







Article

A Comparative Study of the Fatty Acids and Monosaccharides of Wild and Cultivated *Ulva* sp.

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Abstract: There is a need to find new possible raw food sources with interesting nutritional values. One of the most unexploited sources are seaweeds. Thus, *Ulva* sp. is a green edible seaweed that shows a high growth rate in nature and can support drastic abiotic changes, such as temperature and salinity. This work aims to determine the main nutritional compounds, fatty acids (FAs) and monosaccharides profiles of *Ulva* sp. (collected from Mondego estuary, Portugal), to identify the potential of this seaweed as a food source. The present study also highlights the potential of controlled and semi-controlled cultivation systems in *Ulva* sp. profiles. The results showed that the controlled cultivation systems had higher essential FA and monosaccharide content than the semi-controlled cultivation systems. However, they are in some cases identical to wild individuals of *Ulva* sp., supporting that cultivation of *Ulva* sp. can be a key for food safety. It is crucial to control the associated risks of contamination that can occur in wild specimens.

Keywords: seaweed cultivation; *Ulva* sp.; fatty acids; carbohydrates; food safety



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1. Introduction

Nowadays, we live with a deceptive abundance of food, in which we are essentially surrounded by fast food that is rich in calories, saturated fats, and lacking in essential nutrients [1]. Seaweeds, or marine macroalgae, are considered as an alternative natural food resource, with a high nutritional value and low-fat content. Seaweeds are extraordinary dietary supplements because of their high content of minerals (iron and calcium), vitamins, proteins (which contain crucial amino acids for human welfare), and structural polysaccharides (consistently considered dietary fiber). Seaweed aquaculture arises as a potential solution to human food safety problems [2,3].

Macroalgae of the genus *Ulva* (Chlorophyta) is a renewable natural resource, existing in large quantities along the coast around the world. In this genus, 84 *Ulva* species have been discovered, which are widely distributed [4]. *Ulva lactuca* and *Ulva rigida*, commonly known as sea lettuce, exhibit a wide spectrum of nutritional composition, which makes them excellent candidates for healthy eating in human nutrition, with a high protein content and relatively high levels of essential amino acids such as histidine, which presents levels comparable to those found in vegetables and eggs [5,6].

The genus *Ulva* is one of the most abundant marine and estuarine species and has ease of adaptation to the fluctuation of abiotic and biotic factors [7]. It has been cultivated for human consumption since the 1990s, and the cosmopolitan distribution of the genus *Ulva* makes it suitable for cultivation practically anywhere [8]. *Ulva* sp. is considered a highly interesting seaweed due to its tolerance to a wide range of environmental changes; it can

multiply easily and grows rapidly to be cultivated to obtain biomass [6]. Furthermore, this species can be a useful raw source for human food. However, this species is also in ecological danger due to its rapid growth rate. If the aquatic estuarine system or sea is entropized, *Ulva* sp. can outgrow other species and become an environmental problem [9–12]. Thus, to exploit this seaweed species, it is necessary to control carefully the cultivation system so as not to pressure the ecological status of its ecological surroundings [9–11,13,14].

It is also important to note that the nutritional value may vary according to environmental factors (such as temperature, salinity, light, and nutrients) and mineral availability. The chemical composition of this seaweed has been found to change drastically [15].

Ulva sp. has only 1% of lipids per dry weight (d.w.) [16]. Despite this low quantity, the FA composition in seaweeds has gained interest because of the higher percentage of the seaweeds' lipid content that is constituted by monounsaturated and also of polyunsaturated fatty acids (MUFAs and PUFAs) [17–19]. The genus *Ulva* is a source of valuable long-chain polyunsaturated fatty acid (LC-PUFA) profiles, especially the biologically important eicosapentaenoic acid (C20:5 n-3, EPA) [14]. *Ulva* sp. also shows the presence of trace amounts of docosahexaenoic acid (C22:6 n-3, DHA); however, more abundant LC-PUFA include the DHA precursor, docosapentaenoic acid (C22:5 n-3, DPA), along with the more unique C18:4 n-3 and C16:4 n-3 PUFAs [20]. Nevertheless, despite the low total FA content in *Ulva* sp., it has a good qualitative profile with great benefits [21,22]. Green seaweeds have simple carbohydrates (monosaccharides, commonly known as sugars), which are nutrients quickly absorbed by the human organism, being the main source of energy for the human body. These compounds deliver energy to the central nervous system as well as power for working muscles [2]. Moreover, these carbohydrates avoid the consumption of protein as a power source and instead use adipocyte tissue metabolism (which are mainly fat reserves). In addition, carbohydrate monomers (mainly rhamnose and xylose) can be present in ulvan, which is the sulfated polysaccharide present in *Ulva* sp. This sulfate polysaccharide has bioactivity benefits for human welfare, such as anti-tumoral, treatment of digestive diseases (for example, gastric ulcers), and anti-obesity [20].

Overall, *Ulva* sp. has great economic potential, particularly if its application to human and animal nutrition is taken into consideration [16].

