial fields, as well as a critical overview of the suitability of the quantification methods and challenges related to their effect in results evaluation.

Keywords: Macroalgae, Phaeophyceae, Rhodophyta, pigments, chlorophyll, fucoxanthin, Spain

1. Introduction

Seaweed biomass represent and abundant, sustainable and renewable source of great value in the European bio-based economy because of their richness in valuable compounds with multiple applications in several industries such as feed and food supplements, nutraceuticals, cosmetics, and pharmaceuticals (Holdt & Kraan, 2011; Silva et al., 2020; Martínez et al., 2021). Currently, consumer concerns about potential side effects in health due to the use of synthetic additives, are leading food industry trends towards minimal processed food products and the use of ingredients as natural as possible. In particular, colourants market play a relevant role in economic success and consumer acceptance, being projected to be worth 3.75 billion USD by 2022 (Aryee, Agyei, & Akanbi, 2018). In this context, the search for new natural colour sources represents a challenge and macroalgae could take part on this projection as a great source of pigments (Pangestuti & Kim, 2011).

Macroalgae contain different and efficient light harvesting and photoprotective pigments able to turn sunlight energy into biological energy. Brown algae (Phaeophyceae) pigment profile is mainly composed by chlorophyll a, chlorophyll c and fucoxanthin (Stengel, Connan, & Popper, 2011), whereas reddish protein-pigment complexes (phycobiliproteins) of hydrophilic nature are the main photosynthetic pigments in red algae (Rhodophyta) (Dumay, Morançais, Munier, Le Guillard, & Fleurence, 2014). Moreover, macroalgae composition varies significantly among species, individuals, and communities as a result of the influence of great number of factors. Intraspecific variability may be linked to vegetative and reproductive stages and age-related traits, but also it is a result of the influence of local environmental conditions, being sunlight a significant factor that exerts noticeable variations in pigments content. Light transmission decreases along the seawater depth in a wavelength-dependent manner. Thus, long wavelengths (the red-yellow light, 570-750 nm) are absorbed by water molecules or particulate organic matter, whereas shorter wavelength (blue-green light, 450-570 nm) penetrate deepest into the seawater, being the only spectral wavelength available to marine algae at depths of several meters (Kita, Fujii, Cogdell, & Hashimoto, 2015).

Fucoxanthin is a xanthophyll with an unusual allenic bond and a 5,6-monoepoxide in its chemical structure that accounts for more than 10% of the estimated production of carotenoids in nature (Peng, Yuan, Wu, & Wang, 2011). It is the main carotenoid pigment found in brown seaweeds, responsible for their characteristic golden-brown colour (Kumar, Hosokawa, & Miyashita, 2013), being commercially produced mainly from macroalgae species such as *Laminaria japonica*, *Eisenia bicyclis*, *Undaria pinnatifida* and *Hijikia fusiformis* (Petrushkina et al., 2017). Nevertheless, it can be also found in microalgae, which are considered as a promising source because of their richness in fucoxanthin, but they have not been yet implemented globally due to their high costs of production systems (Mohamadnia, Tavakoli, & Faramarzi, 2022; Sun et al., 2022; Wang et al., 2021). In the algal

cell, it enhances light harvesting due to its ability to absorb blue-green range light (450-570 nm) which is poorly absorbed by chlorophylls (Kim, Shang, & Um, 2011). It also acts as a photoprotective agent under overexposure to radiations (Kuczynska, Jemiola-Rzeminska, & Strzalka, 2015). Fucoxanthin has been described as a potential natural colourant (Zahrah, Amin, & Alamsjah, 2020), however, currently its commercialization is still scarce, but it can be found in varying content and quality as supplements (Xanthigen® and FucoVital™) from health stores, and in seaweed extracts for food producers. Even though an evaluation by food regulatory policies has not been performed, the intake of fucoxanthin extracts has not been associated with potential toxic effects (Iio, Okada, & Ishikura, 2011).

Chlorophylls are greenish, non-polar pigments composed by a porphyrin ring co-ordinated to a central atom of magnesium. They can absorb blue (450-495 nm) and red (620-750 nm) ranges of the electromagnetic spectrum, playing a role ensuring algal tissue integrity against oxidative stress due to excessive UV radiation (Biris-Dorhoi et al., 2020). They are commonly used as colouring agents in food and beverages since some of these products can lose their original colour during their processing. Likewise, chlorophylls and its derivates have been authorised as natural green colorants in the EC legislation under the Regulation (EC) No. 1333/2008, either the extracted from natural sources by solvent extraction (E140) or their hydrophilic derivates obtained as a result of further saponification processing (E141).

In addition, the application of these pigments could represent an added value as they also show a great variety of biological characteristics, such as a powerful antioxidant activity, that could contribute to improve food attributes and quality, as well as exert beneficial effects for health (Peng et al., 2011; Hosikian, Lim, Halim, & Danquah, 2010; Morais, Cotas, Pacheco, & Pereira, 2021). However, the high costs of production and their susceptibility to oxidation in their native conformation are probably responsible of its underuse in food and other industries (Jurić et al., 2020), though new technological approaches will overcome these challenges, facilitating opportunities for their commercial exploitation.

Despite worldwide demands for value-added products from macroalgae are increasing, the European seaweed production is underdeveloped. Since seaweed cultivation generally begins with the harvesting of wild individuals (Barbier et al., 2019), the European aquaculture sector needs to promote a global search for hitherto untapped natural algae resources and the specific traits of local flora need to be stablished.

In Spain, the North Atlantic Ocean comprises a wide extension of coastline that harbours a high diversity of macroalgae dominated by the presence of brown algae species belonging to the Phylum

Ochrophyta, class Phaeophyceae, such as fucoids (Fucales) and kelps (Laminariales and Tilopteridales) that form dense forests in cold-temperate waters of Galicia; and red algae species belonging to the Phylum Rhodophyta such as species of Gelidiales, and fucoid algae of the genus *Cystoseira sensu lato* found mostly in warm-temperate waters of Cantabrian Sea coasts (Casado-Amezúa et al., 2019). Among seaweed species from these groups, the fucoid *Himanthalia elongata*, the kelps *Laminaria sensu lato* (including *Laminaria* species and *Saccharina latissima*) and *U. pinnatifida,* and as well as the red alga *Chondrus crispus* (known as Irish moss) are harvested from wild populations (García-Tasende & Peteiro, 2015). On the other hand, seaweeds can be also exploited in aquaculture systems, being cultured at large scale mainly kelp (*Undaria*, *Laminaria*, *Saccharina* and *Sacchoriza*) (Kerrison et al. 2015; Peteiro et al., 2016) and *Sargassum* species (Le et al., 2018; Liu et al., 2021); whereas in laboratory or pilot-scale are the *Dictyota* species (Bogaert et al., 2020), *Fucus* species (Meichssner et al., 2020), and *Halopteris scoparia* (Patarra et al., 2016). In the case of red algae has been cultivated commercially *Chondrus crispus* (Bidwell et al., 1985; Zertuche-González et al., 2001) and experimentally *Halophytis incurva* (Vega et al., 2020). Nonetheless, despite of macroalgae exploitation is still in its early stage in the European Union and Spain and only kelp species have been commercially cultured on a small scale so far, it is expected that seaweed aquaculture will grow significantly during the coming years and great efforts are currently being made to advance in this sense (Peteiro, Sánchez & Martínez, 2016; Araújo et al., 2021).

