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Nutritional Composition of the Atlantic Seaweeds *Ulva rigida*, *Codium tomentosum*, *Palmaria palmata* and *Porphyra purpurea* †

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Abstract: Macroalgae are regarded as a healthy food due to their composition and nutritional properties. In this work, nutritional composition of two green (*Ulva rigida*, *Codium tomentosum*) and two red (*Palmaria palmata*, *Porphyra purpurea*) edible seaweed was studied. Total lipids were measured gravimetrically as evaporated mass after petroleum-ether Soxhlet extraction of samples. In addition, fatty acid profile was determined by gas chromatography coupled to a flame ionization detector (GC-FID). Results showed that all studied species were accounted for very low levels of lipids (<1% dw), but levels of unsaturated fatty acids oleic, linoleic, and linolenic acids were present at high concentrations, with *P. palmata* displaying the highest quantities (>200 mg C18:1/g extract). In parallel, proteins were quantified following the macro-Kjeldahl method. In this analysis, red algae, especially *P. purpurea*, showed significant protein content up to 30% DW. Total organic acids were found by ultra-filtration liquid-chromatography coupled to an amperometry detector (UFLC-PAD) after an acid extraction, *P. purpurea* being the algae with the higher organic acid content (10.61% dw). Minerals were identified and quantified by inductively coupled plasma atomic emission spectroscopy (ICP-OES), suggesting that both algae groups are rich in K and Mg (>15 g/kg), but *U. rigida* also displayed a remarkable iron content (>1 g Fe/kg). Other detected minerals in minor concentrations were Ca, P or F. Altogether, results corroborate that these edible algae are a good source of nutrients in accordance with literature.

Keywords: macroalgae; nutrition; composition; chromatography; minerals

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1. Introduction

Seaweeds (macroalgae) are common ingredients in East Asian cuisine, of which many species such as wakame (*Undaria pinnatifida*), sweet kelp (*Saccharina latissima*) or nori (*Porphyra purpurea*) are used in different dishes. Seaweeds have long been recognized as healthy foods owing to their low caloric index and high content in dietary fiber, minerals and antioxidant molecules such as their cell wall polysaccharides [1,2] Besides, in recent years, there has been an increasing consumer interest in vegetarian food sources. In this context, algae could be a valuable alternative source of essential macronutrients. Indeed, seaweeds have been proposed as an alternative ingredient for the formulation of nutritional supplements that could cover various dietary needs [3]. One of the key

nutritional components is protein and the amino acid composition of a food protein. It is known that red seaweeds account for a protein content between 20 and 47% of its dry weight (dw), while green algae generally contains about 9–26% and brown seaweeds 3 to 15% [4]. From a nutritional perspective, seaweed proteins are also valuable since their content in essential amino acids is generally higher (~50%) than legumes (~40%) [5]. Seaweeds have also been generally described to contain very low levels of lipids usually between 1 and 4% dw, but nonetheless rich in polyunsaturated fatty acids (PUFA) [6]. Considering their mineral composition, seaweeds tend to hold much higher content of potassium, magnesium or calcium than several terrestrial plants. However, they are also described to generally accumulate iodine in great amounts of which an excessive intake could be hazardous to thyroid function [7]. In some cases, hazardous levels of arsenic have also been reported, which requires monitoring and assessment upon consumption of certain species [8]. Nonetheless, the nutritional composition of several seaweed species considering their growing region remains to be described, especially considering traditional methods for determining proximate compositions. In this work, nutritional composition of edible seaweed *Ulva rigida* (UR), *Codium tomentosum* (CT), *Palmaria palmata* (PA) and *Porphyra purpurea* (PU) widely distributed in Atlantic shores was studied using standardized analytical methods.

2. Material and Methods

2.1. Sample Preparation

Algae samples were provided by Algas Atlánticas Algamar S.L company (www.algamar.es, accessed on 1 July 2021) located in Pontevedra, Spain. The algae samples were collected from the coasts of the Galician region, Pontevedra province (NW Spain), washed with distilled water, frozen at −80 °C and freeze-dried afterwards. The seaweed samples were then crushed and grinded to obtain a homogeneous matrix, which was stored at −20 °C for further analysis.

