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Assessment of Nutritional Profile of *Sargassum muticum* alga from the Spanish Coastline †

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Abstract: Using macroalgae, or seaweed, in human diets has a long history, especially in Eastern nations. However, the present interest in these species is driven by their remarkable bioactive and nutritional qualities and their great availability and underutilization, making them incredibly alluring to people following alternative dietary patterns like vegetarianism and veganism. *Sargassum muticum*, also known as Japanese wireweed or Asian seaweed, is considered edible and has been consumed in some cultures, popular as a soup ingredient in Korea. This brown macroalgae found in marine environments has been introduced in various regions outside its native range, including Europe and North America. Moreover, this species could be helpful to feed animals or as soil fertilizer. In this study, the nutritional properties of this marine macroalga were investigated. Nutritional parameters such as protein, sugar, and fiber content were analyzed using classical techniques. In addition, it was also determined the proximate composition in terms of moisture, fixed and volatile carbon, and ash content by thermogravimetry, and the major minerals, including calcium, potassium, and magnesium, using the ICP-OS technique. In terms of its mineral content, it was found to have a high mineral content (21% of ash), which consisted mainly of calcium (9g/kg dw potassium (77g/kg dw) and magnesium (12g/kg dw). In addition, the study determined the presence of iodine by ICP-MS, and 106 mg/kg dw of this essential element was quantified in these algae. The results of this study highlighted the potential nutritional benefits of the tested marine algae. Their composition revealed significant concentrations of vital elements, making them highly advantageous for human/ animal dietary requirements with possible health benefits.

Keywords: *Sargassum muticum*; macronutrients; proximate composition; minerals; nutritional value

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1. Introduction

Sargassum muticum (Yendo) Fensholt is a brown alga belonging to the class Ochrophyta and the order Fucales, it originated in Japan and, as an aggressive invasive species, is now one of the most common macroalgae species on European coasts [1]. Various bioactive properties are attributed to this marine species, including antioxidant, cell protection, and antimicrobial capacity[2,3]. Nevertheless, *S. muticum*'s primary use in Europe is to fertilize soil and as animal feed [4], and the alga is considered simultaneously an environmental threat and an unexploited resource. [2].

Yet, in some Asian cultures *S. muticum*, also known as Japanese wire algae or Asian seaweed, is considered edible, and seaweeds have a long history in nutrition and cuisine. In Korea, it is popular as a soup ingredient [5]. Although it is very abundant, being present along the European shoreline and Mediterranean Sea [6][7], the use of this alga as food/feedstock is not very widespread. It is a species that compares well with others in terms of protein and fat content, and is also rich in polysaccharides such as fucoidan and alginates [6].

The current interest in these species is driven by their remarkable bioactive and antioxidant properties [2] and their abundant availability and underutilization. This makes this species particularly appealing as a source of potential nourishment during periods of food insecurity caused by environmental changes. Also, there is an urgent need to promote food diversification, so that more diversified forms of food with higher nutritional content can be integrated into dietary patterns. This becomes especially relevant when local alternative food sources can contribute to food security and reduce the ecological footprint of food consumption [8].

Furthermore, these environmental and nutritional properties are particularly appealing to individuals who follow alternative diets, such as vegetarians and vegans.

In this framework, the nutritional profile of *S. muticum* harvested in the coastline of Galicia (NW Spain), was analyzed for macronutrients including total fat, protein, sugars, and fibers. The mineral content was also assessed to evaluate the potential nutritional value of this species, whether as food, food ingredient or supplement.

2. Materials and methods

2.1. Alga material

S. muticum was collected (15 specimens) from the coast of Galicia in the winter of 2019. The algal material was sorted, identified by our expert biologists against available literature [9,10], and thoroughly washed to remove sand and other impurities. Finally, the algae were freeze-dried, finely ground, mixed to form a representative sample, and stored at -80 °C until use.

2.2. Proximate Analysis

Proximate analyses were performed thermogravimetrically using a Netzsch STA 449 F3 (Netzsch Gruppe - Wittelsbacherstrasse Germany). Residual moisture content was determined by the change in weight when samples (5-15 mg) were heated from 40 to 105 °C 10 °C/min under an inert atmosphere. The mass change between 105-600 °C (10 ∘C/min) determined the volatile fraction under a nitrogen flow of 40 mL/min. Finally, the ash content was determined until to constant weight after complete combustion of the sample at 900 °C in the presence of oxygen (airflow of 40 mL/min). The fixed carbon content was estimated by subtracting the combined percentages of moisture, volatiles, and ash from 100 % [11,12].