The aim of this study was to evaluate the content of the main major lipophilic pigments of brown (20) and red (4) seaweeds from North coasts of Spain. From the best of our knowledge, scarce information about pigments composition in algae from this region is available. Selected seaweeds represent the most common and dominant species in northern Atlantic coasts of Spain which are a potential source of commercially valuable pigments with interest in food as natural colourants. Furthermore, an assessment of different quantification methods, spectrophotometric and HPLC detection has been carried out, contributing in this sense to the knowledge of their suitability to evaluate the content of these commodities.

2. Materials and methods

2.1. Seaweed samples and collection

A total of 20 species belonging to brown algae (Phaeophyceae) and red algae (Rhodophyta) were collected from northwest Atlantic coasts (Galicia region) and Cantabrian coasts (Cantabria region) in the North of Spain. Species, seasons, and locations of collection are listed in Table 1.

The taxonomic identification of seaweeds was done by an experienced researcher in the field using standard taxonomic keys. Collected specimens were washed using sterile seawater to eliminate residual sand, salt, and attached particles to their surface. The remaining epiphytes and epizooties were carefully picked out. The selected fresh and clean fronds were wrapped in sterile cloths moistened with seawater and kept in dark and cool with ice packs (<15°C) to preserve the alga alive and healthy until transport. In the laboratory freeze algal biomass were ground to a fine powder in a cryogenic homogenizer (SPEX SamplePrep, USA) and stored at -80 °C in darkness under vacuum conditions until the analysis was performed. A sub-group of the collected samples, including *Bifurcaria bifurcata*, *Cladostephus spongiosus*, *Ericaria selaginoides, Fucus guiryi*, *Halopteris scoparia*, *Pelvetia canaliculata* and *Gongolaria baccata*, was dried at 45ºC, pulverized, and stored at room temperature. Moreover, some of the species were collected in different stage of their life cycle, vegetative or reproductive.

2.2. Chemicals

Fucoxanthin and chlorophyll a were from Sigma-Aldrich (USA). Acetone, hexane (HPLC-grade) and acetonitrile (HPLC-grade) were from VWR Chemicals (USA). Isopropanol was from Merck (Germany). Ultra-pure water was obtained by a Milli-Q system (Millipore, USA). The choice of solvents was performed according to the rules from the Directive 2009/32/EC, as well as the current ICH Guideline for Industry ICH Q3C of the Food and Drug Administration (FDA) (2-propanol, class 3; Hexane, class 2).

2.3. Pigments extraction

Samples were analysed in triplicate. 500 ± 0.01 mg of sample were placed in 5 mL amber conical tubes and extracted three times with a solution of 3 mL of hexane-isopropanol-water (10:80:10) at room temperature in an orbital homogenizer for 60 minutes. The extracts were centrifuged at 5000 rpm for 10 minutes at 4 °C (Eppendorf, Germany) and supernatants were pooled in a 10 mL amber volumetric flask and made up to volume with the extraction media.

2.4. Pigments measurement

2.4.1. Spectrophotometric measurement

The absorption spectrum of the extracts was recorded before and after adding 0.1 N HCl within the wavelength range of 350-850 nm in a Varioskan Flash spectrophotometer (Thermo Fisher Scientific, USA). The absorbance intensity at different wavelengths, 666 nm for chlorophyll a and 480 nm for fucoxanthin, was also recorded.

2.4.2. HPLC analysis

Prior the HPLC assays, all the extracts were filtered (0.22 µm, PTFE) and an aliquot (10-25 µL) was injected into an HPLC system (Agilent, USA). The separation of the pigments was performed in a Kinetex EVO C18 column 5 µm, 250 x 4.6 mm (Phenomenex, Germany) in a gradient mode, from water (phase A) to 98 % acetonitrile-isopropanol (50:50, v/v; phase B). The gradient elution was set as follows: 5 min from 25% to 55% B; 20 min from 55% to 98% B; 5 min 98% B; 5 min from 98% to 25% B; 3 min 25% B. The elution rate was set at 1 $mL \cdot min^{-1}$ and the column temperature at 25ºC.

Regarding the spectra of seaweed extracts, the detection was carried out at 666 nm and 434 nm for chlorophyll a and fucoxanthin, respectively. Stock solutions of commercial pigments at 20 $ng \cdot \mu L^{-1}$ were made to assess linearity. Linear ranges were established using the least squared regression analysis (Table 2). The limits of detection (LOD) and quantification (LOQ) were calculated according to Long & Winefordner (1983), assuming k values of 3 and 10, respectively.

2.5. Statistical analysis

The statistical analysis was performed using the software JMP® version 16.0.0 (SAS Institute Inc.). All results were shown as means of three replicates. Data were expressed as means ± standard deviations. One-way ANOVA was used to assess significant differences between samples, followed by the Tukey's comparison test to carry out pairwise comparisons between means. Differences were considered significant at $p < 0.05$.

3. Results and Discussion

3.1. Macroalgae pigment profile

Standardized analytical procedures have not been currently established for the obtention of macroalgae extracts and several approaches have been described in literature. Based on the solvent extraction profile previously evaluated, a solvent mixture of hexane, isopropanol and water was used in this study to enhance the extraction of mid-polar compounds. The evaluation of an apolar organic extract was discarded because previous results revealed that represented a minor fraction in most of the collected seaweeds (van Oirschot, 2018).

In brown seaweeds, the obtained mid-polarity extracts were rich in lipophilic pigments (Table 3), whereas red algae tested exhibited minor content in these compounds (Table 4). Within the four red algae tested, chlorophyll a content was higher in *Halopithys incurva* (193.02 ± 26.85 $\mu g.g^{-1}$ dw), and together with *Centroceras clavulatum*, showed minor amounts of fucoxanthin (14.11 ± 2.02 $\mu g.g^{-1}$ dw); while fucoxanthin was not detected in *Plocamium cartilagineum* and *Chondrus crispus*. These results are consistent with previous observations on the major resistance to extraction of most red

algae that could be related with the structure of their cell walls (Seely, Duncan, & Vidaver, 1972; Domozych, 2019). The occurrence of fucoxanthin in some species of red seaweed belonging to Corallinaceae, Champiaceae, Endocladiaceae, Gigartinaceae, Gracilariaceae, Lithophyllaceae, Solieriaceae, Rhizophyllidaceae and Rhodomelaceae families has also been reported in previous studies (Susanto, Fahmi, Hosokawa, & Miyashita, 2019) but its origin is still uncertain. In general, minor values were detected such as in *H. incurva* from Turkey coasts (2.97 ± 0.05 $\mu g.g^{-1}$ dw) (Yalçın et al., 2020), though noticeable amounts (677.6 ± 96.3 $\mu g.g^{-1}$ dw) were reported in Japanese *Chondria crassicaulis* (Susanto et al., 2019). It was postulated that the epiphytic biota, like diatoms, microalgae or brown seaweeds, present on the surface might be responsible for the detected levels of fucoxanthin in red seaweeds; however, this fact has not been revealed by microscopic examination of *Corallina officinalis, Corallina elongata* and *Jania* sp. (Lourenço-Lopes et al., 2021).

