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Macroalgae as an Alternative Source of Nutrients and Compounds with Bioactive Potential †

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Abstract: The consumption of macroalgae has increased in occidental countries, favored by the excellent nutritional properties of their food products and the bioactive properties attributed to them. The present work aims to analyze the nutritional values, the composition in fatty acids, organic acids and carotenoids of several macroalgae species: *Codium tomentosum*; *Himanthalia elongata*; *Laminaria ochroleuca*.; *Saccharina latissima*; *Undaria pinnatifida*; *Porphyra* sp. and *Palmaria palmata.* Furthermore, the antioxidant and antimicrobial properties were assessed. Regarding the composition of the macroalgae, the levels of proteins (which ranged between 6 and 30 g/100 g of dry weight (DW)) and the low levels of lipids (below 1 g/100 g DW for all the species) stand out. In the case of carotenoids, lycopene and chlorophyll a were detected in all samples. Regarding antioxidant activity, OxHLIA assay was employed. EC50 values varied between 1.7 and 650 μg/mL for *L. ochroleuca* and *P. palmata*. *H. elongata* presented the greatest antibacterial potential (0.5–2 mg/mL) while *L. ochroleuca* showed the best antifungal effects (2–4 mg/mL). These species have good nutritional values and present interesting bioactivities. Thus, the incorporation of this macroalgae into the daily diet could provide nutritional and health benefits to the consumers. In addition, they could be used as a source of compounds for the nutraceutical, cosmetic and pharmaceutical industries.

Keywords: macroalgae; nutritional value; chemical characterization; bioactive potential

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1. Introduction

Macroalgae have been consumed by humans since ancient times, principally in the oriental countries, such as China, Japan or Indonesia. However, in the last decades, the consumption of edible algae in western countries has been increasing exponentially, mainly due to the current consumer preference and demand for organic products with high nutritional values and health benefits [1,2]. In general, dried macroalgae are foods with a low-calorie content, being rich in non-digestible polysaccharides, proteins and essential amino acids, vitamins, interesting minerals (such as sodium, chlorine, potassium and iodine) and phenolic compounds. Despite the low lipid content, macroalgae are rich in polyunsaturated fatty acids [2–4].

According to the literature, macroalgae are described as a source of bioactive compounds, with nutritional and pharmacological benefits on human health, such as dietary fibers, carotenoids or phenolic compounds [4–6]. However, the chemical and nutritional

properties of the seaweed product may differ depending on different factors, such as species, the harvest conditions (season and environmental factors), geographical region and the manufacturing process of the product [1–3].

In this study, a complete evaluation of the nutritional and chemical composition of seven commercial algae (*Porphyra* sp. C.Agardh, 1824; *P. palmata* (L.) Kuntze, 1891; *L. ochroleuca* Bach.Pyl.; *S. latissima* (L.); *H. elongata* (L.) S.F. Gray; *U. pinnatifida* (Harvey) Suringar, 1873 and *C. tomentosum* Stackhouse, 1797) was done, as well as the determination of bioactive potential (antioxidant and antimicrobial), in order to explore their potential as functional foods.

2. Material and Methods

2.1. Sample Preparation

Commercial samples of the abovementioned macroalgae were provided by the Algas Atlánticas Algamar S.L. (based in Pontevedra, Spain) company. The macroalgae were recollected in the natural environment in Pontevedra province coasts (Galicia, Spain) and washed with distilled water in a mechanical roller system. Then, the samples were dried in a force air stove (Pazos de Borbén, Pontevedra, Spain) for 48 h at 40 °C, and finally, were reduced to a fine dried powder (~20 mesh), mixed to obtain a homogeneous sample and package-protected from light, until further analysis.

2.2. Nutritional Characterization of Macroalgae

The contents of protein, fat, carbohydrates and ash, were determined in the seven commercial macroalgae species according the AOAC methods [7] and following a protocol previously reported by [8]. The total carbohydrates were calculated by difference and the energic value was calculated using the equation: Energy (kcal) = 4 × (g protein + g carbohydrates) + 9 × (g fat).