2.3. Proteins and carbohydrates

Nitrogen content was measured by the Kjeldahl method and multiplied by a factor of 5 to estimate the total protein content [13].

To determine the carbohydrate content, the phenol-sulfuric method was applied. For that, 1 g of macroalga was stirred in 20 ml of an ethanol water solution (80:20) for 15 minutes at 80°C. Subsequently, 100 μL of the sample was placed on a 96-well microplate. An equal volume of 20 % phenol solution and 500 μL concentrated sulfuric acid were added. Absorbance was read at 490 nm and results were expressed as mg of glucose equivalent/g dw [14].

2.4. Lipids

The lipidic content of the alga was determined using the Soxhlet method. The extraction was carried out with hexane for two hours, and the lipids were determined gravimetrically.

2.5. Fibers

The fiber content was determined following a method described in previous works [15]. Briefly, 0.5 g of sample was added to 25 mL of CTAB reagent and boiled for one hour.

Afterward, the residue was filtered, washed with hot water and acetone, and dried to constant weight. Results are presented as g/100g dw [15].

2.6. Macro, micro-nutrients and other elements

The macro (Na, Mg, Ca, K) and micro nutrients (Cu, Fe, Zn) and toxic elements (Hg, Pb, As quantification were made by the methodology developed by Millos et al.[16] , and already described thoroughly in a previous work [12] using a Perkin–Elmer Optima 4300 DV spectrometer (Shelton, CT, USA)

Mercury (Hg) was determined by cold vapor atomic absorption spectrometry (CVAAS) (Fims 400 Perkin Elmer Massachusetts, EUA). Iodine (I), arsenic (As), and lead (Pb) were quantified by ICP -MS (Thermo Elemental X7 Series). Isotope 115In was used as an internal standard for quantification of these elements. All determinations were performed at least in triplicate to achieve a coefficient of variation below 5%. The results were expressed as mg/kg dw. These studies were conducted in the Food Security and Sustainable Development Laboratory, Scientific and Technological Support Centre for Research (SSADS-CACTI, University of Vigo, Vigo, Spain). The quantification limits (LOQ) are present in Table 1.

Table 1. Detection limits of the elements analyzed.

	Ca	Cu	Fe	K	Mg	Na	Zn	Hg	As	I	Pb
Analytical technique	ICP-OES	ICP-OES	ICP-OES	ICP-OES	ICP-OES	ICP-OES	ICP-OES	CVAAS	ICP-MS	ICP-MS	ICP-MS
LOQ mg/kg	10.00	0.60	1.00	20.00	10.00	20.00	0.20	0.040	2.50	5.00	1.25

3. Results and discussion

The alga was tested to determine the proximate composition, nutrient profile, and elements present. The results are shown in Figure 1.

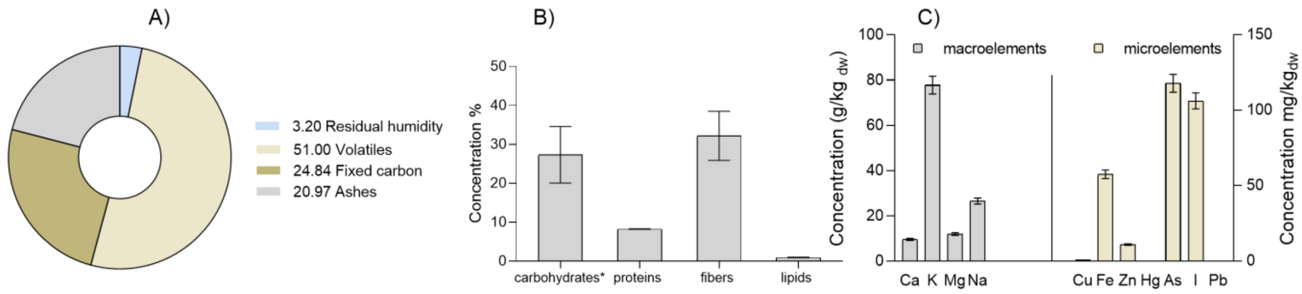

Figure 1. Characterization of *S. muticum* algae A) Proximate composition: B) nutritional profile, C) mineral composition. Bars represent the standard deviation n=3, and *carbohydrates are given in mg glucose equivalents/ g dw

Proximate composition indicated that some residual water remained after lyophilization. Volatile compounds are the most critical group, accounting for 50.99±1.31% of the algal material. However, the experiment revealed a high percentage of ashes, 20.97 ±1.37, corresponding to significant mineral content. These results align with previous findings [6] and reported seasonal variations in the ash content, ranging from 13.2% to 40.54 %, for this alga species [17,18].