A wide range of fucoxanthin and chlorophyll a content was observed in collected species of brown seaweed; nevertheless, these differences among species could not be associated with taxonomic orders or families. The amount of extractable chlorophyll a and fucoxanthin ranged from 377.97 ± 97.48 to 2924.73 ± 97.48 $\mu g.g^{-1}$ dw of chlorophyll a and from 791.78 ± 148.81 to 3903.18 ± 148.81 $\mu g.g^{-1}$ dw of fucoxanthin (Table 3). Detected amounts of fucoxanthin were higher than the outlined in brown seaweed collected from the Atlantic Ocean. Generally, fucoxanthin content varied mostly between 222 and 852 $\mu g.g^{-1}$ dw, but some species from Madeira Archipelago reached up 1190 $\mu g.g^{-1}$ dw in *Sargassum vulgare* and declined to lower than 50 $\mu g.g^{-1}$ dw in *Ascophyllum nodosum*, *Fucus vesiculosus* and some specimens of *Dictyota dichotoma* (Afonso et al., 2021; Marinho, Sørensen, Safafar, Pedersen, & Holdt, 2019; Nunes et al., 2019; Nunes, Valente, Ferraz, Barreto, & Pinheiro De Carvalho, 2020). Additionally, chlorophyll a content was higher than the reported levels in brown seaweed collected from Denmark coasts (170 - 655 $\mu g.g^{-1}$ dw) (Marinho et al., 2019), but was lower than those described from Madeira Archipelago (4530 - 11840 $\mu g.g^{-1}$ dw) (Nunes et al., 2020).

Although significant differences in the fucoxanthin content due to a seasonal effect were not possible to establish in this study, the effect of life cycle as well as a spatial and temporal effect have been observed in some collected samples. Life stage of adult specimens affected the lipophilic pigment profile of *P. canaliculata* and *B. bifurcata*. Fertile specimens exhibited higher content in chlorophyll a (Table 5), whereas the similar content of fucoxanthin observed between the specimens of *B. bifurcata* was attributed to a less advanced stage of reproduction. The photosynthetic activity of brown algae life cycle coupled to the effect of temperature and light has been suggested to be related to the effect of seasonal variations in the fucoxanthin content of *Sargassum horneri* and *Cystoseira hakodatensis* (Nomura et al., 2013). A seasonal effect on fucoxanthin content of Irish *Fucus serratus*

(1587 - 5198 μg.g^{-1} g dw extract) and *Laminaria digitata* (450 - 1403 μg.g^{-1} dw extract) was also reported by Heffernan et al. (2016) in hexane:acetone (70:30) extracts, even though the highest fucoxanthin content of *F. serratus* has been observed in summer, and in winter and spring for *L. digitata*.

An interannual variation between samples of *F. guiryi*, *E. selaginoides* and *C. spongiosus* collected in summer 2017 and 2019 was also observed in the present study. In summer 2019, chlorophyll a content was higher in the specimens of *F. guiryi* and *E. selaginoides*, while the amount of fucoxanthin was lower in *F. guiryi* and higher in *C. spongiosus* (Table 6). These results are in accordance with the content variation in fucoxanthin of *D. dichotoma* collected from Porto Santo in spring 2017 (12.2 ± 0.4 μg·g^{-1} dw) and Madeira Island in summer 2018 (514 ± 5 μg·g^{-1} dw) (Nunes et al., 2019), which also reached 770 ± 30 μg.g^{-1} dw in samples collected in spring 2017 around the Madeira Archipelago by free-diving to a maximum depth of 10 m (Nunes et al., 2020). In addition, the amount of fucoxanthin differed also between the specimens of *Fucus spiralis* collected from Comillas (1489.99 ± 71.91 μg.g^{-1} dw) and As Xubias (1086.23 ± 71.91 μg.g^{-1} dw).

3.2. Inter-specific variations

The species-specific content of pigments is well documented, as well as the predominance of fucoxanthin in the pigment profile of brown seaweed (Holdt & Kraan, 2011). However, the number of intra- and inter-specific factors affecting the lipophilic pigment profile of seaweed makes difficult the comparison with the reported data. Variations might be related to genetic differences among and within species. Nowadays, the introduction of molecular tools provides new criterions for identifying and delimiting species, traditionally based on their morphological characteristics. Thus, the high diverse family Sargassaceae has experienced several taxonomic rearrangements based on morphological and molecular tools, such as the species *Cystoseira baccata* and *Cystoseira tamariscifolia,* which were recently reinstated in the genera *Gongolaria* Boehmer and *Ericaria* Stackhouse, respectively (Molinari-Novoa & Guiry, 2020). Whereas *F. guiryi* has been recently separated from *F. spiralis*, and studies concerning on its geographical distribution and the influence of environmental factors are on course (Prinz, 2020).

Content variations in lipophilic pigments were also related to the taxonomical arrangement in community structure. Moreover, despite the comparable extractability of both compounds in *F. spiralis*, and the lower amount of fucoxanthin observed in *Saccorhiza polyschides*, a linear relationship between the extractability of chlorophyll a and fucoxanthin ($p \leq 0.0001$, R^2=0.840) was established in this study (Figure 1), which agrees with the unchanged profile of lipophilic pigments by seasonal variations (Marinho et al., 2019). Since cultivation in deep seawater has several advantages such as a convenient low temperature, abundance of nutrients, and a minor extent of

pollution and occurrence of potential pathogen, the effect of the location in the water column is nowadays a subject of interest for seaweed production. Gordillo et al. (2006) reported a high content in chlorophyll a in specimens of *Fucus distichus* (5592 - 6456 μg.g^{-1} dw) and *S. latissima* (2499 - 3367 μg.g^{-1} dw) collected at 9 m deep from the Norwegian Arctic Ocean (Gordillo, Aguilera, & Jiménez, 2006). Furthermore, collected specimens of *D. dichotoma* by free diving around the Madeira Archipelago to a maximum depth of 10 m reached 7820 μg.g^{-1} dw (Nunes et al., 2020).