2.3. Chemical Composition of Macroalgae

The fatty acids were evaluated according to a methodology previously described by [8]. The determination was performed through a gas chromatography coupled with a flame ionization detector (GC-FID, DANI model GC 1000, Contone, Switzerland) and were identified by comparing the relative retention times of FAME peaks from samples with commercial standards (FAME reference standard mixture, standard 47885-U, Sigma-Aldrich, St. Louis, MO, USA).

The content of organic acids of seven macroalgae samples was determined following a methodology previously described by [8], using an Ultra-Fast Liquid Chromatography (UFLC, Shimadzu 20A series, Kyoto, Japan) and a photodiode array detector. The quantification of compounds was made using calibration curves obtained from commercial standards.

The concentration of carotenoids were determined used a method previously described by [9].

2.4. Bioactive Evaluation

2.4.1. Preparation of Extracts

The studied extracts, obtained from dried macroalgae, were prepared through a maceration, adding 50 mL of ethanol/water (80:20 *v*/*v*) to 1 g of dried sample. The mixture was left under stirring at room temperature for 1 h and then filtered. The residue was re-extracted with additional 50 mL of the same solution, under the same conditions. Both extracts were evaporated at 40 °C in a rotary evaporator (Buɔchi R-210, Germany) to remove the alcoholic fraction. Finally, the aqueous phase was frozen and lyophilized (FreeZone 4.5, Labconco, Kansas City, MO, USA) to obtain a dry extract [10].

2.4.2. Evaluation of Antioxidant Activity

For the evaluation of antioxidant activity, dried extracts were re-dissolved (100 mg/mL) in ethanol/water (80:20 *v*/*v*) solution and successively diluted to determine their respectively EC_{50} values. The oxidative hemolysis inhibition assay (OxHLIA) was carried out using sheep blood samples, as previously described by [11].

2.4.3. Evaluation of Antimicrobial Activity

The dried extracts obtained from macroalgae were dissolved in water (10 mg/mL) and the antibacterial potential was evaluated applying a methodology previously described by [12]. In this assay, three Gram-negative bacteria strains: *Escherichia coli* (ATCC 25922), *Salmonella typhimurium* (ATCC 13311) and *Enterobacter cloacae* (ATCC 35030) and three Gram-positive bacteria strains: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate) and *Micrococcus flavus* (ATCC 10240), were used. The minimum inhibitory (MIC) and minimum bactericidal (MBC) concentrations were determined, using streptomycin and ampicillin as positive controls.

For the antifungal activity, the methodology described by [13] was applied and six micromycetes were used: *Aspergillus fumigatus* (human isolate), *Aspergillus niger* (ATCC 6275), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC11730), *Penicillium funiculosum* (ATCC 36839), and *Penicillium verrucosum var. cyclopium* (food isolate). The MIC and minimum fungicidal concentration (MFC) were evaluated, using ketoconazole as positive control.

3. Results

3.1. Nutritional Characterization

The nutritional composition of the seven dried macroalgae samples (*Porphyra* sp., *P. palmata*, *L. ochroleuca*, *S. latissima*, *H. elongata*, *U. pinnatifida* and *C. tomentosum*) is showed in Table 1.

Table 1. Nutritional composition of seaweed species analyzed. The values are present as mean ± SD.