2.2. Proximate Composition

Proximate composition was studied following AOAC guidelines (1995) [9]. All thermogravimetric analyses were carried out with a SETSYS Evolution thermobalance (Setaram, Caluire-et-Cuire, France). Results are expressed as g per 100 g dw.

Humidity was determined by a gravimetric method. 1 g of sample was deposited in a previously dried (104 °C/24 h) ceramic crucible. Next, the crucibles with fresh sample were placed in an oven at 104 °C for 24 h. After that time, they were weighed again, and humidity was calculated as the weight difference.

2.2.1. Inorganic Material

To find the content of inorganic material (Ash), 250 mg were placed in a porcelain crucible (previously weighed) and the samples were incinerated at 600 ± 15 °C for 5 h. The crucible was then weighed with the resulting sample content. The obtained difference in weight was calculated as the ash value for each sample.

2.2.2. Protein Content

Protein content was determined according to the macro-Kjeldahl method [10]. Briefly, 500 mg of sample were placed in a Kjeldahl tube, then adding a catalytic tablet (Sigma Aldrich, St. Louis, MO, USA) and 20 mL of sulfuric acid. The tubes were placed in a digestive block and the temperature was gradually increased to 400 °C for 70 min. The tubes were then removed, allowed to briefly cool, and 25 mL of distilled H_2O was added. The nitrogen (N) converted to ammonia was measured with a macro-Kjeldahl distiller. The resulting N value was multiplied by a correction factor of 6.25 to obtain the estimate of the protein content, an extensively used correction factor for algae N-to-protein determinations [11].

2.2.3. Lipids

For total lipids determination, 3 g of sample were placed inside a paper cartridge. An extraction with petroleum ether was then conducted through a ST 243 SOXTEC Soxhlet extraction system (Foss, Hillerød, Denmark) at a constant temperature of 120 °C for 7 h. The resulting product was transferred to a ground test tube, previously weighed, and placed in the oven for evaporation of the solvent. After solvent evaporation, the tube was weighed again to obtain, by difference, the total lipids content.

2.2.4. Fiber and Hydrocarbons

Fiber was determined following the gravimetric AOAC method [9]. Briefly, 1 g of dried sample was sequentially treated with α-amylase from *Bacillus licheniformis* (pH 6, 30 min, 37 °C), protease from *Bacillus licheniformis* (pH 7.5, 30 min, 37 °C) and amyloglucosidase from *Aspergillus niger* (pH 4.5, 30 min, 40 °C). The obtained residue was precipitated with 4 times its volume in ethanol and filtered through a 0.45 µm paper syringe filter. The obtained difference in weight was calculated as total fiber.

Total hydrocarbons were calculated as the difference of the rest of the components, following Equation (1) and the results expressed as % (g/100 g dw) [12]:

$$\text{Hydrocarbons} = 100 - (\text{Lipids} + \text{Proteins} + \text{Ash} + \text{Fiber}) \quad (1)$$

2.3. Organic Acids

To determine the organic acid content, 1 g of each sample was weighed, and an extraction was carried out with 25 mL of 4.5% metaphosphoric acid, while stirring for 20 min. It was then filtered through paper and nylon (0.22 µm) to be able to work in ultra-fast liquid chromatography coupled to a photodiode array detector (UFLC-PAD).

The analysis was performed using a Shimadzu 20A series UFLC (Shimadzu, Kyoto, Japan) Separation was achieved on a SphereClone (Phenomenex, Torrance, CA, USA) reverse phase C_{18} column (5 µm, 250 × 4.6 mm) at 35 °C. Sulfuric acid 3.6 mM was used as mobile phase with a flow rate of 0.8 mL/min. Detection was carried out using wavelengths between 215 and 245 nm. Detected organic acids were quantified by comparison of the area of their peaks with calibration curves obtained by comparison to an ascorbic acid standard (Sigma Aldrich, St. Luois, MO, USA).