Even though the pigment content of the species belonging to the Fucaceae family are among the less studied, the highest content in lipophilic pigments was observed in some species from genus *Fucus*, and their wide content variation was in accordance with the specie location on the shoreline. High contents were observed in *F. serratus* and *F. vesiculosus* (close or higher to 3000 μg·g^{-1}) followed by *Fucus ceranoides*, which were collected in the low (4 m depth), mid (2 m depth) and on the estuary area in the upper (2 m depth) intertidal zones, respectively. Minor contents were accounted in species collected in the subtidal or upper intertidal zone (up to 1 m depth), closer to the water surface (up to a maximum of 1m deep) where the immersion-emersion cycles are more frequent. Even though *F. spiralis* lives below the zone of *P. canaliculata* (0.5 m deep), it accounted higher content of chlorophyll a. Non-significant variations in the pigment profile were outlined among this group (*P. canaliculata*, *A. nodosum* and *F. guiryi*). Though the obtained results were higher than those reported in *F. vesiculosus* (22 ± 1 μg·g^{-1} dw) and *A. nodosum* (21.6 ± 0.9 μg·g^{-1} dw) from Ireland (Nunes et al., 2019), they were in the range of those reported by (Terasaki et al., 2009) in Japanese *F. distichus* (900 ± 300 μg.g^{-1} dw) and *Silvetia babingtonii* (700 ± 200 μg·g^{-1} dw).

Except for *B. bifurcata*, less variation in the content of lipophilic pigments was observed within the members from Sargassaceae family since all were collected in the lower intertidal zone at maximum of 4 m depth, also including 1 m of the infralittoral zone. The lower content in chlorophyll a detected in *Halidrys siliquosa* may be attributed to the different genus included within this group of algae. However, the minor content of *B. bifurcata* agreed with its collection at 3 m deep in low intertidal zone. These results are in the range of the chlorophyll a reported in specimens of *Sargassum* collected from eastern countries (Japan and India) between 2107 and 2688 μg.g^{-1} dw (Susanto et al., 2019; Verma, Kumar, Mishra, & Sahoo, 2017), and were comparable to those reported for fucoxanthin from Indonesian (Susanto et al., 2016; Susanto, Fahmi, Hosokawa, & Miyashita, 2019), Japanese (Airanthi, Hosokawa, & Miyashita, 2011; Nomura et al., 2013; Susanto et al., 2016, 2019; Terasaki et al., 2009), Iranian (Fariman, Shastan, & Zahedi, 2016) and Malaysian (Agatonovic-Kustrin & Morton, 2017) species, mostly accounting between 1020 and 4490 μg.g^{-1} dw. Surprisingly, marked differences have been observed with respect to the lipophilic profile of specimens of *Cystoseira sensu lato* and *Sargassum* collected from Madeira Archipelago (Nunes et al., 2020), where

the values of chlorophyll a reached up to 4530 - 11840 μg.g^{-1} dw and of fucoxanthin decreased to 400 - 1190 μg.g^{-1} dw (Nunes et al., 2019, 2020). Furthermore, minor contents of fucoxanthin (7.10 - 375 μg.g^{-1} dw) were reported in Indian specimens (Raji et al., 2020; Verma et al., 2017; Vimala & Poonghuzhali, 2015), while both compounds exhibit low amounts in Turkish species (Yalçın et al., 2020).

The content in lipophilic pigments of *Himanthalia elongata* was set among the seaweed group with minor content, in comparison with those observed in fucoids species collected in the subtidal and low intertidal zones (*A. nodosum*). Lower contents in chlorophyll a (60 - 157 μg.g^{-1} dw) as well as in fucoxanthin (3 - 9 μg.g^{-1}) were reported in specimens collected from Galicia (Ferraces-Casais, Lage-Yusty, de Quirós, & López-Hernández, 2012; Osório et al., 2020). Nevertheless, significant higher contents (18.6 mg.g^{-1}) have been achieved in cultured Irish specimens (Rajauria, Foley, & Abu-Ghannam, 2017).

A great amount of lipophilic pigments within the order Laminariales was observed in *U. pinnatifida.* Their high content agrees with the fact that most of commercially available fucoxanthin is extracted from species grown in deep waters of eastern countries (Billakanti, Catchpole, Fenton, Mitchell, & Mackenzie, 2013). In Japanese specimens, reported values ranged between 728 - 3090 μg.g^{-1} dw (Sugimura et al., 2012; Susanto et al., 2019), while the amounts of chlorophyll a accounted in this study were higher than those reported (546.7 ± 146.0 μg.g^{-1} dw) by Susanto et al. (2019).

Both specimens, *Saccharina latissima* and *Laminaria hyperborea*, were collected from the intertidal (4 m deep) zone and including 1 m of the infralittoral area, where sunlight reaches the ocean floor. This should be the reason for their low content in chlorophyll a (< 600 μg.g^{-1} dw), including the lowest value detected in this study (377.97 ± 103.00 μg.g^{-1} dw), and despite that the content variation in fucoxanthin was 2.35 times higher in *S. latissima*. The lipophilic pigment profile observed in this study also agreed with the values outlined in specimens collected from the North Atlantic (Afonso et al., 2021; Marinho et al., 2019) and North Pacific coasts (Seely et al., 1972) as well as in Japanese *Saccharina japonica* (Mori et al., 2004; Susanto et al., 2019) and *Kjellmaniella crassifolia* (Airanthi et al., 2011; Terasaki et al., 2009).

The members of the taxonomic order Dictyotales are encompassed in just one family, Dictyotaceae, and together with the order Fucales, constitute two groups characterized by their richness in secondary metabolites. *D. dichotoma* grows in subtidal zones where sunlight reaches and is commonly found besides some species from Sargassaceae family. The location of collected specimens within 1 m deep range might explain their content in chlorophyll a and fucoxanthin, comparable to those species also found in subtidal zones (*Sargassum muticum* and *H. siliquosa*).

Chlorophyll a content was lower than the reported values in India and Madeira Archipelago (Nunes et al., 2019, 2020; Verma et al., 2017), while the amount of fucoxanthin was set between the reported levels in these locations (12.2 - 770 μg.g^{-1} dw) and Malaysia coasts (4337 - 6205 μg.g^{-1} dw) (Agatonovic-Kustrin & Morton, 2017).

The content of chlorophyll a and fucoxanthin of *C. spongiosus* was about twice than the observed in *H. scoparia*. Similarly, to results previously reported in *D. dichotoma*, the content in chlorophyll a of the specimens of *H. scoparia* collected in this study was lower to those reported in specimens from Madeira Archipelago (8150 ± 160 μg.g^{-1} dw), while the contents of fucoxanthin were comparable (10.1 - 340 μg.g^{-1} dw) (Nunes et al., 2019, 2020).