	C. tom	*H. elo*	*L. och*	*S. lat*	*U. pin*	*Por*	*P. pal*
Ash (g/100 g DW)	33.5 ± 0.8	29.1 ± 0.4	46.1 ± 1.3	16.1 ± 0.5	33.08 ± 1.07	7.8 ± 0.04	22.4 ± 0.6
Proteins (g/100 g DW)	16.3 ± 0.5	11.8 ± 0.2	9.5 ± 0.2	6.7 ± 0.1	10.9 ± 0.3	30.2 ± 0.1	21.7 ± 0.7
Fat (g/100 g DW)	3.12 ± 0.13	0.63 ± 0.02	0.55 ± 0.01	0.66 ± 0.01	0.59 ± 0.02	0.43 ± 0.01	0.29 ± 0.01
Carbohydrates (g/100 g DW)	47.1 ± 0.3	58.4 ± 0.4	43.9 ± 0.8	76.6 ± 0.3	55.4 ± 0.5	61.6 ± 0.1	55.7 ± 0.9
Energy (kcal/100 g DW)	281.6 ± 1.9	286.7 ± 1.0	218.5 ± 3.8	338.8 ± 1.4	270.6 ± 3.0	370.9 ± 0.1	311.9 ± 1.7
Energy (kJ/100 g DW)	1739 ± 10	1906 ± 8	1445 ± 23	2350 ± 8	1802 ± 17	2284 ± 2	1971 ± 15

C. tom: Codium tomentosum; *H. elo*: Himanthalia elongata; *L. och*: Laminaria ochroleuca; *S. lat*: Saccharina latissima; *U. pin*: Undaria pinnatifida; *Por*: Porphyra sp.; *P. pal*: Palmaria palmata.

In general, the macronutrient present in the highest concentration were carbohydrates, ranging in the values between 76.6 ± 0.3 and 43.9 ± 0.8 g/100 g DW for *S. latissima* and *L. ochroleuca*, respectively. In contrast, the evaluation of lipid content showed the lowest concentrations in all studied species, with evidenced values oscillating between 0.29 ± 0.01 and 3.12 ± 0.13 g/100 g DW in *P. palmata* and *C. tomentosum*, respectively. The protein content showed great heterogeneity between the algae species, with higher concentrations in *Porphyra* sp (30.2 ± 0.1 g/100 g DW) and lower concentrations in *S. latissima* (6.7 ± 0.1 g/100 g DW). The amount of ash showed concentrations between 7.8 ± 0.04 and 46.1 ± 1.3 g/100 g DW for *Porphyra* sp. and *L. ochroleuca*, respectively. For the energic value, amounts between 218.5 ± 3.8 (for *L. ochroleuca*) and 370.9 ± 0.1 Kcal/100 g DW (for *Porphyra* sp.) were obtained.

In general, the nutritional results obtained in the present study are similar to those reported previously [2,3,14–17]. Red macroalgae had the highest protein content, followed

by green and finally, brown algae, except *U. pinnatifida*, which has been reported to have intermediate protein levels [2]. In the present study, the protein content of *U. pinnatifida* was lower than the expected. Regarding lipid content, most of the studies agreed with the results obtained, demonstrating the low lipid content of the algae. Ash content and carbohydrate content agree with the mentioned studies, except for the carbohydrate content of *S. latissima*, which was higher than previously reported. The differences between studies could be associated to diverse factors that affect macroalgae composition, such as the region, season or the environmental factors during the harvest [1,3].

3.2. Chemical Composition

The fatty acid content of the macroalgae shows a clear heterogeneity, varying both compounds and quantities between species. Eleven different fatty acids have been identified. The most common fatty acids detected above 5% were linoleic and eicosatrienoic acids. Linoleic acid was present in *C. tomentosum* (11.5%), *H. elongata* (14.12%), *L. ochroleuca* (10.5%), *S. latissima* (9.63%) and *U. pinnatifida* (11.52%), while eicosatrienoic acid was present in *C. tomentosum* (7.92%), *H. elongata* (25.4%), *L. ochroleuca* (22.7%), *U. pinnatifida* (18.35%) and *Porphyra* sp. (30.36%). On the other hand, the less common fatty acids were the miristic, stearic, eicosenoic and erucic acids, found only in *S. latissima*, *P. palmata*, *Porphyra* sp. and *C. tomentosum* with values of 18.83, 6.7, 10.86 and 12.0%, respectively. As mentioned before, the composition of macroalgae varies according to several factors, including the season of harvest [18]. Most of the results agreed with other studies previously cited [14,17,18].

