Review

Red Seaweed Pigments from a Biotechnological Perspective

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Abstract: Algae taxa are notably diverse regarding pigment diversity and composition, red seaweeds (Rhodophyta) being a valuable source of phycobiliproteins (phycoerythrins, phycocyanin, and allophycocyanin), carotenes (carotenoids and xanthophylls), and chlorophyll a. These pigments have a considerable biotechnological potential, which has been translated into several registered patents and commercial applications. However, challenges remain regarding the optimization and subsequent scale-up of extraction and purification methodologies, especially when considering the quality and quantity needs, from an industrial and commercial point of view. This review aims to provide the state-of-the-art information on each of the aforementioned groups of pigments that can be found within Rhodophyta. An outline of the chemical biodiversity within pigment groups, current extraction and purification methodologies and challenges, and an overview of commercially available products and registered patents, will be provided. Thus, the current biotechnological applications of red seaweeds pigments will be highlighted, from a sustainable and economical perspective, as well as their integration in the Blue Economy.

Keywords: Rhodophyta; phycobilins; chlorophylls; carotenoids; pigment extraction; pigment purification; biotechnological applications

1. Introduction

Photosynthesis is a biochemical process inherent to photosynthetic organisms, who capture solar energy at wavelengths in the region between 400 and 700 nm, and convert them into chemical energy that is essential for the organism growth and development [1]. The fundamental role of harvesting sunlight and transforming it into that essential energy belongs to pigments present in the living cells of every photosynthetic organism. Depending on their specific role, these pigments are classified as photosynthetic pigments (chlorophylls) or accessory pigments (phycobiliproteins and carotenoids), that capture light energy for chlorophyll a [2]. Ultimately, with this biochemical process, the photosynthetic organism provides atmospheric oxygen to the planet, it being thus fundamental to ensure and preserve all aerobic life within.

Natural pigments come in a great variety of colors and have been extensively used across time in everyday life. Food production, textile industry, paper industry, water science and technology, agriculture research and practice, are just a few examples worth mentioning [3]. Pigments have a number of essential characteristics that render them adequate in these several industrial contexts, and they also show beneficial biological activities, as antioxidants and anticancer agents [3]. Therefore, they have great potential to fulfill recent market demands, that have been increasingly targeting the health and biotechnological sectors, in a quest for natural compounds and products with proven beneficial effects on human health [4]. In marine algae, Pangestuti and Kim [5] compiled a list of the natural pigments with health benefit effects, from a wide range of seaweed
species. Natural pigments are also structurally diverse, which in turn determines their stability against a range of environmental and technological conditions. The analysis of pigments in a matrix is performed by liquid chromatography, which is the most adequate method to perform a qualitative determination and identification of a given pigment [3].

In coastal waters, the visible radiation interacts with sediments and macrophytic components, and thus there are variations in light spectral composition and irradiance, when compared to the incident light. In seaweeds, it is these variable spectral proportions that shape their relative pigment composition [6]. Essentially, it is the pigment abundance and distribution found in a seaweed that determines its color and even its classification into one of the three main phyla. Green seaweeds (phylum Chlorophyta), have high content in chlorophyll \(a\) and \(b\) besides smaller quantities of \(\beta\)-carotene and xanthophylls. Brown seaweeds (phylum Ochrophyta, class Phaeophyceae), which in truth have colors that range from yellow and olive-green to brown, present high concentrations of the xanthophyll fucoxanthin, and low quantities of other xanthophylls, chlorophyll \(a\) and \(c\), and \(\beta\)-carotene. Red seaweeds (phylum Rhodophyta) present high content in phycoerythrin and phycocyanin, and low quantities of chlorophyll \(a\), \(\beta\)-carotene, and xanthophylls [7,8].

There are a number of reviews pertaining to pigment availability, characteristics, and applications, but they are mostly focused on those provided by microalgae [9–12], and other microorganisms [13]; an historical example is the fungi \textit{Monascus} (phylum Ascomycota) [14], whose pigment “red koji” or “angkak” is explored in Asia by the oldest industry within this sector. For centuries, “red koji” has been providing color to red rice wine and red soybean cheese, among other food products [10].

Industrial processes using fungi, bacteria, or microalgae are already existent, providing carotenoids and phycocyanins on a large scale [13]. On an industrial scale, the cosmetic sector covers 870 patent families, where pigments from microalgae occupy a respectable share of 31%; the human nutrition sector covers 2356 patents where pigments have the second largest share, with \(\beta\)-carotene and astaxanthin standing in the top of development lines. Together with proteins, they are the compounds experiencing the strongest growth in both sectors [15]. Table 1 presents an overview of the three main pigments that can be found within Rhodophyta, and the number of patents registered for each of them in Patentscope (search performed in the search field “front page”), regardless of the source they were obtained from (being either macroalgae or any other biological source). Chlorophyll \(a\), whose prime source lies in every oxygenic photosynthetic organism, holds the highest number of patents (5949 patents). A close second is held by astaxanthin (4236 patents), while allophycocyanin ranks in last, being featured in 67 patents only [16].

| Pigment | Number of Patents |
|---------|-------------------|
| PBP     |                   |
| PE      | 314               |
| PC      | 573               |
| APC     | 67                |
| Carotenoids |             |
| \(\alpha\)-C | 178           |
| \(\beta\)-C  | 2589           |
| Xanthophylls |            |
| Lutein  | 2591             |
| Zeaxanthin | 1333          |
| Astaxanthin | 4236         |
| Chlorophylls |        |
| Chlorophyll \(a\) | 5949     |

Table 1. Number of registered patents in Patentscope for Phycobiliproteins (PBP; PE: Phycoerythrin, PC: Phycocyanin, and APC: Allophycocyanin), Carotenoids (\(\alpha\)-C: \(\alpha\) carotene, \(\beta\)-C: \(\beta\) carotene), and Chlorophylls.

The present review focuses, however, on pigments produced by macroalgae, specifically, red seaweeds (phylum Rhodophyta); as this group also share a number of pigments found in microalgae, opportunities do not go amiss when endorsing red seaweeds as an
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additional and valuable source of pigments. This review has thus the objective to provide an insight to recent information and research, concerning the pigments found within Rhodophyta. A brief outline of the role and chemical biodiversity within each pigment group will be provided, and the topics to be explored include current extraction and purification methodologies and challenges, and edge biotechnological applications under a sustainable and economic perspective. Commercially available products and registered patents will also be outlined.

2. Phycobiliproteins

Most plants and algae filo contain chlorophyll \( a \) and chlorophyll \( b \) to harvest light energy. As chlorophyll \( a \) is active at wavelengths 430 and 680 nm, and chlorophyll \( b \) at wavelengths 450 and 660 nm, these plants and algae are photosynthetically active within this range. However, since red seaweeds only have chlorophyll \( a \), light is only harvested within the blue and red region of the visible spectrum, and there would be an absorption gap in the spectra region in between [17]. In order to fill this gap and optimize light harvests, red seaweeds assemble phycobilisomes (PBS) in the thylakoid membrane. PBS are highly efficient, supramolecular complexes, with an absorption range of 500–660 nm, which capture solar energy and transfer it to photosystems. The role of PBS as the main light-harvesting chromoproteins was discovered in 1883 by Theodor Wilhelm Engelmann [18], through the study of the Cyanobacteria Oscillatoria. Nowadays, however, it is known that the PBS role extends beyond light-harvesting, it being also acknowledged to hold an important task as photo-protectors against high irradiances, as well as a nutrient source in times of nitrogen and phosphorus insufficiency [19].