3.3. Methodological and technological issues

3.3.1. Measurement methods

Aside from the potential effect of the genetic lineages and environmental factors, some differences can be at least partly due to the assayed experimental procedure, either the solvent extraction or the detection system, high performance liquid chromatography *versus* spectrophotometric detection. The spectrophotometric detection represents a fast, sensitive, and inexpensive method for the lipophilic pigment profile, useful for a screening evaluation. Whereas high-performance chromatography is a time-required methodology that allows the identification and quantification of specific compounds with high accuracy. Both detection systems have been commonly used in most reviewed data (Lourenço-Lopes et al., 2020). A comparison between both methodologies was performed in this study, and to avoid potential biases caused by differences in sample treatment either solvent extractions and measurements were performed on the same day of treatment. Empirical equations at pigment-specific peak wavelengths from a calibration curve measured under the same conditions (solvent and instrument) of the unknown concentration extracts were also attained before and after the extract acidification (Table 2). The comparison of the estimation content performed by both detection systems had shown higher correlation for the chlorophyll a ($p \leq 0.930$) than for fucoxanthin ($p \leq 0.820$). The discrepancy extent was mainly determined by either the content or the specie traits (Figure 2), since seaweeds comprise a complex matrix of compounds with multiple interactions giving support to the cellular structure that can difficult the extraction procedure, irrespective of the solvent affinity.

On the other hand, the extractability based on the solubility of supramolecular structures, more than the solvent affinity of the specific compounds can help to explain the extraction efficiency of the wide range of solvents assayed in the analytical procedures reported in the reviewed studies; which includes the single use or mixtures of polar (water, alcohols), non-polar (acetone, ether, ethyl acetate,

hexane), halogenated (chloroform, dichloromethane) and dipolar (dimethyl sulfoxide and N,N-dimethylformamide) solvents (Lourenço-Lopes et al., 2020). Moreover, the linear relationship between the extractability of chlorophyll a and fucoxanthin previously mentioned support the idea of fucoxanthin–chlorophyll protein complex may be the key molecular complex for light harvesting, not only in diatoms, but also in several kinds of seaweeds (Apt, Clendennen, Powers, & Grossman, 1995; Gelzinis et al., 2015; Susanto et al., 2019). Furthermore, it could also partly explain the simultaneous extraction of other phytochemicals with an apparently divergent polarity in the assayed extracts (Rubiño et al., submitted).

3.3.2. Effect of dry processing in pigments stability

Besides the choice of commercially important species, the preservation of post-harvest seaweed is also a crucial stage in the value-chain. Drying is the oldest and most commonly used technique to avoid biomass decomposition, increasing shelf-life and allowing its storage for further conversion processes. However, the applied temperature and the exposure to sunlight during long-time period can significatively affect their biochemical composition as well as their nutritional benefits and health effects (Amorim, Nardelli, & Chow, 2020).

An unspecific effect on the lipophilic pigment profile has been observed in this study (Table 7), which should be taken in account to avoid inaccurate estimation of contents depending on the post-harvest processing and the detection system performed. Thus, the occurrence of lipophilic pigments mostly disappeared or decreased significantly in Fucales species, being more significant in chlorophyll a content. Whereas fucoxanthin exhibited a relative high stability during the drying process. The loss in chlorophyll a content is assumed to be consequence of the effect of the exposure to light and temperature in drying process, since chlorophylls are easily degraded due to enzymatic reactions and non-enzymatic reactions influenced in processing conditions (Yilmaz & Gökmen, 2016). The disparities between samples outcomes, as well as the results from both detection systems, might result in a different balance between the influence of conditions set in post-harvesting process, and the endogenous content of specific metals and the enzymatic activities occurring in samples. As a result of this balance, the potential formation of chlorophyll-derivate compounds more resistant to heat treatment that still retain the green colour, such as chlorophyllide or metallo-chlorophyll complexes, will occur being responsible of the measured spectrophotometric absorbance (Indrasti, Andarwulan, Purnomo, & Wulandari, 2018).

Regarding the estimation by HPLC-DAD detection, a decrease in fucoxanthin amounts in dried samples of Fucaceae species was set between 35-50 %, which were not significantly detected by spectrophotometric detection. The stability pattern in Sargassaceae and Sphacelariales species was

irregular. The fucoxanthin amount was not detected in dried samples of *G. baccata*, decreased by 20% in *B. bifurcata*, and not significant effect was detected in *E. selaginoides*. The extent of fucoxanthin degradation could be limited by the inactivation of the endogenous oxidase enzymes besides the occurrence of endogenous antioxidants in samples, which will prevent the oxidative damage caused by the free radical scavengers originated from the synergic effect of light and temperature, together with the exposure to the atmospheric oxygen (Susanto, Fahmi, Agustini, Rosyadi, & Wardani, 2017; Wang et al., 2018). Surprisingly, irrespective of the detection system, not significant effect of drying process was observed on the content in chlorophyll a and fucoxanthin of *C. spongiosus*, whereas both pigments content increased in *H. scoparia*. The major content in lipophilic compounds previously observed in Sphacelariales species (van Oirschot, 2018) was assumed to have a beneficial effect in the stability of lipophilic pigment profile. Thus, it was pointed out that the stability and bioaccessibility of carotenoids can be improved when they are consumed with lipids, whereas their solubilization in mixed micelles is required for absorption by intestinal cells (Kaur, Gurpreet; Khattar, J.I.S; Singh, D.P.; Singh, Yadvinder and Nadda, 2009; Peng et al., 2011).

4. Conclusions

Algal lipophilic major pigments are considered valuable compounds with health beneficial properties, and current trends regarding to consumers and manufacturers preferences towards the use of natural ingredients make them a great alternative source. Nevertheless, their commercialization is scarce since extraction and scale-up procedures has not been standardized. In this study, a solvent extraction procedure was developed to perform a comprehensive screening of the content variation in lipophilic major pigments in macroalgal flora from Northern coast of Spain. Regarding the heterogeneity among macroalgal genetic linages spread worldwide, it is of great interest to screen and evaluate their content to stablish the most profitable species. Results obtained in this research have revealed high diversity between the content of brown and red macroalgae. Despite the large differences observed among the brown macroalgae, this study also highlights that the specimens from Northern coast of Spain constitute a great reservoir of lipophilic pigments. However, the highest content in either fucoxanthin and chlorophyll a was mainly detected in *F. serratus* and *F. vesiculosus*, together with *U. pinnatifida* from Laminariales, being suitable candidates for the development of functional foods and other products with high-added value.

Nevertheless, for exploitation purposes, further research is required to clearly ascertain the effect of depth, life stage and season on their content, as well as the most suitable post-harvesting technologies that preserve the functionality of macroalgae biomass. Furthermore, the application of detection techniques (spectrophotometric and HPLC-DAD) should be taken into consideration since

they can mislead the content estimation. Overall, this study place marine natural pigments of brown seaweeds species from Spanish North Atlantic coast as a rich resource for various fields as food, cosmetics and pharmacological.

