Diversity of monosaccharides in marine macroalgae from the Eastern Mediterranean Sea

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ABSTRACT

Macroalgae are primary producers bearing key roles in the normal functioning of marine environments. The critical energy carriers that macroalgae produce are carbohydrates, which support marine ecosystems and are used in biorefineries. In this work, we quantified the monosaccharide content and diversity of macroalgal species common to the Eastern Mediterranean shores representing the three major seaweed divisions, namely, Chlorophyta (Ulva sp. and Cladophora pellucida), Rhodophyta (Nemalion helminthoides, Galaxaura rugosa and Gracilaria sp.) and Ochrophyta (Padina pavonica and Sargassum vulgar). We found that the most abundant monosaccharide was different in 5 out of the 7 investigated species. The monosaccharide diversity profile was specific to each taxonomic group, especially in the first two orders of diversity, which correspond to Shannon entropy and Simpson concentration. The content of monosaccharides released by acid hydrolysis varied by 153% between Ulva sp., C. pellucida, G. rugosa, N. helminthoides, Gracilaria sp., P. pavonica, and S. vulgar collected from the same site. Ulva sp. collected at different sites at different months showed up to 79% variance in the total released monosaccharides, with up to 270% variance in the content of individual monosaccharides. The cultivation of Ulva sp. in a photobioreactor under more stable conditions reduced the diversity and the variability of the total carbohydrates to only 2%. By comparing amounts and types of monosaccharides derived from macroalgae, microalgae and terrestrial plants, it appears that Ulva sp. has the highest economic potential of all photosynthetic organisms, between $1733 kg⁻¹ and $3140 kg⁻¹ of Ulva biomass.

1. Introduction

Marine macroalgae (seaweeds) are primary producers and along the photic zone support life and diversity of numerous marine organisms such as protista, bacteria [1–3], and grazers [4]. In addition, large-sized seaweeds provide habitat and protection against environmental stressors and predators for seaweed-dependent multicellular heterotrophic organisms [5]. Besides their ecological role in the marine environment, macroalgae provide ecosystem services to numerous coastal communities. Indeed, the demand for seaweeds and seaweed-derived products doubled in the last decade and reached approximately 30 million tons in 2016 [6]. Global seaweed aquaculture accounts for approximately 20% of the total world marine aquaculture production by weight, with an annual value of about US $7 billion in 2013 with more than 80% produced in Asia [7]. Wild harvests only account for about 5% of the total seaweed production, while cultivated seaweed production has grown by about 50% in 10 years [8].

Moreover, macroalgae high biomass productivity [9–11] of polysaccharides and proteins [12] makes them an exciting feedstock for emerging biorefineries [13,14]. There is a large and diverse array of applications and uses of macroalgae products. Naturally harvested and cultivated macroalgae have many applications, such as in pharmaceutical, cosmetic, fertilizers, feed and food processing industries [13,15–17]. Besides the existing products and applications, macroalgae play a significant potential role in the emerging low carbon economy by providing food and biofuels [13,18,19].

Additional ecosystem services from macroalgae include coastal protection against erosion, carbon sequestration, nursery for fishes, feeding and breeding ground, nutrients cycling, and socio-economic values [20]. Furthermore, some species of seaweeds (kelps, fucoxids, encrusting corallines, rhodoliths) act as ecosystem engineers by modifying the spatial structure of the environment in which they live and influencing associated organisms [21,22]. Moreover, kelp forests and seaweed beds play a significant role in climate change mitigation by providing CO2 sinks [20].

An important parameter to determine and quantify ecosystem services is diversity [23–26]. Diversity describes the different states that the system under examination takes. In life sciences, diversity describes an
ecosystem in terms of number and abundance of living species, genes or biochemicals [27]. Most recently, the true diversity was described as the number of equally-common species [27]. In this approach, the diversity can be described in terms of an equivalent system — one that has the same value of the diversity index as the system in question, which is composed of equally-common system states [27]. Importantly, differently from other diversity indices, useful for system composition complexity measurements, diversity is always measured in units of number of species.

Biodiversity, as the diversity of life at all levels, is scientifically defined as “the variety of life in a particular habitat or ecosystem, and relates to genetic and biochemical diversity” [28–31]; referring directly to ecosystem services [23,32–34] as different species provide different services that affect ecosystems processes [35] impacting the overall system function [35]. Specifically in the marine ecosystems, species richness and abundance increases ecosystem function and services [24]. Many recent studies show that marine biodiversity improves the ocean’s capacity to provide food, maintain water quality, and recover from perturbations [36]. In addition, biomass feedstock and the diversity of potentially derived products are promising for feedstock availability, accessibility, affordability and economic viability [37,38]. Chemical diversity is known to drive ecosystem processes, especially in complex system such as forest ponds [39] and coral reefs [40]. Besides complex ecosystems, chemical diversity shows special intraspecific variations [30].