2.1. Distribution, Properties and Structure

Phycobilisomes can be classified into three types according to their morphology (hemi-ellipsoidal, hemi-discoidal, and bundle shaped) [1] and are composed by phycobiliproteins (PBP). PBP present an intrinsic brilliant color being highly fluorescent [20], and can be found not only in Rhodophyta (comprising up to 50% of all water-soluble proteins) [21] but also in Cyanobacteria (comprising up to 60% of all water-soluble proteins) [22] and in Cryptomonads (phylum Cryptophyta) [23]. PBP are classified in different families, according to absorption properties, and present a distinctive color conveyed by the chromophores: the red–pink phycoerythrin (PE, \( \lambda_{\text{max}} = 540–570 \text{ nm} \)), the blue phycocyanin (PC, \( \lambda_{\text{max}} = 610–625 \text{ nm} \)), the blue–green allophycocyanin (APC, \( \lambda_{\text{max}} = 650–660 \text{ nm} \)), and the blue–pink phycoerythrocyanin (PEC, \( \lambda_{\text{max}} = 560–600 \text{ nm} \), not present in red seaweeds) [4,17,24], along with hydrophobic linker peptides [1]. All these PBP are generally composed of an \( \alpha \) and a \( \beta \) subunit, and among these, PE also holds a \( \gamma \) subunit [25]. These subunits may contain about 160 to 180 amino acid residues, and are connected with prosthetic group chromophores, which are essentially linear tetrapyrrole groups that bind an apoprotein to cysteine residues through a thioether bond [4,26]. The protein to which this attachment occurs determines the PBP absorption spectrum, and the prosthetic group of the chromophore. A phycobilin can be categorized as phycoerythrobilin (pink–red compound), phycocyanobilin (blue compound), phycourobilin (yellow compound), and phycoviolobilin (purple compound) (Figure 1) [4]. PE and PEC are rich in blue-absorbing phycoerythrobilin, phycouribilin, and phycoviolobilin, whereas PC and APC contain phycocyanobilin only [27].
PBPs have noteworthy spectroscopic properties, such as high absorption coefficient, high excitation and emission spectra, high quantum yield, low interference, high quenching stability, and water solubility [4,31]. Therefore, they have been widely considered in several applications.
and well documented applications, namely, in biomedical research, clinical diagnostics, therapeutic science, and cosmeceutical and pharmaceutical industries [1,28,32,33]. Namely, these pigments have been widely applied as fluorescent probes in flow cytometry, immunofluorescence microscopy [34], immunomodulation [35], and as photosynthesizers in cancer therapy [36].

However, the primary commercial interest in PBP stems from the fact that these proteins offer health benefits as antioxidants and free-radical scavengers [5,37–39], having a therapeutic and nutraceutical effect [40–43], as well as being effective neuroprotective, anti-bacterial [37], anti-viral [44], anti-inflammatory [45], anti-allergic [46], anti-tumoral [37,47–50], anti-ageing [46], anti-Alzheimer, hepatoprotective [39], immunomodulatory [51], and hypcholesterolemia agents [4,19,42].

In addition to their antioxidant power, PBPs are non-toxic and non-carcinogenic natural dyes. Therefore, they are also earning crescent interest over synthetic colorants within the food and cosmetic industry [9,10,21], following consumer demands in their pursuit for a healthier lifestyle. In food products, these pigments also play a role in appealing to the consumer, by conveying eye-catching colors to milk-based products, soft drinks, ice-creams, desserts, candies, milkshakes, and cake decoration, and holding the color for at least one month in room temperature [19]. In cosmetics, they act as photoprotective agents in sunscreens, and give color to a range of make-up products such as lipsticks and eyeshadows [19]. On a commercial level, PBP (especially PC) are mainly obtained from the cyanobacteria Arthrospira sp., known as Spirulina, that is widely acknowledged in itself as a functional food due to its noteworthy nutraceutical properties [52].

2.3. Extraction and Purification Methods

PBPs are not easy and straightforward obtained. Traditionally, the methods to obtain PBP extracts present a challenge by themselves since, as mentioned, PBP are located within the phycobilissome inside the chloroplast, and thus, the algae must be pretreated with appropriate solvents, and the cells must be homogenized and disrupted using suitable methods, to release the contents within [18]; this must be achieved while avoiding any step or method that involves high temperatures, as these pigments are highly thermosensitive. Afterwards, the PBP must be purified to remove every other cell compound from the end product. The PBP purity determines its final application and, henceforth, its market value. Low purity (\(A_{\text{max PBP}}/A_{280} \approx 0.7\)) is assigned to food grade, purity with \(A_{\text{max PBP}}/A_{280}\) around 3.9 is assigned to reagent grade, and high purity (\(A_{\text{max PBP}}/A_{280} \geq 4.0\)) is assigned to analytical grade [18]. In recent years, the increasing market demands and the amount of new possible applications for these pigments, has encouraged huge efforts from the scientific community to improve and develop cost-effective methods to get PBPs with maximum yield and purity [19].

There are different methods to perform the extraction, whose choice is a critical step to attain a maximum PBP recover, as it has a significant impact on activity and purity of the obtained pigment [1]. Specific factors include biomass conditioning (fresh versus dry algae), biomass/solvent ratio, cellular disruption method, solvent, extraction time and number of steps taken [18], storage method prior to extraction [53], and even factors unconnected to the methodology itself, such as the harvest season [18], and species where the pigment was extracted from [54]. Therefore, it is naturally expected to find a certain degree of variation among works on this topic.

Traditionally, the abundance and diversity of the different PBP in an organism has been commonly estimated by means of light absorption assessment at distinct wavelengths, performed upon the PBP extracted from the biological matrix [55,56]. However, authors such as Saluri et al. [57], who lists several other works that reply on this approach to assess the PBP R-PE (a subtype of PE obtained from Rhodophyta [28]) content in red seaweed species, defend a more reliable method being needed, so that the most promising algae species regarding R-PE content can be targeted. According to the authors, the traditional method of absorbance reading is both quick and widely used; however, it can
produce misleading results whenever impurities remain present in the samples, and it is unreliable overall, regardless of whether it is assessed upon crude extracts or following their purification. An example offered in order to circumvent this is found in Saluri et al. [57]’s work, which describes an alternative approach of employing the High Performance Liquid Chromatography technique of Size Exclusion Chromatography (HPLC-SEC) method with fluorescence and photo-diode array detectors, not only to separate PBP from interfering compounds, but also to reliably quantify their yields.

2.4. Production and Commercialization

The wide commercialization and implementation of PBP in food and cosmetics is not only hindered by the low yields obtained during production, as mentioned, but also stalled by the limited chemical instability that characterizes these compounds, as these compounds tend to suffer denaturation, especially under heat and light conditions. In this sense, strategies to improve yield and chemical stability of both phycocyanin and phycoerythrin have been compared and reviewed [17]. However, and to this day, the purified PBP is the result of expensive methodologies, and more effective extraction and purification methods need to be considered [58], especially on a large scale. Lastly, most laboratorial protocols share a few points in common, such as a meticulous preparation of biomass, and use of highly specialized equipment. This, coupled with the wide range of fine-tuned differences between extraction protocols hinders the attempt to universalize an efficient procedure, able to sustainably process large quantities of algae at a minimal cost. Therefore, while researchers report amazing yields in the laboratory, they would be far from being considered as such on a commercial perspective. As pointed out by research [18,59], research must focus on a much larger scale setting and involving industrial parameters, to develop a commercial protocol able to deliver a pigment with high yield and purity. This still holds true to this day.