Ulva sp. is used in Asia as a food condiment, medicinal herb, and as a nutritional supplement in Japan, China, and other southeast Asian countries, as well as in North and South America (especially in Chile), Oceania, and France [23,24]. It is consumed in traditional Hawaiian cuisine and in Japan, being included in a variety of dishes such as salads, soups, and condiments [25]. In recent years, *Ulva* has been investigated to develop functional foods such as fermented foods and drinks, and therefore can be considered a good source of dietary fiber and a potential source of prebiotics [26,27]. These macroalgae exhibit a wide spectrum of nutritional composition, which makes them excellent candidates for healthy human food and animal nutrition [25]. Because of the above-mentioned *Ulva* sp. characteristics, it is considered an excellent candidate for large-scale aquaculture [28].

The location of the species can vary the nutritional value and its cultivation success [3]. *Ulva* is a cosmopolitan genus and can easily adjust its metabolism under specific environmental conditions, wherein the FAs and the lipids have an important role in this adaptation [29]. Seaweed cultivation contributes to reducing environmental pressure in wild habitats, securing food safety, and promoting a blue-green economy [2]. However, little has been done beyond the laboratory scale in the development of controlled commercial production systems. Therefore, it is important to demonstrate the level to which culture conditions can be controlled to obtain the desired composition profiles. Thus, the objective of this study was to improve and to analyze *Ulva* sp. aquaculture techniques in a laboratory and in an inshore aquaculture system. From a biochemical and a nutraceutical point of view, the cultivated seaweed biomass was compared with the biomass of wild specimens. The study also aimed to understand whether the change in the location of the algae has an impact on its nutritional profile, so it was decided not to add nutrients to the culture medium (Mondego River estuarine water).

2. Materials and Methods

2.1. Seaweed Collection

Specimens of *Ulva* sp. (morphologically identified as *U. lactuca* using the electronic guide with seaweed occurrence [30]) (Figure 1) were collected during the winter of 9 January 2020 from Mondego estuary, on Morraceira Island (41°57'56" N, 8°44'30" W), Figueira da Foz, Portugal (Figure 2).

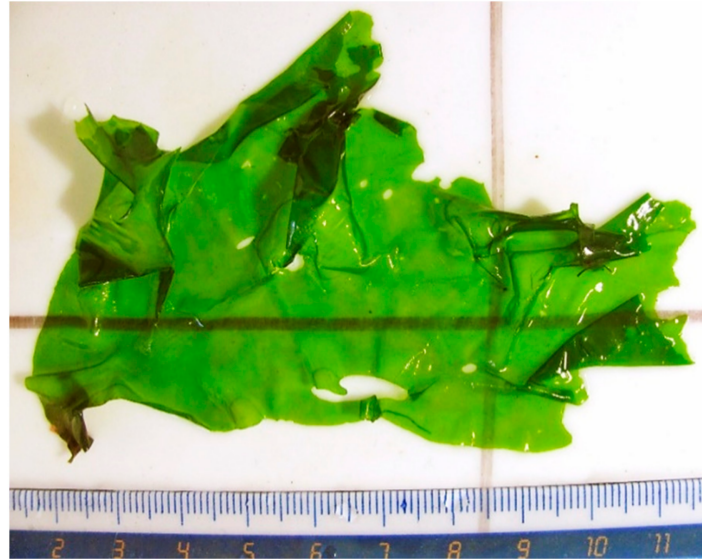


Figure 1. *Ulva* sp. specimen collected from Mondego River estuary in January 2020.



Figure 2. Sampling site: Morraceira Island (41°57'56" N, 8°44'30" W), Figueira da Foz, Portugal.

Samples were harvested by hand from well-established *Ulva* sp. patches and without epiphytes or degradation visible to the eye, to later be taken in cold boxes to the laboratory. Samples were cleaned with filtered seawater to remove sands, epiphytes, and other impurities. After this cleaning process, the specimens were separated according to distinct ends:

some specimens were weighed to go through the cultivation systems, and the rest of the biomass was washed with distilled water, cleaned with paper, and stored at -80°C (New Brunswick, UK) until the further proceedings.

2.2. Seaweed Cultivation Method

Ulva sp. small specimens and in new cyclic patches (≤ 5 cm) (which in trials have higher growth rate and less death rate) were chosen in the course of the primary seaweed collection, conveyed to the laboratory in plastic bags with seawater, and stored in iceboxes. To help in the process of washing the samples, as well as being the medium of the first stage of *Ulva* sp. in the indoor culture, seawater was collected and then filtered with filter papers.

2.2.1. Indoor Cultivation (Aquaria)

The majority of the specimens, with lower growth area (<5 cm), were utilized for cultivation and were chosen for the indoor controlled cultivation conditions at the MAREFOZ (MARE UC) laboratories, located in Figueira da Foz, Portugal, as shown in Figure 3.