2.4. Mineral Content

For mineral detection and quantification, 2 g samples were subjected to a metaphosphoric acid digestion for 10 min and analyzed afterwards with optic emission spectrometry with inductively coupled plasma (ICP-OES) using an Optima 4300 DV instrument (Perkin Elmer, Whaltman, MA, USA) [9]. Briefly, quantification of minerals was determined following detection in specific wavelengths for Ca (317.9 nm), Mg, (285.2 nm), Cl, (134.7 nm), Fe (248.6 nm), Mn (279.4 nm), Zn (206.2 nm), K, (769.9 nm), I (178.2 nm), F, (685.6 nm), As (188.9 nm), P (213.6 nm) with RF power of 1450 W and at an argon plasma flow of 15 L/min. Results are expressed as g/kg dw.

2.5. Fatty Acid Profile

To carry out this determination, the product resulting from the lipids Soxhlet extraction was used and a derivatization process was carried out to obtain fatty acid methyl esters (FAME). 5 mL of reagent A (MeOH, H_2SO_4 and C_7H_8) was added in a 2:1:1 ratio and they were kept in a bath at 50 °C while stirring at 160 rpm for 12 h. Afterwards, 3 mL of distilled H_2O was added, then adding 3 mL of diethyl ether under vigorous and continuing stirring until a homogeneous sample was obtained. Later, the two phases separation was allowed to occur, and the supernatant was transferred to a vial with sodium sulfate. The contents of the vial were filtered through 0.22 µm nylon prior to their chromatographic analysis by gas chromatography coupled to an infrared detector (GC-FID). The GC system was an Agilent 7820A and an Agilent HP-88 (60 m, 250 µm × 0.25 µm) column was used (Agilent

Technologies, Santa Clara, CA, USA). Helium was used as carrier; 1µL of sample was injected. Oven temperature program started at 120 °C, increasing to 175 °C at 10 °C pre min. rate and hold for 10 min. Then, temperature was further increased to 220 °C at 3 °C increase per min. and kept at 220 °C for 5 min.

Different fatty acid levels were determined by comparing the relative retention times of the FAME peaks of the algae samples with respect to a commercial standard of FAME mix (Supelco 37 Component FAME MIX, Sigma Aldrich, St. Luois, MO, USA).

3. Results and Discussion

3.1. Proximate Composition

All studied species were accounted for very low levels of lipids (<1% dw), which could be due to the harvesting season or an incomplete extraction, as other studies report generally higher lipid content in some closely related species [7].

Green seaweeds displayed protein levels around 15% dw (Figure 1). Red algae, especially *P. purpurea*, showed a significant protein content, up to 30% dw. This is in contrast with other analyses reported on both *P. purpurea* and *P. palmata*, which displayed higher protein content [7,13]. Organic acids content was significantly heterogeneous, with only *P. purpurea* showing a high content (10.61% dw), half of which was determined as citrate. No organic acids were detected in *U. rigida*. Almost all the analyzed species showed more than 40% dw of insoluble fiber, with similar results to those generally reported in literature [5,13]. On the other hand, inorganic matter was somehow homogeneous for all seaweeds except for *C. tomentosum*, which showed a 37% dw ash content.