As the production of PBP from natural sources requires a high investment in large-scale cyanobacteria or algae cultures, alternative approaches to obtain this pigment for biotechnological purposes has been investigated. Specifically, the production of recombinant PBP in Escherichia coli, while retaining all the qualities of a pigment obtained from native organisms, has been studied by a number of authors [33,60,61].

PBP are applied in the industry in their water-soluble protein form, and their commercial utilities and applications lie on inclusive knowledge and technology backgrounds, which are yet scarce [62]. Therefore, the great majority of the novel applications involving the use of PBP are only reported in patents to this day [62,63]. Nevertheless, a few examples can be found on a commercial level, where there are a few companies profiting from PBP commercialization in the form of natural dyes yet featuring prominently microalgae as the source for their products. For example, the commercially available blue colorant Linablue® Spirulina Extract is sold as a food product decoration component, and several companies promote their microalgae-based natural dyes to be introduced in cosmetic products, namely, phycocyanin from Arthrospira sp. And phycoerythrin from Porphyradium sp., or distribute R-Phycoerythrin as a laboratory consumable (from Neopyropia tenera or Neogastroclonium subarticulatum) for biotechnological/biomedical research.

2.5. Phycoerythrin

Phycoerythrin (PE), the red pigment, owes part of its name to “erythros”, which means “red” in Greek. PE is a photosynthetic pigment, present in red macroalgae [35], red microalgae [23] and cryptomonads [64]. The original source where they are found determines their further classification into R-PE (PE obtained from Rhodophyta, \( \lambda_{\text{max}} = 545-565 \) nm, Figures 3 and 4), C-PE (PE obtained from Cyanobacteria, \( \lambda_{\text{max}} = 540-570 \) nm), and B-PE (PE obtained from Bangiales, a specific family of filamentous red macroalgae, \( \lambda_{\text{max}} = 546-565 \) nm) [4,28]; besides their origin, these compounds have among them slight variations in their absorption characteristics [62]. PE stands as the major soluble protein produced by the cell of red algae kept growing under low light but high nutrient load [65].
Gracilaria gracilis have commercial applications such as decorations, soft drinks, and alcoholic beverages that fluoresce under UV light or specific pH values [10].

Regardless of the purification methodology specifics, it is noticeable that species that results (all PE Purity values above 5% were obtained through one of these approaches), sodium phosphate buffer or, less frequently, distilled water, yields the highest Purity Index. Table 2. Through the analysis of this table, it is noticeable that the combination of grinding and/or freeze-thaw, specifically with the red macroalgae Ceramium ciliatum.

Additionally, as it is able to emit a strong yellow fluorescence, PE extracted from the red macroalgae Porphyridium sp. (phylum Rhodophyta) has been extensively explored and tested in the formulation of foods with “special effects”, such as visually appealing cake decorations, soft drinks, and alcoholic beverages that fluoresce under UV light or specific pH values [10].

In the last decades, PE has been extracted and purified from a wide range of species, and adopting a variety of methodologies, listed in Table 2. Through the analysis of this table, it is noticeable that the combination of grinding and/or freeze-thaw, specifically with sodium phosphate buffer or, less frequently, distilled water, yields the highest Purity Index results (all PE Purity values above 5% were obtained through one of these approaches), regardless of the purification methodology specifics. It is also noticeable that species that have commercial applications such as Gracilaria gracilis, Grateloupia turuturu, and Palmaria

Figure 3. Crude extract of R-PE obtained from the red macroalgae Ceramium ciliatum.

Figure 4. Typical absorption spectra of phycobilin’s (R-phycoerythrin and R-phycocyanin) extracted from the red macroalgae Gracilaria gracilis. The red arrows represent the absorption maxima that characterizes the spectrum of R-phycoerythrin, whereas the blue and the blue–green arrows represent the plateau where the absorption maxima of R-phycocyanin and allophycocyanin can be found.
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*palmata* (phylum Rhodophyta) have drawn more attention research-wise. Moreover, while this review focuses on pigments obtained from red macroalgae, it is noteworthy to point out the role of the microalgae *Porphyridium purpureum* (formerly *Porphyridium cruentum*), also a Rhodophyta, in the recovery of PE (specifically B-PE), it being the focus of several research endeavors [55,66–68].

**Table 2.** Examples of extraction and purification methods, yields, and purity of PE obtained from red seaweed species.