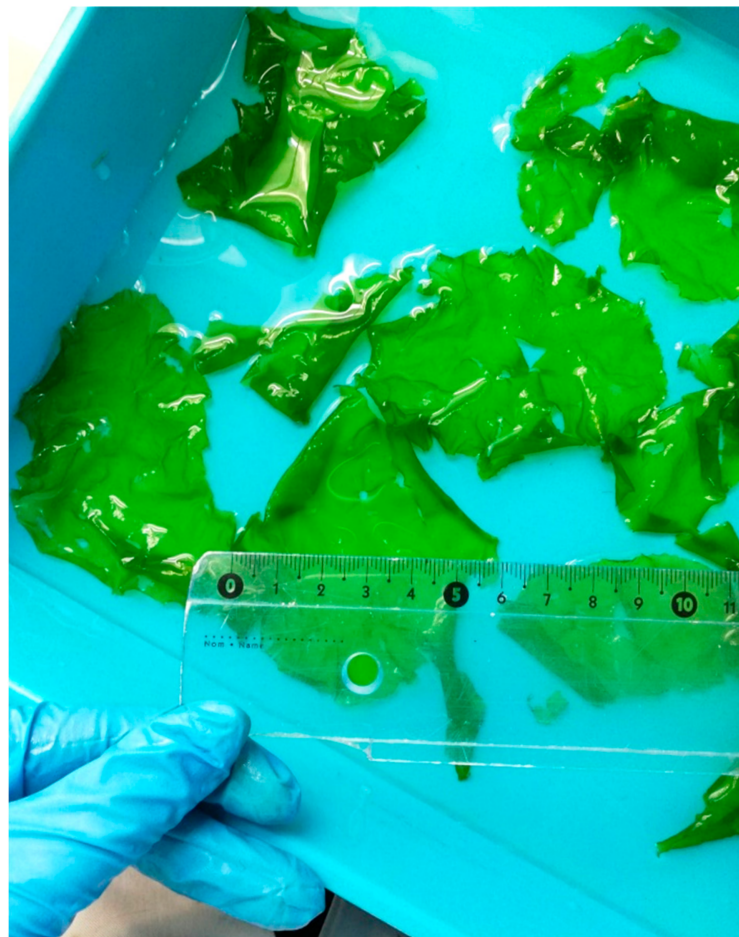


Figure 3. *Ulva* sp. specimens cultivated under controlled conditions at the MAREFOZ laboratories.

The initial cultivation apparatus (closed controlled cultivation) was rounded flasks with a volume of 2 L, holding 2 L of filtered seawater (collected on seaweed harvest location), with the coupling of artificial light (LED light 24 W 6500 K) and aeration from a diaphragm aquarium pump, as shown in Figure 4a. After 14 days (2 weeks), the specimens were transferred to aquaria (total volume capacity 15 L) (open controlled cultivation) (Figure 4b) with 6 L of seawater within 21 days and 12 L in the last two weeks (14 days) of the experiment, due to biomass growth (to maintain the biomass per L between 2 and 4 g).

Specimens were maintained under photoperiod conditions (16 h^L:8 h^D), the light intensity was 5 klux (around 100 μmol photons m⁻² s⁻¹). The room temperature was 20 ± 2 °C and 60% ± 10% humidity. This cultivation experiment was performed in triplicate, with seaweed initial weights of 2 g ± 0.5/L of seawater, which is recommended by the work of Vandermeulen and Gordin [31]. The seawater was renewed every two days (three times per week). One of the three days, when the water in the flask and aquaria was renewed, was used to weigh and measure the macroalgae. So, once a week, the seaweed was removed from the flask/aquarium, drained, and weighed on a PS.R2 series semi-analytical precision scale to analyze their growth rate over time.

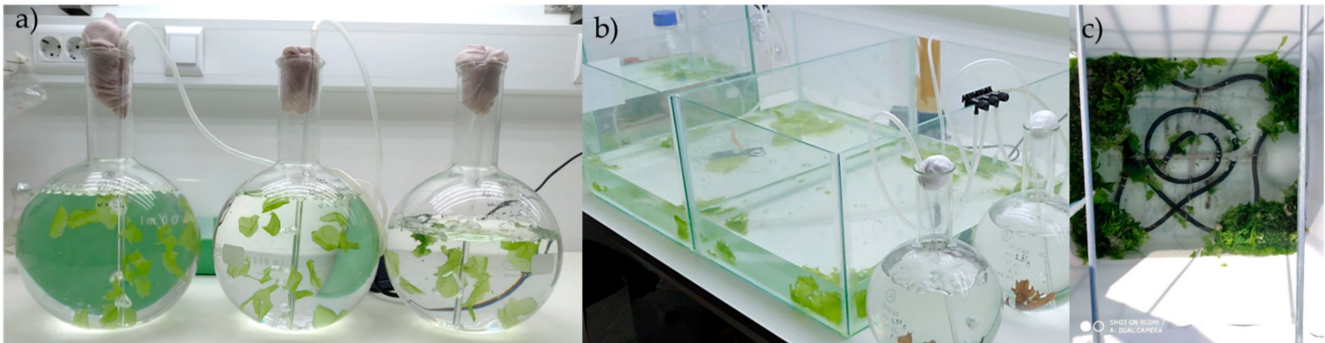


Figure 4. Seaweed cultivation techniques: (a) Volumetric flasks with *Ulva* sp. specimens in closed controlled cultivation; (b) aquaria with *Ulva* sp. specimens in closed controlled cultivation; (c) prototype cultivation tank (1000 L) with *Ulva* sp. from Lusalgae Lda.