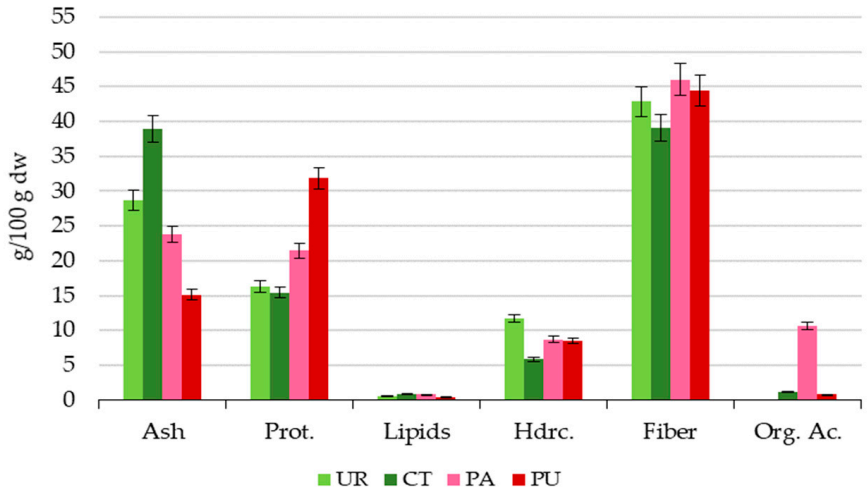

Figure 1. Proximate composition of the studied seaweed species (% dw). UR, *Ulva rigida*; CT, *Codium tomentosum*; PA, *Palmaria palmata*; PU, *Porphyra purpurea*; Prot, proteins; Hdrc., hydrocarbons; Org. Ac., organic acids.

3.2. Mineral Composition

The main minerals detected were Cl, K and Mg, of which *P. palmata* significantly outstand with as much as 100 g/kg dw of Cl and K, followed by *U. rigida* (Figure 2A). Indeed, the latter was accounted for the highest levels of Mg (22.9 g/kg dw), whereas *C. tomentosum* displayed the lowest mineral concentrations for Cl, Mg or K. *P. purpurea* displayed 33.5 g/kg dw of K and 12.1 g/kg dw of Cl, but the rest of its minerals were found at levels below 10 g/kg dw.

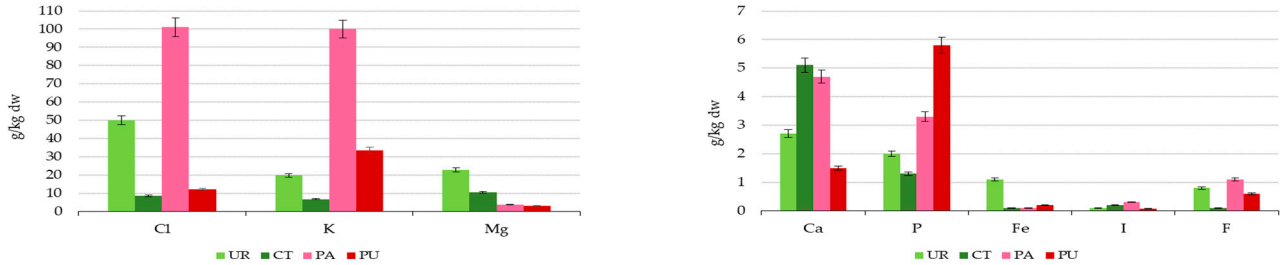

Figure 2. Mineral composition of the studied seaweed species in: (**A**), in major concentrations (g/kg dw); and (**B**), in minor concentrations (g/kg dw). UR, *Ulva rigida*; CT, *Codium tomentosum*; PA, *Palmaria palmata*; PU, *Porphyra purpurea*.

Minerals detected at minor concentrations are displayed in Figure 2B. Ca levels for *C. tomentosum* and *P. palmata* were higher than those found in terrestrial plants. Conversely, out of the four studied species, *P. purpurea* displayed the highest P content, a feature that may be related to its higher protein content (Figure 1). It is worth noting the remarkably high Fe content of *U. rigida*, as it is several times higher than those reported in terrestrial plants. Considering this result, *U. rigida* may contain higher iron quantities than legumes, which are considered one of the main sources of this mineral [3]. On the other hand, iodine levels from the studied sampled species appear lower [14]. Although this could be due to experimental errors, it is plausible that it is related to the their growing region, since it seems that seaweeds from Galician waters accumulate less iodine [15]. It is also noteworthy that *C. tomentosum*, despite showing the highest ash content, did not display significantly high levels of any of the test minerals, except for Ca (5.1 g/kg dw). This could be due to the presence of other minerals or metals not tested in this work. On the other hand, other minerals and metals searched for like Mn, As and Zn were detected at trace amounts, below 0.01 g/kg dw (data not shown). F was also detected in all species, reaching up to 1.1 g/kg dw in *P. palmata*, although *C. tomentosum* had the lowest levels of it (0.1 g/kg dw).