| Species                  | Extraction Method | Isolation and Purification Steps                                          | PE Concentration (mg g⁻¹ or mg mL⁻¹) or Yield (%) | PE Purity (%) | Reference                  |
|--------------------------|-------------------|--------------------------------------------------------------------------|-----------------------------------------------------|---------------|----------------------------|
| *Amphiroa anceps*        | Grinding with PBS(+) | (NH₄)₂SO₄ salting-out Q-Sepharose IEXC Sepharose CL-6B GFC                | 25 mg 35 g⁻¹ dw (PEx) n/a                           |               | Kawasar et al. [69]        |
| *Carradoriella elongata* | Extraction with PB | n/a                                                                      | 3.70 mg g⁻¹ fw n/a                                  |               | Ismail and Osman [70]      |
| *Ceramium isogonum*      | Washing with KPB Freeze-thaw with Sodium Nitrate French Press | (NH₄)₂SO₄ salting-out Sephadex DEAE IEXC Ultrafiltration                   | 0.383% dw (PE) 2.10                                  |               | Kaixian et al. [71]        |
| *Ceramium tenuicorne*    | Cryogrinding and suspension in SCB | Phenomenex HPLC-SEC                                                         | 2.13% dw⁻³ (PE) n/a                                 |               | Saluri et al. [57]         |
| *Cottotylus truncatus*   | Cryogrinding and suspension in SCB | Phenomenex HPLC-SEC                                                         | 0.95% dw⁻³ (PEx) n/a                                 |               | Saluri et al. [57]         |
| *Colaconema formosanum*  | Grinding in PBS(+) | (NH₄)₂SO₄ salting-out HiTrap DEAE IEXC                                      | n/a                                                 | 5.79          | Lee et al. [46]            |
| *Constantinea rosa-marina* | Cryogrinding and suspension in SCB | Phenomenex HPLC-SEC                                                         | ≈0.22% dw⁻²⁻³ (PEx) n/a                             |               | Saluri et al. [57]         |
| *Corallina officinalis*  | Cryogrinding and homogenization in SPB | (NH₄)₂SO₄ salting-out Sepharose 4B CL-200 SEC DEAE-cellulose IEXC Sephacryl S-200 SEC | n/a                                                  | 4.7           | Hilditch et al. [72]       |
|                          | Extraction with PB | n/a                                                                      | >2 mg g⁻¹ fw ² n/a                                   |               | Ismail and Osman [70]      |
| *Dasypiphonia japonica*  | Ultrasonication with PB | (NH₄)₂SO₄ salting-out Sepharose CL-4B GFC Phenomenex HPLC-SEC             | n/a                                                 | 4.89          | Sun et al. [73]            |
| *Ellisolanda elongata*   | Grinding with NaPi⁺ and NaCl | HAC Superdex 75 GFC                                                      | 15 mg 25 g dw (PEx) 6.67                             |               | Rossano et al. [35]        |
|                          | Extraction with PB | n/a                                                                      | >1 mg g⁻¹ fw ² n/a                                   |               | Ismail and Osman [70]      |
| *Furcellaria lumbricalis*| Cryogrinding and suspension in CB | Superdex 200 SEC Phenomenex HPLC-SEC                                      | 0.13% dw (CEx) 1.41                                  |               | Saluri et al. [74]         |
|                          | Cryogrinding and suspension in SCB | Phenomenex HPLC-SEC                                                         | 0.59% dw⁻³ (PEx) n/a                                 |               | Saluri et al. [57]         |
| *Gelidium elegans*       | Cryogrinding and suspension in SCB | Phenomenex HPLC-SEC                                                         | ≈0.17% dw⁻²⁻³ (PEx) n/a                             |               | Saluri et al. [57]         |
| *Gelidium pacificum*     | Cryogrinding and suspension in SCB | Phenomenex HPLC-SEC                                                         | ≈0.18% dw⁻²⁻³ (PEx) n/a                             |               | Saluri et al. [57]         |
| *Gelidium pusillum*      | Grinding and enzymatic hydrolysis in PB | n/a                           (NH₄)₂SO₄ salting-out DEAE Cellulose AEXC | 0.29 mg g⁻¹ dw (CEx) n/a 57%                       |               | Mittal and Raghavarao [75] |
|                          | Grinding and ultrasonication with PB | Ultrafiltration                                                             |                                                     | 1.1           | Mittal et al. [76]         |
| *Gracilaria canaliculata*| Grinding with distilled water | (NH₄)₂SO₄ salting-out DEAE Cellulose AEXC                                    | 0.50 mg g⁻¹ fw (CEX) 3.79                            |               | Sudhakar et al. 2015 [77]  |
| Species                        | Methodology                                                                 | Protein Concentration | % Yield | Reference                          |
|-------------------------------|------------------------------------------------------------------------------|-----------------------|---------|------------------------------------|
| *Gracilaria corticata*        | Grind with PB, PB (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> salting-out     | 0.024% (PEX)          | 1.10    | Sudhakar et al. 2014 [78]          |
| *Gracilaria domingensis*      | Cryogrinding and suspension in PB, PB (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> salting-out | 7.69 mg g<sup>-1</sup> dw (CEX) | n/a     | Pereira et al. 2012 [79]           |
| *Gracilaria gracilis*         | Suspension in distilled water, grinding and suspension with acetic-acid-sodium acetate | 0.141 mg g<sup>-1</sup> fw | 4.4     | Wang et al. [80]                   |
| *Gracilaria longa*            | Cryogrinding and suspension in K-Pi and EDTA                               | 1 mg g<sup>-1</sup> fw (PEX) | 4.5     | D’Agnolo et al. [82]               |
| *Gracilaria tenuistipitata*   | Grinding and freeze-thaw with distilled water                              | 5.34 µg g<sup>-1</sup> (PEx) | 4.2     | Zhao et al. 2020 [83]              |
| *Gracilaria vermyculophylla*  | Grinding and freeze-thaw with EDTA                                         | 0.14 mg g<sup>-1</sup> fw | 4.4     | Francavilla et al. [81]            |
| *Gracilaria longa*            | Grinding and suspension in K-Pi and EDTA                                   | 10 mg 100 mg<sup>-1</sup> (PEx) | 6.5     | Gu et al. [85]                    |
| *Halymenia floresii*          | Freeze-thaw with PB                                                       | 0.91 mg g<sup>-1</sup> fw | 0.43    | Ismail and Osman [70]              |
| *Mastocarpus stellatus*       | Grind with PB, PB (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> salting-out     | 2.79 mg g<sup>-1</sup> dw (CEX) | 1.14    | Denis et al. [86]                  |
| *Neodilsea yendoana*          | Grinding and freeze-thaw with distilled water                              | 3.69 mg g<sup>-1</sup> dw | 0.93    | Munier et al. [53]                 |
| *Neopyropia elongata*         | Grinding and freeze-thaw with PB                                           | 1.4 mg g<sup>-1</sup> (PEX) | 5.29    | Niu et al. [91]                   |
| *Neopyropia kinositae*        | Grinding in PB                                                            | 2.22 µg cm<sup>-2</sup> | n/a     | Sano et al. [93]                   |
| *Neopyropia yezoensis*        | Grinding and freeze-thaw with PB                                           | 3.69 mg g<sup>-1</sup> dw | 3.30    | Pan et al. [50]                   |
| *Palmaria decipiens*          | Cryogrinding and freeze-thaw with KPB                                       | 732 µg g<sup>-1</sup> fw | n/a     | Lüder et al. [96]                 |
Endeavors such as the aforementioned examples recurrently produce a fair number of valuable publications promoting novel, simple, fast, and/or optimized methods of phycoerythrin extraction from red seaweeds. However, these advantages, endorsed by such research and certainly replicable on a laboratory scale, do not always hold the same effectiveness, cost level, and sustainability when considering an industrial scale. Dumay et al. review the existing methods of extraction and purification of PE from marine red algae [21], while Fleurence [28] reviews extraction methods to obtain PE from red seaweeds, as well as their potential in biotechnological applications.

Most procedures to extract PE from red macroalgae are summarized by a range of simple and fast steps, where the challenge lies in breaking the cellular wall and recovering the water-soluble PE with an appropriate solvent. Old, classical processes consist in a simple maceration with mortar and pestle upon fresh or frozen samples have been proven effective in a range of red seaweed taxa [58, 81, 102–104]. Authors often choose to complement this conventional mechanical grinding with freeze thawing cycles [83, 84, 91],

| Table 2. Cont. |
|----------------|
| **Phycology** 2022 |
| **Palmaria palmata** |
| Cryogrinding and suspension in PB |
| Preparative PAGE |
| 1 mg l⁻¹ (CEx) |
| 3.2 |
| Galland-Irmouli et al. [97] |
| Cryogrinding, suspension in AB and enzymatic hydrolysis (xylanase) |
| n/a |
| 11.27 mg g⁻¹ dw |
| 0.74 |
| Dumay et al. [98] |
| Grinding, suspension in distilled water, (NH₄)₂SO₄ precipitation, and digestion in thermolysin |
| n/a |
| 54.3 mg g⁻¹ dw |
| n/a |
| Lee et al. [45] |
| **Porphyrha sp.** |
| Grinding and suspension in SCB Phenomenex HPLC-SEC |
| 0.36 mg mL⁻¹ |
| 3.18 |
| Huang et al. [59] |
| Enzymatic hydrolysis (xylanase) |
| n/a |
| ≈ 2 mg g⁻¹ fw² |
| n/a |
| Sfriso et al. 2018 [64] |
| **Polysiphonia morrowii** |
| Grinding and freeze-thaw with EDTA |
| Phenyl-Sepharose EBAC |
| 0.34% (CEx) |
| 3.90 |
| Niu et al. [100] |
| Q-Sepharose AEC |
| HAC |
| **Portiera hornemannii** |
| Grinding and freeze-thaw with (NH₄)₂SO₄ salting-out Q-Sepharose AEC |
| 1.23 mg g⁻¹ fw (CEx) |
| 5.21 |
| Senthilkumar et al. [101] |
| **Pterocladiella capillacea**¹ |
| Extraction with PB |
| n/a |
| ≈ 2 mg g⁻¹ fw² |
| n/a |
| Ismail and Osman [70] |
| **Rhodomela confervoides** |
| Grinding and suspension in SCB Phenomenex HPLC-SEC |
| 1.33% dw⁻¹ (PEEx) |
| n/a |
| Saluri et al. [57] |
| **Vertebrata fucoides**¹ |
| Grinding and suspension in SCB Phenomenex HPLC-SEC |
| 0.52% dw⁻¹ (PEEx) |
| n/a |
| Saluri et al. [57] |