2.2.2. Inshore Cultivation (Tank)

With the aim of expanding the information, during the “MENU—Marine macroalgae—alternative recipes for a daily Nutritional diet” (Project Reference: FA_05_2017_011) project, we used a prototype cultivation tank from Lusalgae Lda. (Figueira da Foz, Portugal) to examine the potential of *Ulva* sp. with the method used by this seaweed aquaculture company, as it can be seen in Figure 4c. The methods are based on the methodology of Araujo et al. [32]. In this stage, *Ulva* sp. grew in a water tank exposed to direct sunlight with aeration during the day (normally, the aeration was during 14 h) with the goal to obtain enough biomass for the extraction of compounds with biological importance and to contrast them with seaweed grown under controlled situations. To avoid the difference in biochemical composition due to the growth status of the seaweed, the cultivated species were identical to those of the indoor culture (collected in the same pools and dates, with a length less than 5 cm). Therefore, the culture method was the main difference controlling factor of the biochemical profile.

The cultivation medium was estuarine seawater (29–34%) collected in the Mondego River estuary, in Figueira da Foz, Portugal, without the addition of nutrients. The cultivation tank had a capacity of 1000 L, containing 800 L of mechanically filtered estuarine seawater [32].

The cultivation began from an opening quantity of 878 g of wet biomass in a unique tank. Approximately 75% of the volume of water in the tank was changed three times per week, and after three weeks all the biomass was collected for later analysis. The seaweed cultivation method was carried out by the Lusalgae cultivation standardized method to the best growth rate of *Ulva* sp. in the cultivation systems.

The air temperature during this outdoor experiment was 9.58 °C ± 6 and water temperature was stable at 14.5 °C ± 1.5.

2.2.3. Quantitative Analysis of the Seaweed Growth

The specific growth rate (SGR) was calculated based on the equation below, according to Pérez-Mayorga et al. [33] and regularly used as a standard for determining the growth rate of algae:

$$SGR \left(\frac{\%}{day} \right) = \frac{\ln|m_2| - \ln|m_1|}{t_{final} - t_{initial}} \times 100 \quad (1)$$

where

- m_1 represents the initial wet mass in g;
- m_2 represents the final wet mass in g;
- $t_{initial}$ the initial day when culture started;
- t_{final} the final day that the culture ended.

2.3. Samples Preparation for Biochemical Analysis

To access biochemical composition, the biomass collected from the field and the biomass resulting from the cultivation systems were prepared for the analysis. All the samples were weighed and placed in, duly identified, aluminum trays. The samples were dried in the oven at a constant temperature of 60 °C during 48 h. Finally, the samples were milled using a commercial mill (Taurus aromatic, Oliana, Spain) and the dried biomass was stored, protected from the light, for further analysis.

2.3.1. Fatty Acids Analysis by GC/MS

Samples were split into three replicas and were treated in order to obtain the extracts. As per the following methodology described by Gonçalves et al. [34], lipids were extracted and methylated to fatty acids methyl esters (FAMES). To quantify the FAs, to each sample, the internal standard nonadecanoic acid C19 (Fluka 74208, Sigma-Aldrich, Steinheim, Germany) was added. FAMES identification was performed through Gas chromatography–Mass Spectrometry (GC-MS), equipped with a 0.32 mm internal diameter, 0.25 µm film thickness, and 30 m long TR-FFAP column. The sample (1.00 µL) was injected with a splitless mode. The column temperature was programmed to increase from 80 to 230 °C, with helium as the carrier gas, at a flow rate of 1.4 mL min⁻¹. Integration of FAMES peaks was carried out using the equipment's software. The identification of each peak was performed by retention time and mass spectrum of each FAME, comparing each one to the Supelco®37 component FAME mix (Sigma-Aldrich, Steinheim, Germany). Quantification of FAMES was performed as previously described in Gonçalves et al. [34].

2.3.2. Carbohydrate Analysis by GC-FID

Hydrolysis occurred first, from which the profile of the three replicas were extracted, followed by reduction and acetylation [34]. Samples were incubated with sulfuric acid, and later distilled water was added as described by Coimbra et al. [35]. For the quantification of sugars, the internal standard 2-desoxiglucose was added. The samples were run through a Thermo Scientific Trace 1310 chromatography equipment (Waltham, MA, United States) equipped with a flame ionization detector (GC-FID). A TG-WAXMS A (30 m length, 0.32 mm i.d., 0.25 µm film thickness) GC column was used and the oven was programmed to an initial temperature of 180 °C, following a linear temperature increase of 5 °C min⁻¹ until the final temperature of 230 °C was reached, maintaining this temperature for 12 min. The carrier gas was helium at a flow rate of 2.5 mL min⁻¹. Through the comparison of the retention time with standards, the monosaccharides were identified. Quantification of sugars was performed by comparison of the sugar chromatographic peaks to the peaks obtained for the internal standard used (2-desoxiglucose).