3.3. Fatty Acid Profile

Regarding fatty acid profile (Figure 3), the proportion of PUFA was notably high, with *P. palmata* displaying the highest relative quantities. *P. palmata* levels of oleic acid were higher than 200 mg/g extract and more than 150 mg/g extract for palmitoleic acid. Moreover, *P. palmata* denoted more than 100 mg/g extract of eicosatetraenoic acid. *U. rigida* however, accounted for the most linoleic acid content (>150 mg/g extract).

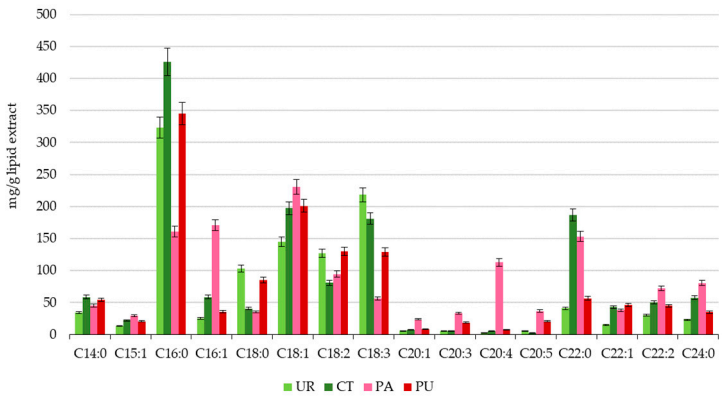

Figure 3. Fatty acid profile of the studied species. UR, *Ulva rigida*; CT, *Codium tomentosum*; PA, *Palmaria palmata*; PU, *Porphyra purpurea*.

In contrast, *P. purpurea* showed higher proportions of saturated fatty acids, foremost palmitic (>320 mg/g extract) and stearic acids (>70 mg/g extract). Nonetheless, *C. tomentosum* accounted for the highest proportion of saturated fatty acids, like palmitic acid (>400 mg/g extract) and behenic acid (>170 mg/g extract). Altogether, results corroborate that these edible algae are a good source of nutrients and analytical methods are suitable, in accordance with literature [7,16].

4. Conclusions

Seaweeds have been traditionally consumed for their nutritional value and availability. Moreover, in recent years they have been proposed as an alternative source of proteins and minerals, in contrast to terrestrial plants. It is of interest to accurately determine their nutritional composition to critically assess their value. In this work, standardized and recognized AOAC analytical methods for nutritional composition were employed to determine the major elements of four edible Atlantic seaweed species. Additionally, their mineral and fatty acid profile was investigated. Results unveiled that their protein and mineral content makes them notable sources of these nutrients, especially in red algae species. In these red seaweeds, protein content reached more than 20% for *P. palmata* and even more than 30% for *P. purpurea*. Whereas lipid content was particularly low (possibly due to specific environmental growing conditions), fatty acid analysis denoted high proportions of unsaturated fatty acids, i.e., oleic and linoleic acids in both *U. rigida* and *P. palmata.* Considering the reported results, these seaweed species growing in NW Spain could be a potential food and/or feed ingredient, especially owing to their high contents in minerals. Among the studied species, *P. palmata* stands out due to its PUFA and mineral composition, as well as its mineral content. *P. porphyra* on the other hand, was shown to be especially rich in proteins and P. Taking present results, seaweeds could be proposed as alternative supplementation ingredients for food and feed instead of animal or terrestrial plant sources; since not only they are rich in valuable nutrients, but also currently underexploited for this purpose. Further research should analyze other nutritional aspects from these widespread seaweed species in more depth. This would allow to assess with more accuracy the nutritional value of these Atlantic seaweeds.

Supplementary Materials: The poster presentation is available online at https://www.mdpi.com/article/10.3390/CSAC2021-10681/s1.

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