Regarding the organic acids, oxalic, malic and citric acids were identified. However, only oxalic acid was present in all the species with values between 0.2 g/100 g (DW) and 4.3 g/100 g (DW) for *U. pinnatifida* and *L. ochroleuca*, respectively. Malic acid was present in *H. elongata* and *Porphyra* sp., with values of 0.42 g/100 g (DW) and 3.66 g/100 g (DW), respectively. Finally, citric acid was found in *H. elongata* (1.57 g/100 g (DW)), *U. pinnatifida* (0.83 g/100 g (DW)) and *Porphyra* sp. (5.47 g/100 g (DW)). The total concentration of organic acids of the macroalgae fluctuate between 0.59 g/100 g (DW) for *U. lactuca* and 10.61 g/100 g (DW) for *Porphyra* sp. According to the research in the literature, there are no other studies that report the organic acid content in the macroalgae selected in this study, except for *C. tomentosum* [19] and *Porphyra* sp [20].

The β-carotene, lycopene and chlorophyll a and b content were evaluated in the seven macroalgae. β-carotene was only detected in the brown macroalgae *U. pinnatifida*, with a value of 0.78 mg/100 g DW. In the case of lycopene, the compound was detected in all the samples, ranging between 11.2 and 0.32 mg/100 g DW for *C. tomentosum* and *Porphyra* sp., respectively. Finally, *C. tomentosum* showed the highest content of chlorophyll a and b (56.3 and 47 mg/100 g DW), while *P. palmata* had the lowest (1.5 and 0.58 mg/100 g DW). Chlorophyll b was not detected in *U. pinnatifida.* Several studies have evaluated the content of β-carotene, chlorophyll a and b content of the selected macroalgae [21–24]. The differences observed with previous studies may be attributed to the fact that, like other previous parameters, the pigment content varies throughout the year, depending on environmental factors, such as light, salinity and temperature. Other factors may have affected, such as the extraction solvent [23]. Finally, although it has been described that some macroalgae may contain lycopene [23], to our knowledge, there are no studies that have reported the presence of lycopene in the selected species.

3.3. Bioactive Potential

The EC_{50} values ranged between 1.7 and 650 μg/mL for *L. ochroleuca* and *P. palmata*, respectively. *S. latissima* and *U. pinnatifida* did not show antioxidant effects, as no reduction of the hemolysis was observed. As it could be observed, *C. tomentosum*, *H. elongata* and *L. ochroleuca* displayed great antioxidant activity, presenting a EC_{50} much lower that

the antioxidant control (46 μg/mL). To our knowledge, no previous studies have employed OxHLIA to evaluate the antioxidant activity of the seven selected macroalgae.

Regarding antibacterial activity, the tested extracts of all macroalgae showed antibacterial activity with inhibitory and bactericidal potential against several studied strains. The MBC values ranged between 2 and 4 mg/mL for *C. tomentosum*, 1 and 4 mg/mL for *H. elongata*, 2 and >8 mg/mL for *L. ochroleuca*, 1 and 8 mg/mL for *S. latissima*, 2 and 8 mg/mL for *U. pinnatifida* and *Porphyra* sp. and 2 and >8 mg/mL for *P. palmata*. Finally, antifungal results showed that MFC values ranged between 4 and 8 mg/mL for *C. tomentosum, H. elongata, U. pinnatifida, Porphyra* sp. and *P. palmata*, while *L. ochroleuca* and *S. latissima* showed values ranging between 2 and 4 mg/mL and 1 and 8 mg/mL, respectively. The antimicrobial properties of *C. tomentosum* [25], *H. elongata, S. latissima* and *P. palmata* [26], and *L. ochroleuca* [27] were consistent with previous reports. To our knowledge, no studies have evaluated previously the antibacterial and antifungal properties of *U. pinnatifida* and *Porphyra* sp. extracts.

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