2.4. Data Analyses

Analyses of variance (ANOVA) were carried out for FAs and monosaccharides, one for each component and later Tukey tests were performed in order to contrast the values in the quantifications within the treatment (type of cultivation or field samples). Differences were considered significant when the *p* value was lower than 0.05.

3. Results

3.1. *Ulva* sp. Cultivation Growth

The aquarium’s cultivation (A, B, C, and the total (indoor cultivation)) technique showed that the specimen’s diameter growth was at a constant rate. However, the increase in specimen’s diameter was not always accompanied by an increase in the specimen’s biomass (specimen diameter growth during the cultivation: A = 4.5 cm; B = 6.5 cm; C = 3.5 cm). Shifting cultured specimens from Erlenmeyer’s flasks to larger aquaria (with more seawater volume) allowed higher growth and development rates, which support the obtained results. As noticeable in indoor cultivation, starting from day 21, there was a lower rate of growth of the *Ulva* specimens (Table 1).

Table 1. Results of growth rate of *Ulva* sp. from *Ulva* sp. cultivated in indoor aquaria (A, B, and C) and cultivated in outdoor tank.

ID of Aquarium	Initial Biomass (g)	Closed Controlled Cultivation SGR	Aquarium SGR	Final SGR	Final Biomass (g)
A	4.18	5.08%	4.84%	4.94%	47.07
B	4.01	4.72%	3.90%	4.25%	32.15
C	4.03	5.48%	3.77%	4.49%	36.63
Indoor total	4.03 ± 0.08	5.09 ± 0.31	4.17 ± 0.48	4.56 ± 0.29	38.62 ± 6.25
Tank cultivation	878	-	-	4.37%	2200

The tank cultivation allowed the biomass limit of the aquaculture systems in three weeks (21 days) to be obtained, with a total of 2200 g, where the *Ulva* sp. total growth was 1322 g from the initial inoculum. A daily growth rate of 62.95 g per day and specific growth rate of 4.37% was achieved. These values are within the values obtained from the indoor cultivation, which demonstrates that this seaweed was not impacted by the two different cultivation techniques in the overall growth.

3.2. Fatty Acid Characterization

Table 2 shows the mean values of each FA identified in the profile of *Ulva* sp. and the sum of each group of FA according to the saturation level. All FAs identified were long-chain FAs, especially with 16 and 18 carbons (from zero to four unsaturations). PUFA and saturated fatty acid (SFA) groups, and aquaria A and B showed no statistical significant differences, having the highest concentration of PUFAs (691.40 µg.g⁻¹ d.w. and 742.87 µg.g⁻¹ d.w., respectively) and SFAs (1506.42 µg.g⁻¹ d.w. and 1241.51 µg.g⁻¹ d.w., respectively). Still, field samples (F) were the richest in PUFA content (577.37 µg.g⁻¹ d.w.), followed by the seaweed cultivated in the outdoor tank (T) (316.32 µg.g⁻¹ d.w.). Referring to the MUFA group, field samples (F) had the lowest concentration (304.78 µg.g⁻¹ d.w.) (Figure 5).

The HUFAs (DHA and EPA) samples collected from the field (F) were similar to the samples that were cultivated in the external tank (tank T) (577.37 µg.g⁻¹ d.w. and 316.32 µg.g⁻¹ d.w., respectively), whereas samples cultivated in the laboratory (A, B, and C) were also similar (128.50 µg.g⁻¹ d.w., 170.18 µg.g⁻¹ d.w., and 113.69 µg.g⁻¹ d.w., respectively) but showed significant differences with respect to samples F and T.

Table 2. FA content expressed (in $\mu\text{g}\cdot\text{g}^{-1}$ d.w.) of *Ulva* sp. from different cultivation tanks or wild: indoor aquaria (A, B, and C), outdoor tank (T), and harvested from the field (F). Data as mean \pm SEM; n = 3.

FA	A	B	C	F	T
C14:0	39.66 \pm 3.23	35.79 \pm 3.49	39.15 \pm 2.71	48.34 \pm 1.20	117.27 \pm 3.11
C16:0	1431.98 \pm 107.33	1171.27 \pm 35.27	949.87 \pm 22.10	764.09 \pm 44.19	922.94 \pm 18.43
C18:0	34.78 \pm 8.89	34.45 \pm 7.57	32.43 \pm 6.54	31.27 \pm 10.11	43.39 \pm 2.73
Σ SFA	1506.42 \pm 99.78	1241.51 \pm 42.74	1021.45 \pm 26.23	843.70 \pm 50.05	1083.59 \pm 20.30
C16:1	53.44 \pm 3.24	35.87 \pm 1.39	34.02 \pm 0.46	35.64 \pm 1.82	77.25 \pm 1.54
C18:1	496.17 \pm 17.20	379.09 \pm 3.51	344.95 \pm 6.71	269.15 \pm 16.34	441.69 \pm 15.03
Σ MUFA	549.60 \pm 15.11	414.96 \pm 3.31	378.986 \pm 6.78	304.78 \pm 18.13	518.94 \pm 13.72
C18:2	342.11 \pm 20.05	322.11 \pm 9.04	263.05 \pm 7.01	145.67 \pm 8.54	78.12 \pm 1.48
C16:3	52.69 \pm 10.10	60.97 \pm 6.10	46.59 \pm 1.94	38.83 \pm 1.49	16.77 \pm 1.04
C18:3	296.59 \pm 5.06	359.80 \pm 8.61	256.31 \pm 8.56	437.42 \pm 27.36	173.98 \pm 7.60
Σ PUFA	691.40 \pm 12.67	742.87 \pm 6.03	565.96 \pm 14.97	621.92 \pm 37.23	268.87 \pm 9.95
C16:4	59.50 \pm 4.63	90.19 \pm 7.29	59.78 \pm 0.51	258.35 \pm 10.46	121.36 \pm 9.48
C18:4	69.02 \pm 4.31	79.99 \pm 4.64	53.92 \pm 1.82	319.02 \pm 13.71	194.96 \pm 11.82
Σ HUFA	128.50 \pm 8.69	170.18 \pm 11.22	113.69 \pm 2.10	577.37 \pm 24.17	316.32 \pm 21.28
Σ FA	2875.92 \pm 115.69	2569.51 \pm 61.31	2080.08 \pm 36.49	2347.77 \pm 121.60	2187.72 \pm 63.32