Extraction Method and Purification Method is listed if available and refer to the method that gave the best PE Purity Index (PI) results, regardless of if higher yields (but lower PI) were reported. Therefore, PE data are expressed as the maximum recovery, yield, or purity index the authors obtained, if available, giving priority to data presenting the highest PI per reference. PE Recovery and PE Purity values listed refer to those obtained from the last purification step, even though the intermediate purification steps are listed in Purification Method. AC: Acetate Buffer; PBS(+): Phosphate Buffer Saline; CB: Citrate Buffer; SCB: Sodium Citrate Buffer; PB: Phosphate Buffer; SPB: Sodium Phosphate Buffer; KPB: Potassium Phosphate Buffer; IEXC: Ion-Exchange Chromatography; AEC: Anion Exchange Chromatography; EBAC: Expanded Bed Absorption Chromatography; HIC: Hydrophobic Interaction Chromatography; CPC: Centrifugal Precipitation Chromatography; GFC: Gel Filtration Chromatography; HAC: Hydroxyapatite Chromatography; SEC: Size Exclusion Chromatography; DEAE: Diethylaminomethyl; FPLC: Fast Protein Liquid Chromatography; PAGE: Polyacrylamide Gel Electrophoresis; ATPE: Aqueous Two-Phase Extraction; PI: Purity Index; dw: dry weight; fw: fresh weight; FD: Fluorescence Detection; PDAD: Photo-diode Array Detection; CEx: Crude Extract; PEEx: Purified Extract; n/a: not reported. ¹ The scientific name underwent update(s) since the corresponding reference was published. ² Value estimated from the graphic/image within the corresponding reference. ³ As the authors compare different quantification methods to estimate PE yields, the Beer and Eshel result with baseline-corrected spectrum was chosen to show PE yields for this reference. ⁴ PI is the abbreviation of Phosphate.
or in tandem with liquid nitrogen (cryogrinding) [53,57,79,96], both working effectively in a number of species. Liquid nitrogen can also be used to homogenize red seaweed biomass assisted by an acid-washed neutral sand or diatomaceous earth, coupled with a weak acid buffer such as sodium phosphate [18]. However, it remains an unsuitable process to consider on an industrial scale [28].

Common solvents applied and tested among researchers, to retrieve PE from red seaweeds, are phosphate buffer [35,46,50,58,69,70,73,78,79,89,90,93] and sodium phosphate buffer [53,103], featured prominently in Table 1. However, solvents such as potassium phosphate buffer [96], distilled water [45,77,83,91], acetic acid–sodium acetate [81,102], citrate buffer [57,74], diethyl ether [74], and EDTA [84], have also been found to be effective.

To separate PE from other pigments and compounds in red macroalgae, the pigment can be isolated and purified by either precipitation with ammonium sulfate [50], gel filtration chromatography [103], gel filtration and ion-exchange chromatography [69,72,73], expanded bed absorption and ion-exchange chromatography [80,83,94], centrifugal precipitation chromatography [85], size exclusion chromatography [57], preparative polyacrylamide gel electrophoresis (SDS-PAGE) [89,97,103], and hydroxyapatite chromatography (hydroxyapatite being a low-cost, laboratory-prepared chromatographic resin, which has also been used as an alternative to chromatography to purify PE from red seaweeds) [35,100,104]. A novel high-pressure liquid chromatography method, coupled with fluorescence and photodiode array detection, was developed recently by Saluri et al. [57,74]; however, as seen by Table 1 for Furcellaria lumbricalis, studied by these authors, the results do not stand out when compared to results obtained by other authors; it is noteworthy, though (and already stated), that given the amount of variables pertaining to the method, species, or time of the year when harvesting, it is not unexpected to find a certain degree of variation in the results across the literature.

As mentioned, the conventional means of PE extraction hold huge economic costs when considering industrial applications. Among non-conventional methods, the ultrasound technique has also been used in PE recovery from Grateloupa turuturu [87,105]. Another alternative method that has been suggested by Fleurence et al. [28], consists in the enzymatic hydrolysis of the seaweed cell wall to access the content within, it being possible to achieve yields higher than those generally recorded by conventional methods, without denaturation of the pigment. The biochemical composition of this wall presents variations according to species; therefore, the choice of the enzyme has to be done sensibly. Xylanases and cellulases have been commonly applied, the PE yield being dependent on factors such as enzyme/algae ratio, temperature, pH, and time adopted during the enzyme hydrolysis [28]. Fleurence et al. [28]’s proposal of enzymatic maceration, which would decrease extraction costs as the proposed enzymes are available commercially, has been explored by a number of authors in recent years, to extract PE from several red seaweed taxa, generally reporting substantially higher PE yields when applied to an enzyme or a consortia of enzymes than those obtained with conventional methods, or controls without the enzyme addition (Table 3).

Recent reviews (from 2016 onwards) pertaining to PE are yet scarce, but existent (and discussed together with other PBP), and they seem to be more focused on those obtained from microalgae [4,9,17,19,32,55,106]. These include pigment availability and utilization [9], PBP/PBS structural chemistry [1,27,32,107], production [1,17,19,55], purification [19], and applications [1,4,19,32,62,106]. Recently, Zhang et al. [108] uncovered the entire PBS complex (to 3.5 Å) from the red seaweed Griffithsia pacifica, through Cryogenic Electron Microscopy (cryo-EM).
Table 3. Examples of extraction methods assisted by enzymatic hydrolysis, and corresponding PE yield % increase (in comparison with an assay without enzyme treatment, or in comparison to an assay without condition optimization for extraction) and resulting final yields of the crude extract (unless stated otherwise), obtained by several authors. This list presents examples, and it is not meant to be exhaustive. For extra information (extraction method and steps) regarding each reference, see also Table 1. If more than one enzyme is listed in “Extraction Procedure”, that means a consortium of those enzymes was applied. Xyl: xylanase; Cel: cellulase; Aga: agarase; dw: dry weight; PEx: Purified Extract.

| Species                  | Extraction Enzyme(s) | PE Yield % Improvement | PE Yield | Reference                        |
|--------------------------|----------------------|------------------------|----------|----------------------------------|
| Furcellaria lumbricalis  | Xyl + Cel            | 130 $^2$               | >0.45% dw (PEx) | Saluri et al. [74] |
| Gelidium pusillum        | Xyl + Cel + Aga      | 26 $^2$                | 0.29 mg g$^{-1}$ dw | Mittal and Raghavarao [75] |
| Mastocarpus stellatus    | Xyl                  | 1.8 $^3$               | 1.99 mg g$^{-1}$ dw | Nguyen et al. [90] |
| Neopyropia yezoensis     | Cel + Aga            | 3.33 $^2$              | 6.953 mg g$^{-1}$ dw | Wang et al. [95] |
| Palmaria palmata         | Xyl                  | 62 $^2$                | 12.36 mg g$^{-1}$ dw | Dumay et al. [98] |
| Porphyra sp.             | MA103 + MAEF108      | n/a                    | 0.36 mg mL$^{-1}$ | Huang et al. [99] |

1 The scientific name underwent update(s) since the corresponding reference was published. 2 When compared to assay without enzyme treatment. 3 When compared to assay without condition optimization for extraction. 4 Crude enzyme solutions from the marine bacterial strains Pseudomonas vesicularis MA103 and Aeromonas salmonicida MAEF108.