Acknowledgements

This work was supported by the Spanish Ministry of Economy and Competitiveness (INIA Project: RTA2015-00010-C03-01), and by the Generalitat de Catalunya (CERCA Programme). Susana Rubiño acknowledges the FPI Ph.D. grant from the Spanish Ministry of Economy and Competitiveness (BES-2017-0027).

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Table 1. List of macroalgae and collection data. The taxonomic classification and currently accepted scientific names of the species, including important synonyms (=), are based on Algaebase[1] and following the rules of the International Code of Nomenclature for algae, fungi, and plants (ICN)[2]

Specie	Life stage	Season	Year	Region	Locality	Latitude/ Longitude	Littoral zone
Phaeophyceae							
Dictyotales							
Dictyotaceae							
Dictyota dichotoma (Hudson) J. V. Lamouroux 1809	Fertile and non-fertile	Summer	2019	Cantabria	Comillas	43°23'N/4°17'W	Subtidal (-1 m)
Fucales							
Fucaceae							
Ascophyllum nodosum (Linnaeus) Le Jolis 1863	Fertile and non-fertile	Summer	2019	Galicia	As Xubias, A Coruña	43°20'N/8°23'W	Low intertidal and subtidal (-1 m)
Fucus ceranoides Linnaeus 1753	Fertile and non-fertile	Winter	2019	Galicia	O Burgo, Culleredo	43°20'N/8°21'W	Upper intertidal
Fucus guiryi G.I.Zardi, K. R. Nicastro, E. S. Serrão & G. A. Pearson 2011 (=*Fucus spiralis* var. *platycarpus* (Thuret) Batters 1902)	Fertile and non-fertile	Summer Summer	2017 2019	Cantabria Cantabria	Comillas Comillas	43°23'N/4°17'W	Upper intertidal
Fucus serratus Linnaeus 1753	Fertile and non-fertile	Winter	2019	Galicia	Esteiro, Muros	42°47'N/8°58'W	Lower intertidal
Fucus spiralis Linnaeus 1753	Fertile and non-fertile	Winter Autumn	2019 2019	Galicia Cantabria	As Xubias, A Coruña Comillas	43°20'N/8°23'W 43°23'N/4°17'W	Upper intertidal
Fucus vesiculosus Linnaeus 1753	Fertile and non-fertile	Autumn	2019	Galicia	Esteiro, Muros	42°47'N/8°58'W	Mid intertidal

Pelvetia canaliculata (Linnaeus) Decaisne & Thuret 1845	Fertile and non-fertile	Summer	2017	Cantabria	Comillas	43°23'N/4°17'W	Upper intertidal
	Fertile	Summer	2019	Galicia	Santa Cristina, Oleiros	43°20'N/8°22'W	
	Non-fertile	Summer	2019	Galicia	Santa Cristina, Oleiros	43°20'N/8°22'W	
Himanthaliaceae							
Himanthalia elongata (Linnaeus) S. F. Gray 1821	Non-fertile	Spring	2019	Galicia	Barizo, Malpica	43°19'N/8°52'W	Low intertidal and subtidal (-1m)
	Fertile	Summer	2019	Galicia	Esteiro, Muros	42°47'N 8°58'W	
Sargassaceae							
Bifurcaria bifurcata R. Ross 1958	Non-fertile	Summer	2017	Cantabria	Trasvia, Comillas	43°23'N/4°17'W	Mid intertidal
	Non-fertile	Spring	2019	Galicia	Portiño, Bens, A Coruña	43°22'N/8°26'W	
	Fertile	Spring	2019	Galicia	Portiño, Bens, A Coruña	43°22'N/8°26'W	
	Fertile and non-fertile	Winter	2019	Cantabria	Comillas	43°23'N/4°17'W	
Ericaria selaginoides (Linnaeus) Molinari & Guiry 2020 [=*Carpodesmia tamariscifolia* (Hudson) Orellana & Sansón 2019] [=*Cystoseira tamariscifolia* (Hudson) Papenfuss 1950]	Non-fertile	Summer	2017	Cantabria	Trasvia, Comillas	43°23'N/4°17'W	Low intertidal and subtidal (-1m)
	Non-fertile	Summer	2019	Cantabria	Trasvia, Comillas	43°23'N/4°17'W	
Gongolaria baccata (S. G. Gmelin) Molinari & Guiry 2020 [=*Treptacantha baccata* (S. G. Gmelin) Orellana & Sansón 2019][= *Cystoseira baccata* (S. G. Gmelin) P. C. Silva 1952]	Non-fertile	Summer	2017	Cantabria	Trasvia, Comillas	43°23'N/4°17'W	Low intertidal and subtidal (-1m)
Halidrys siliquosa Linnaeus 1753	Fertile and non-fertile	Winter	2019	Galicia	Santa Cristina, Oleiros	43°20'N/8°22'W	Subtidal (-1 m)
Sargassum muticum (Yendo) Fensholt 1955	Fertile and non-fertile	Spring	2019	Galicia	San Pedro de Veigue, Sada	43°20'N/8°17'W	Subtidal (-1 m)

Laminariales							
Alariaceae							
Undaria pinnatifida (Harvey) Suringar 1873	Non-fertile	Spring	2019	Galicia	San Pedro de Veigue, Sada	43°20'N/8°17'W	Low intertidal and subtidal (-1m)
Laminariaceae							
Saccharina latissima (Linnaeus) C. E. Lane, C. Mayes, Druehl & G. W. Saunders 2006 [= *Laminaria saccharina* (Linnaeus) J. V. Lamouroux 1813]	Non-fertile	Summer	2019	Galicia	Esteiro, Muros	42°47'N/8°58'W	Low intertidal and subtidal (-1m)
Laminaria hyperborea (Gunnerus) Foslie 1885	Non-fertile	Summer	2019	Galicia	Esteiro, Muros	42°47'N/8°58'W	Subtidal (-1 m)
Sphacelariales							
Cladostephaceae							
Cladostephus spongiosus (Hudson) C. Agardh 1817	Non-fertile	Summer	2017	Cantabria	Comillas	43°23'N/4°17'W	Lower intertidal
	Non-fertile	Summer	2019	Cantabria	Comillas	43°23'N/4°17'W	
Stypocaulaceae							
Halopteris scoparia (Linnaeus) Sauvageau 1904 [= *Stypocaulon scoparium* (Linnaeus) Kützing 1843]	Non-fertile	Summer	2017	Cantabria	Trasvia, Comillas	43°23'N/4°17'W	Lower intertidal
Tilopteridales							
Phyllariaceae							
Saccorhiza polyschides (Lightfoot) Batters 1902	Non-fertile	Spring	2019	Galicia	Portiño, Bens, A Coruña	43°22'N/8°26'W	Low intertidal and subtidal (-1m)