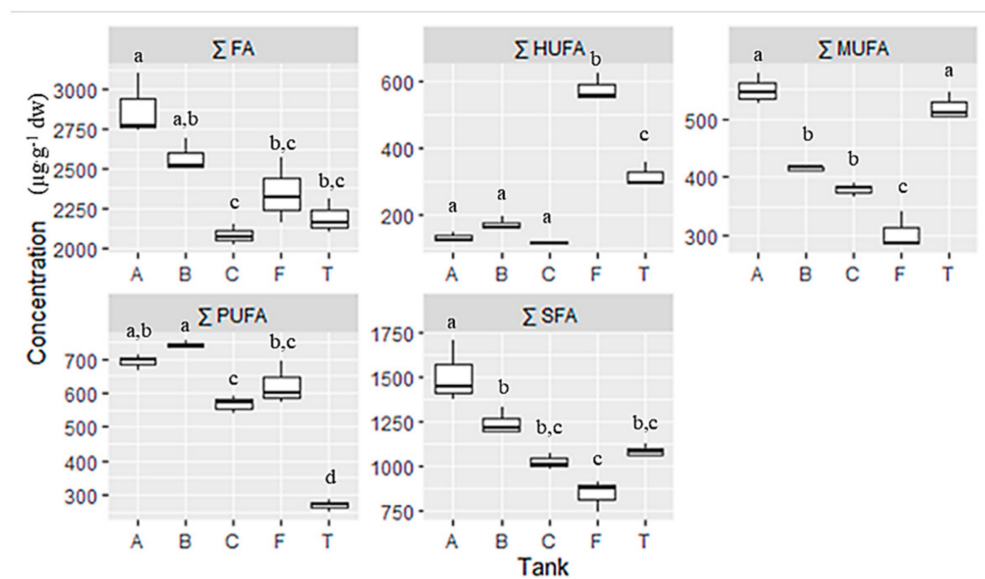


Figure 5. Graphs of each sum of FA group and total FA expressed in $\mu\text{g}\cdot\text{g}^{-1}$ of d.w. of *Ulva* sp. obtained from cultivated in indoor aquaria (A, B, and C), cultivated in outdoor tank (T) and collected from the field (F). In each graph, the same upper letters (a, b, c, and d) mean there are no statistically significant differences between the treatments (cultivation type). Data as mean value distribution and variance; n = 3.

3.3. Carbohydrate Characterization

After hydrolysis of the polysaccharides, the monosaccharides present in the samples were analyzed. The method performed does not allow the correct differentiation and separation of the two hexoses, glucose and galactose, so they appear together, and the results showed the total galactose plus glucose. These two residues (the sum of glucose and galactose) were highly identified in all samples, followed by rhamnose, xylose, and mannose. In terms of monosaccharide concentration, samples collected from the field (F) are similar to the samples that were cultivated in the external tank (tank T), respectively: galactose plus glucose ($333.38 \text{ mg}\cdot\text{g}^{-1}$ d.w. and $345.08 \text{ mg}\cdot\text{g}^{-1}$ d.w.), rhamnose ($133.35 \text{ mg}\cdot\text{g}^{-1}$ d.w. and $208.53 \text{ mg}\cdot\text{g}^{-1}$ d.w.), and xylose ($62.47 \text{ mg}\cdot\text{g}^{-1}$ d.w. and $28.10 \text{ mg}\cdot\text{g}^{-1}$ d.w.).

d.w.), except for mannose in which the concentration was different ($6.37 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$ and $20.55 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$) (Figure 6). In Figure 6, it is possible to see the concentration of each carbohydrate in all types of cultivation tanks compared to the wild conditions. Comparing the three aquaria cultivated in the laboratory, aquaria B and C had similar and higher concentrations of carbohydrate than the aquaria A. The results of the carbohydrate values from aquaria A, B, and C are, respectively: galactose plus glucose ($476.21 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$, $774.55 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$, and $676.88 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$), mannose ($10.12 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$, $13.67 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$, and $11.04 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$), rhamnose ($190.35 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$, $260.68 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$, and $296.68 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$) and xylose ($57.87 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$, $118.34 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$, and $133.41 \text{ mg}\cdot\text{g}^{-1} \text{ d.w.}$).