2.6. Phycocyanin

Phycocyanin (PC), the blue pigment, owes part of its name to “cyan” in English, which in turn derives from “kyanos” in Greek. Each of these words mean, interestingly, their own distinct shade of blue: blue-green and dark blue, respectively. PC is classified according to its source, and thus Rhodophyta contain R-Phycocyanin (R-PC), Cyanobacteria, and Cryptophyta contain C-Phycocyanin (C-PC), and Bangiophyceae contain B-Phycocyanin (B-PC) [109]. These pigments have slight variations among them, in their absorption characteristics [42,62].

PC shares the same applications as PE, with the added fact that it is the most frequently used natural blue pigment in the food industry, to color products such as jelly and bubble gum; in fact, other alternatives, likewise approved as natural blue food colorant, are yet scarce [110]. PC has an extra advantage and versatility over other natural pigments, which lies within its health-promoting abilities, and not as much as a food colorant; this is due to its lower stability under heat and light when compared to gardenia and indigo natural pigments, for example [111].

PC itself, regardless of the biological source, has been recently reviewed as well (from 2016 onwards) in a number of works, regarding its nutritional value as food and feed [112], nutraceutical potential [113], bioactive potential [114] as anti-cancer agent [47,48,115,116], extraction and purification methods [114,117–119], and even in phycocyanin remote-sensing, by scanning entire aquatic systems from space [120].

It is noteworthy to mention, however, that most studies involving PC do not obtain this pigment from red algae, let alone macroalgae. In fact, in an industrial context, this blue pigment is mainly provided by Cyanobacteria, one of the earliest groups of prokaryotic organisms, which keeps a cosmopolitan distribution over a wide range and variety of aquatic and terrestrial environments [4]. Specifically, PC is commonly extracted from the dried biomass of the Cyanobacteria Arthrospira platensis [43,121]. Therefore, it is a widely available, known and researched organism, not only regarding growth conditions [122], but also in a medical and cosmetic scope [123–125], as a nutrient removal and effluent treatment agent [126,127], and as a nutritional and health supplement [128,129]. In the European Union, extracts from this microalgae are used as a food colorant in confectionary products [110]. The advanced state-of-the-art and well-developed culture techniques for this specific cyanobacterium, have perhaps put behind research on this pigment in red
macroalgae, since it can be easily obtained from *A. platensis* in comparison, for further study and investment.

Nevertheless, while cyanobacteria remain to this day as the major provider of PC, this pigment has also been isolated and studied from red algae (although efforts are more incident in red microalgae than in macroalgae). The red microalgae *Cyanidium caldarium*, which are isolated from hot springs under intense sunlight, high temperatures, and an acidic environment, is considered an atypical red alga. Thus, its PC has been isolated and studied under the hypothesis that it may be thermostable and present distinct characteristics compared with cyanobacterial pigments [130], while the organism itself is less prone to be contaminated in its adjusted harsh culture conditions [131]. The B-PC isolated from the Bangiophyceae *Neoporphyrha haitanensis* [132] and *Bangia atropurpurea* [133] has shown potential as an anti-allergic agent and the one isolated from *Porphyra* sp. has potential as an antioxidant agent [99]. R-PC from *Polysiphonia stricta* was isolated, purified, and studied, in order to improve knowledge about R-PC structure and function in red macroalgae [134].

Compared to PE, PC obtained from red macroalgae has seldom been studied, although Fan-jie et al. [135] make reference to existing works present in the literature. Solvents such as phosphate buffer [70,134], potassium phosphate buffer [96], or phosphate buffer with ammonium sulfate [135] were used to extract PC from red seaweeds. Purification methods existent to obtain pure PC from algae extracts include aqueous two phase extraction [136], ammonium sulfate precipitation, polyacrylamide gel electrophoresis [99], gel filtration chromatography, high-performance liquid chromatography [99], and ion-exchange chromatography [20], which can be applied combined (e.g., aqueous two-phase extraction and ion-exchange chromatography [137] or gel filtration followed by ion exchange chromatography [134]). These methods have been mostly applied to recover C-PC from microalgae, and as long as cyanobacteria continue to satisfy the industry as the main provider of PC, red seaweeds will fall behind in comparison, and dedicated studies focusing on PC obtained from these macroalgae may not be easily acknowledged, followed, or promoted in the near future.

PC is reportedly not usable in low-acid beverages due to its sensibility to acid; therefore, the industry is currently seeking natural-derived alternatives [110]. Factors that shape PC stability were studied by Chaiklahan et al. [138], where the authors report temperature and pH as factors responsible for this pigment stability, and showing that glucose, sucrose, and NaCl have potential to maintain it [138]. Alternatively, thermophilic species could provide more thermostable PC variants (such as *Cyanidium caldarium*, mentioned above), but then a different sort of challenges and cost may arise when trying to cultivate these extreme-living organisms on a large scale. Therefore, a solution may pass to produce thermostable PC in non-native hosts, as proposed by Puzoriov et al. [139].

PC is the focus of a high number of patents, surpassing the number of existing patents focused on other PBP. A thorough report [140] regarding current trends and a forecast (up to 2027) of the PC market value mainly attributes this growth and interest to the increasing acceptance of this pigment (in substitution to synthetic alternatives) in the incorporation of therapeutic and nutritional products, but also attributes it to a demand for its unique color by itself. In fact, PC has a dark cobalt blue color, which almost no other natural pigment reproduces [62]. The report also states that (1) phycocyanin in a powder form, or (2) with a food grade, lead the phycocyanin market in 2020, while PC specifically formulated to be applicable as a nutraceutical agent will lead the market within the next years [140].

2.7. Allophycocyanin

Allophycocyanin (APC), the blue-green pigment, owes its name to “allos” (other) and “kyanos” (blue) in Greek. This pigment is situated in the core of phycobilisomes (PBS), where assembled trimers (αβ)₃ and phycocyanobilin’s bind to a α or β phycobilisome (PBP) subunit [26].

Research into APC seems to be yet quite scarce when compared with research targeted on the other PBP found in red seaweeds, and it seems to be more focused on understanding
mechanisms of structure [107,141,142] and synthesis [33,143]. Nevertheless, regarding extraction methods, diethyl ether gave good results, but methanol and ethanol were deemed improper in APC extraction of *Furcellaria lumbricalis* [74]. On the other hand, APC was extracted from a number of Rhodophyta species using phosphate buffer [70] and potassium phosphate buffer [96].

In red seaweeds, recent research aimed to produce high-quality recombinant APC in *Escherichia coli* from *Gracilaria chilensis* [33], to be applied in a biotechnological and biomedical context. However, in a laboratory context, most researchers often choose to study APC from cyanobacteria instead [44,61,144,145].

While APC is the PBP with the lowest amount of patent registrations, its unique blue-green color is reproducible by almost no other natural pigment [62], which probably increases its value as a unique compound.

3. Carotenoids

3.1. Distribution, Properties and Structure

Carotenoids are a family of long conjugated isoprenoid pigments that have a cosmopolitan presence among the vegetal kingdom, as well as in photosynthetic organisms and fungi [146], whereas the animal kingdom lacks the ability to produce them, probably due to the evolutionary loss of the required genes needed to encode the enzymes for carotenoid biosynthesis [147]. These accessory pigments are part of a light-harvesting antenna complex in the thylakoid membrane of chloroplasts [146], and contribute to the photosynthetic process by enhancing light harvest in the blue spectrum, and thus extending the light-absorption range [147]. Additionally, they have a key role as photo-protective and antioxidant agents, by shielding organisms from excess light energy, by performing the thermal dissipation of any energy surplus in the photosynthetic apparatus, and by acting as direct quenchers of reactive oxygen damage [148–150].