Rhodophyta								
Ceramiales								
Rhodomelaceae								
Halophytis incurva (Hudson) Batters 1902	Fertile and non-fertile	Summer	2017	Cantabria	Trasvia, Comillas	43°23'N/4°17'W	Subtidal (-1 m)
Ceramiaceae								
Centroceras clavulatum (C. Agardh) Montagne 1846	Fertile and non-fertile	Summer	2017	Cantabria	Comillas	43°23'N/4°17'W	Mid intertidal
Plocamiales								
Plocamiaceae								
Plocamium cartilagineum (Linnaeus) P.S. Dixon 1967	Non-fertile	Summer	2017	Cantabria	Trasvia, Comillas	43°23'N/4°17'W	Low intertidal and subtidal (-1m)
Gigartinales								
Gigartinaceae								
Chondrus crispus Stackhouse 1797	Non-fertile	Summer	2017	Cantabria	San Román, Pielagos	43°23'N/4°17'W	Mid intertidal

[1] Guiry and Guiry (2021), searched on 9th January 2022, [2] Turland et al. (2018); [3] Geographic coordinates of the sampling locations were obtained from Google Earth Engine

Table 2. Calibration, linearity, accuracy, and precision for chlorophyll a and fucoxanthin determination

Standard	Wavelength	Equations	R^2	Linear range	LOD	LOQ
Spectrophotometric						
Chlorophyll a	666 nm	Abs = 0.206 * µg + 0.111	0.996	0.75 – 12 µg	0.66 µg	2.19 µg
Fucoxanthin	480 nm	Abs = 0.158 * µg - 0.013	0.999	1.25 -12.5 µg	0.26 µg	0.88 µg
HPLC-DAD						
Chlorophyll a	666 nm	Abs = 0.374 * ng - 1.614	0.999	50 - 500 ng	6.09 ng	20.30 ng
Fucoxanthin	434 nm	Abs = 2.245 * ng - 9.435	0.999	50 - 500 ng	3.37 ng	11.22 ng

Table 3. Extractable amounts (μg.g^{-1} dry weight) of lipophilic pigments in brown seaweed assessed by HPLC-DAD detection

Specie	Chlorophyll a[1]			Fucoxanthin[1]		
	μg.g^{-1}		sd	μg.g^{-1}		sd
Fucus serratus	2924.73 ^{a}	±	103.00	3903.18 ^{a}	±	149.63
Fucus vesiculosus	2914.90 ^{a}	±	103.00	3334.04 ^{ab}	±	149.63
Fucus ceranoides	2007.81 ^{b}	±	103.00	2539.40 ^{cde}	±	149.63
Gongolaria baccata	1920.31 ^{bc}	±	103.00	2703.83 ^{bcd}	±	149.63
Undaria pinnatifida	1870.21 ^{bcd}	±	103.00	3175.45 ^{abc}	±	149.63
Sargassum muticum	1677.23 ^{bcde}	±	103.00	2297.33 ^{de}	±	25.74
Ericaria selaginoides	1479.12 ^{cde}	±	72.83	2296.41 ^{de}	±	105.81
Cladostephus spongiosus	1430.85 ^{de}	±	72.83	2183.88 ^{de}	±	105.81
Fucus spiralis	1312.99 ^{e}	±	72.83	1288.11 ^{fg}	±	105.81
Dictyota dichotoma	1229.71 ^{ef}	±	103.00	1888.14 ^{ef}	±	149.63
Halidrys siliquosa	1214.24 ^{ef}	±	103.00	2093.24 ^{de}	±	149.63
Himanthalia elongata	775.55 ^{fg}	±	72.83	950.71 ^{ghi}	±	105.81
Pelvetia canaliculata	748.82 ^{g}	±	59.47	1110.02 ^{g}	±	86.39
Halopteris scoparia	729.55 ^{fg}	±	103.00	954.31 ^{ghi}	±	149.63
Saccorhiza polyschides	669.71 ^{g}	±	103.00	277.59 ^{i}	±	149.63
Ascophyllum nodosum	582.15 ^{g}	±	103.00	920.66 ^{ghi}	±	149.63
Saccharina latissima	557.21 ^{g}	±	103.00	1861.44 ^{ef}	±	149.63
Fucus guiryi	557.14 ^{g}	±	72.83	969.95 ^{gh}	±	105.81
Bifurcaria bifurcata	538.81 ^{g}	±	51.50	618.88 ^{hi}	±	74.82
Laminaria hyperborea	377.97 ^{g}	±	103.00	791.78 ^{ghi}	±	149.63

[1] $p < 0.0001$. Data are presented as mean ± standard deviation (sd) of 3 determinations. Different letters within each row represent significant differences ($p < 0.05$) between the pigment content.

Table 4. Extractable amounts (μg.g^{-1} dry weight) of lipophilic pigment profile of red seaweeds

Specie	Chlorophyll a[1]		Fucoxanthin[2]	
	μg.g^{-1}	sd	μg.g^{-1}	sd
Halopithys incurva	193.02 [a]	± 26.85	14.11	± 2.02
Centroceras clavulatum	70.21 [b]	± 26.85	14.11	± 2.02
Plocamium cartilagineum	34.44 [b]	± 26.85	nd	
Chondrus crispus	27.15 [b]	± 26.85	nd	

[1] $p < 0.0080$; [2] $p < 0.0009$. Data are presented as mean ± standard deviation (sd) of 3 determinations. Different letters within each row represent significant differences ($p < 0.05$) between the pigment content.

Table 5. Effect of life stage on the amount (μg.g^{-1} dry weight) of extractable lipophilic pigments assessed by HPLC-DAD detection

Species	Chlorophyll a				Fucoxanthin			
	Fertile	Non-Fertile	sd	*p*	Fertile	Non-Fertile	sd	*p*
Bifurcaria bifurcata	568.82[a]	439.67[b]	22.63	*	545.73	492.88	40.45	ns
Pelvetia canaliculata	892.77[a]	471.94[b]	53.02	**	1556.98[a]	681.4[b]	79.24	**

Values are means ± standard deviation (n = 3). For each lipophilic pigment, means with different letters in the same files are significantly different (** $p < 0.05$; * $p < 0.01$)

Table 6. Temporal effect on the extractable amount (µg.g^{-1}dry weight) of lipophilic pigment assessed by HPLC-DAD detection

Species[1]	**Chlorophyll a**				**Fucoxanthin**			
	2017	2019	sd	*p*	2017	2019	sd	*p*
Fucus guiryi	501.28^{b}	613.00^{a}	19.67	*	1223.79^{a}	716.10^{b}	31.20	***
Ericaria selaginoides	1252.09^{b}	1706.14^{a}	48.53	**	2111.21	2481.61	95.63	ns
Cladostephus spongiosus	1473.93	1387.77	70.24	ns	1948.46^{b}	2419.31^{a}	100.37	*

[1] samples collected on 25th August 2017 and 2nd August 2019. Values are means ± standard deviation (n = 3). For each lipophilic pigment, means with the different letters in the same file are significantly different (*** $p < 0.001$, ** $p < 0.05$, * $p < 0.01$)