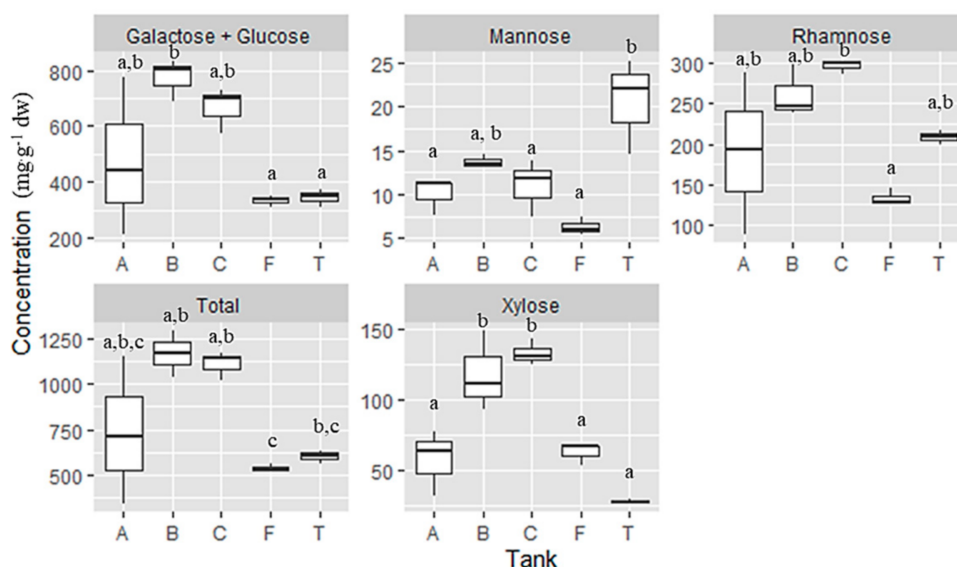


Figure 6. Graphs of monosaccharide composition expressed in $\text{mg}\cdot\text{g}^{-1} \text{ d.w.}$ of *Ulva* sp. obtained from different cultivation tanks or wild: indoor aquaria (A, B, and C) outdoor tank (T) and harvested from the field (F). In each graph same upper letters (a, b, and c) mean there are no significant differences between the treatments (cultivation type). Data as mean value distribution and variance; $n = 3$.

4. Discussion

Recently, *Ulva* sp. biomass has gained attention in various economic sectors due to its multipurpose use in commodities. Therefore, the production of sustainable biomass feedstock in experimental cultivation, or on a pilot scale, is the first step for cultivation on a large scale. Because seaweed can be a problematic species in nature, if we control the inshore cultivation system, we can have an important source of raw material and diminish the potential harm of seaweed blooms in non-controlled seawater cultivation (offshore cultivation), if the water is eutrophicated [24,36]. Moreover, this species can also protect against microalgae blooms in aquatic system [14,37].

We divided the process into two phases. In the first phase, the cultivation occurred in a controlled environment, using a closed system. We opted for this system to minimize the losses due to open system interactions, such as possible infections of the specimens by air (from bacteria or fungi), which gave the specimens ideal growth conditions (acclimatization period). However, the specimens' biomass growth increased, so there was a need to go to the next volumetric system, which was the aquarium with an open area, wherein the specimens presented a lower specific growth rate, which may have been due to a reaction with the different abiotic situation and interactions in a non-septic environment. One possibility was the aeration pattern, which can be similar; however, the aquaria and tank (using the same model in a different scale) may be the key to explain the differences due to aeration being important because of the abiotic pressure in the seaweed cell walls [38–40]. In addition, the aquaria and tank are an open-air system; therefore, bacteria/fungi present

in the air may also be responsible for part of the change [41–43]; thus, further studies are still required. Notably, with the naked eye, the experiment was analyzed every day to discharge possible plant or species contamination, and until the end of the experiment and the analysis, no contamination or interference was found in the seaweed culture [41–43]. Moreover, the lack of natural bacteria present in *Ulva* sp. can be a factor for biochemical changes since the seawater and light in the aquaria were the same between them at all times. Thus, there is the possibility of genetic and metabolic variability between specimens [38–40].

The tank cultivation was outdoors during the winter, which may have impacted the seaweeds' cultivation; although, the growth rate of *Ulva* sp. was identical to the indoor cultivation, supporting that this species can be cultivated all year round with a robust growth rate.

Concerning the FA analysis, the best samples results were obtained from the indoor cultivated aquaria (A, B, and C) for PUFA and SFA content, whereas the samples collected from the field (F) had the highest content of HUFAs. The cultivation tank (T) showed the lowest PUFA content and one of the highest MUFA concentrations. Referring to the monosaccharide concentration, the cultivation tank (T) presented the highest content in mannose and the lowest content in xylose. Rhamnose and galactose plus glucose had a higher concentration in the cultivation aquaria (A, B, and C). Samples obtained from the field presented a lower content of sugar. Similarly, the sugar content was higher in the aquaria cultivation systems, with an identical pattern of FA analysis.