The nutritional profile is shown in Fig. 1.B. The determined protein content was 8.29±0.67 and the lipid content was 0.94±0.06%, in agreement with the values reported by Balboa *et al* [17]. These values fall within the expected range for brown algae which is 0.3-4.5% for protein and 1-24% for lipids [19] but are lower than the ones reported for the same species collected in the coast of Portugal [6]. The total carbohydrates, quantified as

27.32±7.25 mg glucose eq/g dw, and fiber at 32.15±6.3% were also measured. The fibers values are higher than the one described (around 20%) for other sargassum species [20]. Nevertheless, these values are within the expected range of this biomaterial [21].

Brown algae carbohydrates, like fucoidan, laminarans, and alginates have been found to have several bioactive abilities [19]. For instance, alginates not only reduce cholesterol levels but also have antihypertensive effects. Unlike terrestrial plants, these beneficial dietary polysaccharides are uniquely present in marine algae. Furthermore, fibers from these algae play a vital role in cleansing the digestive tract and safeguarding the surface of the stomach and intestines [22].

The results also showed a considerable amount of macrominerals in the algal constitution. Ca and Mg were determined at 9.56 g/kg dw and 11.96 g/kg dw, respectively, deviating from the 2:1 ratio described as optimal for osteoporosis prevention [23]. Ca values are reported to be as high as 69.6 g /kg, and magnesium reaches 15.5 g/ kg in this alga [17], highlighting the potential of this species as a source of these nutrients. Emerging research indicates magnesium's role in many physiological processes, suggesting that adequate magnesium intake is crucial for cardiovascular health, muscle function, sleep, and mood, among other things [24,25]. Sodium was determined at a concentration of 26.5 g/kg dw while potassium was at 77.8 g/kg dw; the Na content agrees whit previous work (20.1mg/g) while K values are quite higher than the 38.8 mg/g reported [17]. The determined concentrations correspond to a Na: K molar ratio of <1, associated with a decrease in cardiovascular disease risk. These macrominerals' recommended daily dietary intake is 375 mg for Mg, 800 mg for Ca, and 2000 mg each for K and Na [26].

Concentrations of other microelements were also assessed. Of these, copper, iron, and zinc were determined at 0.67,57.6, and 10.9 mg/kg dw, respectively. The toxic metals Hg and Pb were not detected in the seaweed sample.

With iodine deficiency reemerging in Europe, it is crucial to ensure adequate intake either through diet or supplements. For *S. muticum*, a value of 101.6 mg/kg dw was identified, consistent with levels reported for other brown algae[12] but much higher than the ones reported in *S. muticum* [17] this differences could be related to different extraction techniques. .

The arsenic concentration was also high at 117 mg/kg dw, similar to levels reported in other studies on Sargassum sp [27]. While algae generally contain higher amounts of arsenic compared to other vegetables and grains, most of the arsenic detected is metabolized and it is present in the non-toxic form known as arsenosugares [28].

In conclusion, a comprehensive nutritional evaluation of *S. muticum* revealed its potential as a dietary source. The alga boasts a low-fat content moderate protein levels, and notably rich in carbohydrates and fiber. Its mineral composition is particularly striking, not only meeting but also aligning with the recommended dietary ratios Na: K. the high iodine content offers potential nutritional benefits, especially given the resurgent iodine deficiency in some regions. While the considerable presence of arsenic, a metalloid commonly found in algae, is noteworthy, it is crucial to underscore the need for detailed speciation. Such analysis will determine the safety and appropriateness of integrating this marine resource into regular human consumption or animal feed.

Seaweeds, particularly the species studied, present numerous opportunities for commercial exploitation due to their rich bioactive compounds and sustainable growth characteristics. To this end, this seaweed shows potential in the food industry as a source of natural food additives or as primary ingredients in plant-based dishes, given their rich nutritional profile; cosmetics and pharmaceuticals by leveraging their bioactive compounds for skincare products or nutraceutical supplements; bioenergy industry by exploring their biomass as a potential renewable energy source.

Furthermore, future research could focus on the scalability of seaweed cultivation, post-harvest processing techniques, and in-depth exploration of the bioactive compounds' potential therapeutic effects. However, for a detailed discussion on industrial applications, a comprehensive up-scaled laboratory and pilot testing would be a prerequisite to validate real-world feasibility and ensure these seaweed resources' sustainable and efficient use.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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