Table 7. Effect of drying process on the amount of extractable lipophilic pigments (μg.g^{-1} dry weight) assessed by HPLC-DAD and spectrophotometric detection

Species	HPLC-DAD				Spectrophotometric			
	Fresh	Dried	sd	p^1	Fresh	Dried	sd	p^1
Chlorophyll a								
Fucus guiryi	501.28	nd	10.68	***	1071.71	nd	29.78	***
Pelvetia canaliculata	881.76	nd	13.68	***	976.62	nd	7.38	***
Bifurcaria bifurcata	498.49	nd	11.31	***	537.20	49.77	41.53	***
Gongoloria baccata	1920.31	nd	30.62	***	2488.46^{a}	656.19^{b}	172.21	***
Ericaria selaginoides	1252.09^{a}	432.75^{b}	42.67	***	-	-	-	
Cladostephus spongiosus	1473.93	1737.15	209.17	ns	2272.93	1317.51	449.19	ns
Halopteris scoparia	729.55^{b}	1742.48^{a}	84.07	***	1078.36^{b}	2024.80^{a}	188.02	**
Fucoxanthin								
Fucus guiryi	1223.79^{a}	622.88^{b}	23.63	***	1698.22	1349.57	94.00	ns
Pelvetia canaliculata	1091.68^{a}	706.01^{b}	12.97	***	1764.43	1717.39	44.97	ns
Bifurcaria bifurcata	647.29^{a}	516.74^{b}	17.10	***	909.72^{b}	1690.90^{a}	26.53	***
Gongoloria baccata	2703.82	nd	52.36	***	3491.08^{a}	2359.59^{b}	96.90	***
Ericaria selaginoides	2111.21	2435.46	139.16	ns	-	-	-	
Cladostephus spongiosus	1948.46	2180.63	283.93	ns	2428.36	3169.73	394.81	ns
Halopteris scoparia	954.31^{b}	3026.18^{a}	125.12	***	1320.0^{b}	4334.55^{a}	155.50	***

[1] *** $p < 0.001$; ** $p < 0.01$; ns: not significant. Samples with different superscript between columns were significantly different.

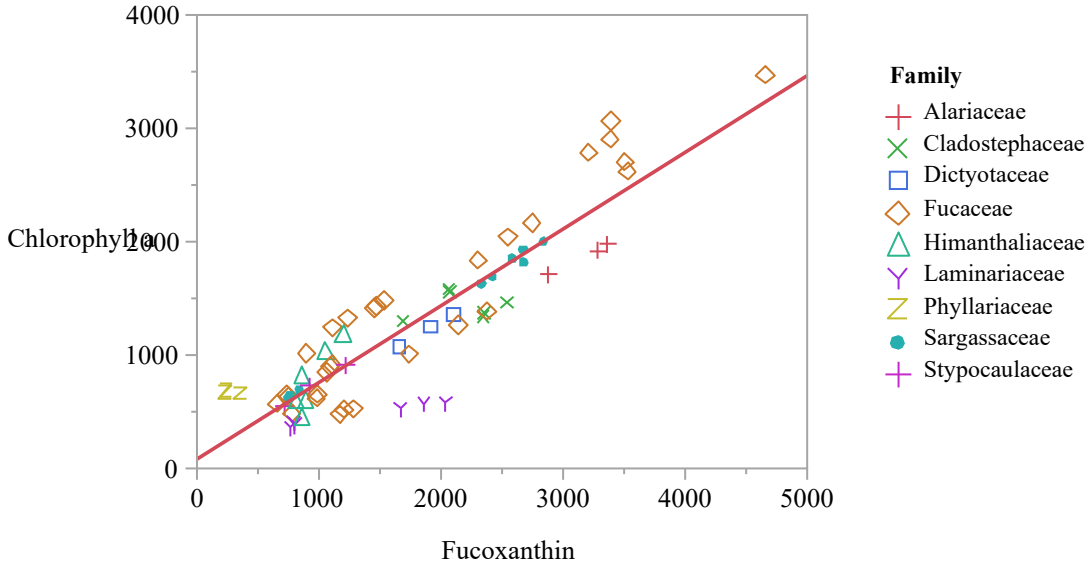

Figure 1. Linear relationship between the extractable content (µg.g^{-1} dw) of chlorophyll a and fucoxanthin

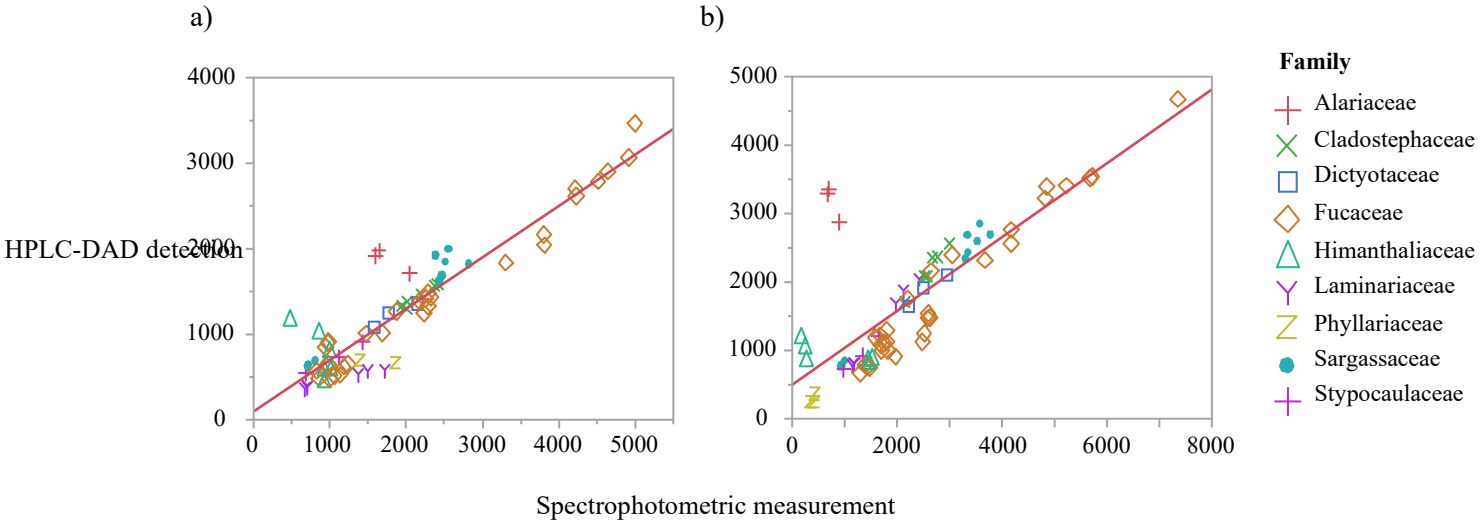

Figure 2. Linear relationship between spectrophotometric and HPLC-DAD detection of (a) chlorophyll a and (b) fucoxanthin