Being red-to-yellow isoprenoid compounds, with eight isoprene units composed of 40 carbon atoms [151], carotenoids have further chemical differences that earned them the main classification as carotenes (pure hydrocarbons) or xanthophylls (oxygenated carotenes) [148]. Examples of carotenes include α-carotene, β-carotene, and lycopene, while examples of xanthophylls include lutein, zeaxanthin, fucoxanthin, and astaxanthin [152].

While Rhodophyta is rich in carotenoid composition, differences can be found within the phylum, in microalgae (family Porphyridiaceae) and macroalgae. While the former contain β-carotene and zeaxanthin only, the latter contain α-carotene and lutein [153]. Additionally, Takaichi et al. [154] found an evident relationship between Rhodophyta phylogeny and carotenoid composition, establishing that there are differences between the macrophytic-type classes Bangiophyceae, Compsopogonophyceae, and Florideophyceae. Specifically, the class Bangiophyceae contains α-carotene, lutein, and zeaxanthins, the class Compsopogonophyceae contains antheraxanthin and zeaxanthins, and the class Florideophyceae presents, in turn, differences according to subclasses.

3.2. Biotechnological Potential and Applications

Historically, carotenoids have been investigated since the beginning of the 19th century [155], and nowadays, we know that these pigments provide a wide range of health benefits to humans [156]. However, since carotenoids cannot be synthetized by the animal kingdom as mentioned, humans must rely on food to get this healthy compound. For instance, vegetables and fruits (and their processed counterparts) are regarded as the best sources for this purpose [152].

Carotenoids enhance the nutritional value of a myriad of natural sources, such as fruit and vegetables, eggs, fish, algae, fungi, and yeasts [157,158]. In the human diet, approximately 50 carotenoids can be found [152].

Additionally, carotenoids are highly acknowledged in the cosmetic industry, by improving skin health by increasing dermal defense against UV [149]. They act as antioxidant agents, working connected with other reducing agents such as polyphenols and vitamins.
Carotenoids are photosynthetic pigments that are involved in primary light absorption [170], but also in the seaweed photoprotective system [171,172]. These orange, yellow, and red pigments are constituted by carbon and hydrogen units and are essential as vitamin A precursors [173]. While several carotenoids can be detected, depending on the algal species, β-carotene is one of the major carotenoids found [174].

The position of a double bond (and so a hydrogen) in the cyclic group at one end differs between the two major isomers of carotene, α-carotene, and β-carotene. The molecule of β-carotene has two rings known as β-rings that are made up of nine carbon atoms, while α-carotene has a β-ring at one end of the molecule chain and an ε-ring on the other end (Figure 5). Thus, carotenoids are tetraterpenoids considered lipophilic hydrocarbons, due to the absence of oxygen [175].

**Figure 5.** Molecular structure of the β-carotene.
Non-provitamin A carotenoid, lutein, and zeaxanthin are structural isomers. Lutein is a polyisoprenoid with 40 carbon atoms with cyclic structures at both ends of its conjugated chain chemically (Figure 6). As a result, it has a structure similar to zeaxanthin, but differs in the location of the double bond in one ring, resulting in three chiral centers compared to zeaxanthin’s [146,197]. Regarding the zeaxanthin chemical structure, it is constituted by a polyene chain with 11 conjugated double bonds and ionone rings (Figure 7). The hydroxyl group on the ionone rings might connect to the fatty acids during esterification [198].

Sample preparation, extraction, and saponification, followed by separation, identification, and quantification, are the steps applied in the general techniques for determining carotene in various matrices [158]. Still, some precautions must be considered when extracting carotenes in order to minimize carotene degradation and/or isomerization. For example, exposure to light, heat, and oxygen can significantly decrease the extraction efficiency, requiring thus a quick handling and processing [176,177].

A variety of methods have been used to extract carotenoids. Liquid–liquid extraction is the most common extraction method. However, this technique has several drawbacks, such as low efficiency of carotene extraction and time consumption and has high solvent requirements [178]. In contrast, many more recently developed extraction procedures have been reported and reviewed by other authors [178–183]. These techniques include ultrasound assisted extraction (UAE), microwave assisted extraction (MAE), enzymatically assisted extraction (EAE), pressurized liquid extraction (PLE), also known as accelerated solvent extraction (ASE), and supercritical fluid extraction (SFE) [178–183]. However, the high temperatures associated with the UAE and MAE techniques can cause carotenoids to degrade, while the SFE technique can be costly due to the need for dried samples and solvents [178]. Subcritical fluid extraction, which operates at lower temperatures and pressures than SFE, has recently been demonstrated to be a promising technique to extract carotene from seaweeds [184–186].

Despite the drawbacks of carotene extraction, the manipulation of abiotic parameters, such as light intensity, can enhance β-carotene production in red seaweeds [187]. For instance, there was observed in Neopyropia yezoensis sporophytes a higher β-carotene synthesis as a response to the increase of light intensity [187].

As stated, both α- and β-carotene have pro-vitamin A activity [188], and have shown potential as an anti-inflammatory agent [189] agent. Still, carotenoids hold other biological activities, such as antioxidant, anticancer, antiaging, and cosmeceutical [190–194]. Thus, these class of pigments represents a high potential for food, cosmetic, and pharmaceutical industries [190,195,196]. However, due to the evolution on microalgae cultivation and extraction technologies, the commercially products with carotene are mainly extracted from microalgae, such as Solgar, which is sold as a food supplement rich in β-carotene extracted from the green microalgae Dunaliella salina.

3.4. Xanthophylls

Non-provitamin A carotenoid, lutein, and zeaxanthin are structural isomers. Lutein is a polyisoprenoid with 40 carbon atoms with cyclic structures at both ends of its conjugated chain chemically (Figure 6). As a result, it has a structure similar to zeaxanthin, but differs in the location of the double bond in one ring, resulting in three chiral centers compared to zeaxanthin’s [146,197]. Regarding the zeaxanthin chemical structure, it is constituted by a polyene chain with 11 conjugated double bonds and ionone rings (Figure 7). The hydroxyl group on the ionone rings might connect to the fatty acids during esterification [198].

Figure 6. Molecular structure of lutein.
Despite the fact that the majority of zeaxanthin is extracted from green microalgae such as Dunaliella salina or Chloridium ellipsoideum, the red seaweed Corallina officinalis was discovered to be a good source of this compound of interest [199,200]. Moreover, the red seaweeds Gracilaria corticata and Grateloupia filicina also exhibited interesting concentrations of the xanthophyll’s lutein (0.26 and 18.38 μg·g⁻¹ dry weight, respectively) and zeaxanthin (0.65 and 2.16 μg·g⁻¹ dry weight, accordingly) [201]. Nevertheless, using the supercritical extraction method, lutein content can be increased through the algal biomass drying process, or ethanol quantity used, as well as the pressure, temperature, and carbon dioxide flow rate too [202]. Moreover, it was found that the high light intensity, as a stressor, can enhance zeaxanthin content on the sporophytes of the red seaweed Neopyropia yezoensis [187], which is a field worth further exploring.

Due to its anti-inflammatory potential, zeaxanthin is considered a tool against tumor and cancer development, and its application on chemotherapy can be beneficial for the patients [203]. This molecule, as a photoprotective agent, can be also employed on eye diseases, such as cataracts, or to mitigate macular degeneration [204,205].