In the present work, the growth rate was lower compared to other studies [33,44]; however, during the winter, the cultivation technique was different where the seaweed growth was lower compared to our results [45]. Moreover, their abiotic and biotic conditions are very different to our cultivation system location because the other studies added nutrients or added wastewater to the cultivation media. Our study only used estuarine water without supplementation to all the cultivation systems, unlike the other studies [46–50]. However, the growth rate of these studies was higher than that obtained by Rodrigues [51], whose species was cultivated in the Azores, the Portuguese island area.

For FA content, the inshore *Ulva* sp. is in concordance with other studies where a higher content of MUFAs than PUFAs was observed [16,22,52]. However, from the previously carried out studies on this species, there was no identical FA profile, and it was seen that cultivated and wild specimens can vary the FA profile immensely, which may be due to the different environmental conditions to which they were exposed. However, if controlled in culture, the unsaturated FAs from this species are exceptionally vital to the human diet, since these compounds have a key role in the prevention, or reduction in the risk, of chronic diseases, such as cardiovascular diseases and cancer [53,54]. However, their cultivation permits a better control of the maintenance of the nutraceutical profile of this species, mainly carbohydrates and fatty acids, which, consequently, prevents the risks involved in collecting wild biomass that might be harmful, due to the high potential of the species accumulating toxins and metals [55,56].

The sugar content from all the analyzed samples is different to that obtained by Yaich et al. [52] where glucose and rhamnose were the principal sugar compounds. Mannose concentration is divergent from various other studies of the *Ulva* sp., mainly in inshore cultivation [23,52].

Therefore, the inclusion of *Ulva* sp. in the human diet contributes to the daily intake of these essential nutrients (Table 3) with a typical daily serving of 5 g d.w. seaweed, which is recommended due to the high concentration of mineral content present in seaweeds [57–59]. In FAs, the PUFA/SFA ratio was normally higher than one (with the exception of aquarium A), which demonstrates similar quantity of SFA and PUFA promoting a balanced food source of FAs [60,61]. *Ulva* sp. has low FA content compared to the Dietary Reference Intake (DRI), whereby 20% of the energy is to be provided by FAs (in an adult diet of 2000 calories per day) [62]. However, the HUFAs yield demonstrates that *Ulva* sp. presents low content in ω -6 and ω -3 FAs, so HUFAs, which are important FAs, have low impact in this seaweed intake (maximum 0.6% of the Dietary Reference Intake). In terms of sugar level,

Ulva sp. gave between 3 and 6.5%, where the tank cultivation content was similar to the wild specimens. Only, the sugar content of the tank cultivation was better than those of wild specimens.

Table 3. Nutritional Profile of *Ulva* sp. in the human diet (daily dosage: 5 g d.w.) according with the PUFAs/SFA ratio based in the literature [63] and with established Dietary Reference Intake (DRI) based in the literature [2,59,62].

Nutrients	A	B	C	F	T
PUFAs/SFA ratio	0.91	1.07	1.06	1.78	1.02
Total FAs	0.03%	0.03%	0.02%	0.03%	0.02%
HUFA (EPA and DHA) (%)	0.3%	0.4%	0.3%	1.4%	0.8%
Sugars (%)	4.1%	6.5%	6.2%	3.0%	3.3%

5. Conclusions

This work supports the cultivation of *Ulva* sp. during the winter in southern European countries with temperate weather; however, in semi-controlled abiotic cultivation system, the nutraceutical compounds might be affected. Thus, a controlled system might be the key to control the initial growth of specimens to achieve higher nutraceutical value; although, the cultivation costs are higher, which can influence this type of cultivation.

Ulva sp. can be used in the food and nutraceutical industries with a high impact regarding food safety, guaranteeing a nutritious food source. Cultivated *Ulva* sp. biomass has the potential to offer a sustainable source of essential nutrients for human and animal consumption. Seaweeds' cultivation can prevent the negative effects of the ecological wild populations and can guarantee the seaweed quality to be used as food source. On the other hand, the inshore cultivation appears to be better evaluated to increase the growth rate without lowering the biochemical compounds by identifying major players to maintain an identical biochemical profile. The system of cultivation needs to be enhanced to accomplish and ensure a high-content seaweed profile, thus improving the nutritional value for human food safety.

Additional and subsequent strategies of the study are to improve and acquire more data about the aquaculture process of this seaweed species. In addition, the full characterization of the nutritional value of wild and cultivated *Ulva* sp. in parallel with the optimization procedure should be performed to ensure that the stakeholders and farmers have high quality cultivation without complex techniques. The biochemical profile should be further analyzed in the quantification and characterization of the polysaccharides, phenols, pigments, and sulfated compounds, along with the protein content and amino acids profile, ash quantification, and mineral concentration, to have a useful tool to promote *Ulva* sp. cultivation and future usage.

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