For another perspective, lutein is already widely employed in industries such as cosmetics, pharma, and food, due to its color and biotechnological applications [206–208]. In fact, several investigations have shown that lutein has anticancer properties and prevents age-related macular degeneration and cataracts [206,209]. Oral lutein supplementation, for example, was found to minimize the impact of ultraviolet (UV) irradiation by reducing initial inflammatory reactions and the hyper-proliferative rebound caused by UV rays [210].

Because of its molecular structure, astaxanthin is a ketocarotenoid with unique chemical characteristics. Two carbonyl groups, two hydroxyl groups, and eleven conjugated ethylenic double bonds constitute astaxanthin [211]. The presence of hydroxyl and keto moieties on each ionone ring explains some of its distinct characteristics, including the capacity to be esterified, increased antioxidant activity, and a more polar nature than other carotenoid compounds [212].

The green microalgae Haematoococcus lacustris (formerly, Haematoococcus pluvialis), Chromochloris zofingiensis (formerly, Chlorella zofingiensis), and Chlorococcum sp., the red yeast Phaffia rhodozyma, and the marine bacterium Agrobacterium aurantiacum have all been shown to contain astaxanthin. Moreover, it was also found that the red seaweed Catenella caespitosa (formerly, Catenella repens) synthesizes interesting concentrations of astaxanthin depending on the sampling site and the harvesting season. For instance, researchers found that the pre-monsoon astaxanthin content of this seaweed collected on different sites of the northeast coast of India was higher than the monsoon and post-monsoon levels [213].

Astaxanthin extraction techniques have improved due to its biotechnological potential, particularly from the microalgae species Haematoococcus lacustris [214,215]. Many reported extraction methods were identified, including solvent extraction, supercritical fluid extraction, and oil extraction [214,216]. Still, astaxanthin isomers are difficult to isolate using conventional methods due to their chemical structure. As a result, many studies have been conducted to separate astaxanthin isomers such 13-cis astaxanthin, 15-cis astaxanthin, and trans-astaxanthin. Astaxanthin is often quantified through spectrophotometry or chromatography [215]. Thus, high performance liquid chromatography (HPLC) is the method of choice for analyzing carotenoids; for instance, one of the most powerful technologies for enantiomeric separation is HPLC using chiral stationary phases [217].

The potential for astaxanthin to be used as a nutritional component in treatment or prevention strategies for a variety of health problems caused by oxidative stress, UV-light
photooxidation or inflammation, cancers, and other pathological conditions has triggered an expansion in clinical trials and increased commercial production [218–224].

4. Chlorophyll
4.1. Distribution, Properties and Structure

Chlorophyll is a green pigment, which plays a key role in capturing energy from the light source, transferring energy and separating charges during photosynthesis [225]. The origin of the name chlorophyll derives from the Greek words “khloros” (green) and “phullon” (leaf) and due to chlorophyll, it is possible to convert carbon dioxide into oxygen, giving the origin to arise of oxygen-dependent organisms [226].

Red seaweeds have only one type of chlorophyll, chlorophyll $a$, which is present in all oxygenic photosynthetic organisms. Chlorophyll $a$ is located in the photosystem cores and in the light-harvesting antennas on the chloroplasts [225]. This molecule is chemically characterized by a porphyrin ring, comprised by a hetero polycyclic planner structure surrounding a central Mg$^{2+}$ ion, that bonds with nitrogen atoms coordinate positioned around it; a long phytol tail is attached to the polycyclic structure by ester linkage [227] (Figure 8). Chlorophyll $a$ is crucial to the algae’s autotrophic system, converting light energy, carbon dioxide, and water into chemical energy. This reaction is essential for the development and growth of all photosynthetic organisms, including algae [228].

![Molecular structure of chlorophyll $a$.](image)

4.2. Biotechnological Potential and Applications

Chlorophylls are naturally strong antioxidants acting as free radical scavengers [229], and have antimitagenic effects [230]. In addition, the study of Lee et al. [231] demonstrated that chlorophyll and chlorophyll derivatives extracted from the red seaweed Grateloupia elliptica have anti-obesity potential. This potential was analyzed by in vitro assays with 3T3-L1 adipocytes, where chlorophyll suppressed lipid accumulation by down-regulating adipogenic protein expression at the intracellular level without being cytotoxic to cells [231].

4.3. Extraction and Purification Methods

The extraction of chlorophylls from red seaweeds is mainly carried out by two methods: the conventional method of extraction by organic solvent (alcoholic or acetone-based solutions) or by cell disruption (mechanical or ultrasonication techniques). However, innovative green techniques demonstrate a high potential to be exploited to extract chlorophyll from red seaweeds, such as microwave-assisted extraction or green solvent extraction methods [232–236]. Heating of the red seaweeds reduces chlorophyll recovery and bioavailability, unlike green and brown seaweeds; however, heating decreases chlorophyll micellization [232]. In addition, during extraction and handling the extract, light is also critical to the quality of chlorophyll, as chlorophyll immediately reacts to the light intensity by absorbing energy [232].
Purification methods based on liquid chromatography are regularly applied. The main technique for purification and characterization of chlorophyll is the HPLC–MS/MS (High Performance Liquid Chromatography–Mass Spectrometry detector and selector) or using a UV/V spectrophotometry detector, due to the two well-known light absorbance peaks that chlorophyll $a$ has (372 and 642 nm) [237–241]. However, there are methods that facilitate the isolation of pigments after extraction and before purification/characterization of chlorophyll. These preparatory methods are thin-layer chromatography or column chromatography, which reduce time and effectively separate the chlorophyll [238,242].

Chlorophyll content and its bioavailability are highly related to the analyzed algal species, regardless of the Phylum. Although red seaweeds have the same chlorophyll $a$, the seaweed composition and biotic and abiotic factors, interfere with the content and quality of chlorophyll. The sensitivity to environmental conditions is due to the fact that chlorophyll may react with minerals, mainly magnesium, originating chlorophyll derivatives (pheophytins), with oxidation or decarboxymethylation reactions [232,238,243,244].

4.4. Production and Commercialization

Chlorophyll $a$ (chemical reference CAS 479-61-8) from red seaweeds is being studied as pigments/colorant for textile, food (EFSA approved food additive with code E140), animal feed, skin care, and cosmetic industry [245–250].

5. Conclusions and Future Perspectives

The photosynthetic pigments held by red seaweeds are highly valuable compounds that are present in noteworthy quantities, having unquestionable value in the biotechnological context, with potential applications currently reviewed. Granted, being natural pigments, they are desired over their synthetic counterparts, by a society that has been gradually growing more conscious regarding the environment and themselves, being increasingly concerned and rigorous about their health, well-being, and lifestyle. However, it is not easy to obtain these pigments from red macroalgae, and while there are listed here a good number of methods to extract, isolate, and purify these compounds, it is also acknowledged that there is no universal, single method that flawlessly works for every Rhodophyta. Intrinsic differences regarding cell structure and composition are found across taxa, which determine the effectiveness of the method, and steer the researcher to find a suitable recipe, and further optimize it. Therefore, the protocols must be fine-tuned, adapted, and ultimately tailored on a species basis, and thus, they all have their merits and virtues. Nevertheless, the grand challenge remains to this day how to convert these small-scaled methods into a single and universal large-scaled and profitable protocol, that must also be approved in a Blue Economy context to foster economic growth and improve livelihoods, while preserving the oceans, being eco-friendly and sustainable. This protocol can then be exploited by biotechnological industries, which in turn may provide high-quality natural pigments for humankind.

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