Monosaccharides are known to play important metabolic roles such as energy storage, molecule restructuration and energy transfer from the phototrophic to the heterotrophic worlds. Although used for species characterization [41], the diversity of monosaccharides for plants and algae, defined in terms of number of equally-common species [27], has not been determined yet. The question of how monosaccharide diversity affects functionalities from the organism level to the community is largely unknown.

We aim to understand the links between biochemical diversity and energy flows in marine environments and their ecosystem services outputs. In this study, we aimed at determining the diversity of monosaccharides in selected macroalgal species collected in the Eastern Mediterranean Sea, along Israeli shores. Therefore, we developed a methodology for rapid monosaccharide profile determination with high pressure ion chromatography (HPIC) and, thereafter, we established the major monosaccharide diversity profile in the three major seaweed divisions (Chlorophyta, Rhodophyta and Ochrophyta), including comparisons of intra-species chemical diversity spatially and seasonally in the green alga Ulva.

We suggest that the chemical diversity profiling of macroalgae species based on monosaccharides would provide new insights to the macroalgae ecosystems services and feedstock potential for biofuel industries, altogether contributing to biodiversity conservation, sustainable development and climate change mitigation. Moreover, we believe that this approach would provide insights into diversity changes, especially community interaction modeling, improving our knowledge on primary producer diversity, emphasizing the reliance on macroalgae as primary chemical producers.

2. Materials and methods

2.1. Algal collections and their taxonomic identification

Fresh macroalgal specimens were collected during the Spring time of 2016, from the tidal zone at four sites in the Eastern Mediterranean Sea: Rosh-Haništra (33°02′00″ N, 35°06′00″ E), Haifa (32°35′5″ N, 34°9285′ E), Mikhmoret, three sites [1–3], (32.4065° N, 34.8716° E), and Tel Aviv (32°07′00″ N 34 49′00″ E). The algal material was transported as quickly as possible to the lab. The species were identified using morphologically features as verified in Algae Base (http://www.algaebase.org) and published literature for the local marine flora [42–45], as follows (Fig. 1a): two distinct (leaf-shaped and wide filaments) morphotypes [46], collectively defined as Ulva sp. (as Ulva species also change their morphology as a function of environment [46], we decided to report the results on the genus level), and Cladophora pellucida from the division Chlorophyta (green seaweeds), Galaxaura rugosa, Nemalion helminthoides and Gracilaria sp. from the division Rhodophyta (red seaweeds) and Padina pavonia and Sargassum vulgare from the division Ochrophyta (brown seaweeds).
2.2. Indoor cultivation of Ulva sp.

Thalli of leaf-shaped Ulva sp. collected from Haifa were cultivated for up to 4 months (March–June 2016) in 40 L, 200 μm thick polyethylene sleeves (Polyvit, Israel) embedded with anti-UV protection as described in reference [47]. The cultivation was done in nutrient-enriched seawater under natural illumination. Nutrients were added as ammonium nitrate (NH₄NO₃, Haifa Chemicals Ltd., Israel) and phosphoric acid (H₃PO₄, Haifa Chemicals Ltd., IS) to maintain 6.4 g m⁻³ of nitrogen and 0.97 g m⁻³ of phosphorus in the cultivation media. Depending on specific sleeve location, illumination varied between reactors in the range of 238–348 μmol photons m⁻² s⁻¹. Seawater circulated continuously with an exchange rate of 40–80 L h⁻¹ (all water was exchanged 1–2 times per hour). Mixing was done using an air column inserted in the sleeves at 2–4 L min⁻¹ air flow rates. Sleeves were manually cleaned with soap and bleached to remove the contaminating epiphytes prior to cultivation.

2.3. Acid hydrolysis of algae tissues

The collected biomass was dried at 40 °C until constant weight and crushed manually to powder in liquid nitrogen using a mortar & pestle. Most chemicals and standards were purchased from Sigma-Aldrich (Israel). Sulfuric acid (2 mL) was added into the tubes and the mix was vortexed to make the powder evenly distributed in acid. Thermochemical deconstruction [48] (2% sulfuric acid, 1:20 solid to solvent ratio, 30 min, 121 °C) was conducted in 10 mL centrifuge tubes (Nalgene™ Oak Ridge High-Speed PPCO Centrifuge Tubes (Thermo-Fisher Scientific, CA)) in autoclave (Tuttnauer 2540MLV, Netherlands). Dried samples of biomass (50 mg) were weighed on an analytical balance (Mettler Toledo, Switzerland). Hydrolysates were stored at −20 °C after centrifugation (5 min, 12,000 rpm, using a benchtop centrifuge (Eppendorf, Germany).

2.4. Determination of monosaccharides in acid hydrolysis by high pressure ion chromatography (HPIC)

For monosaccharide analysis, hydrolysates were thawed and an aliquot was diluted 50 times in ultrapure water before being filtered through a 0.22 μm syringe-filter (Millipore, USA) in HPIC vials (Thermo Fischer Scientific, MA, USA). Monosaccharide contents in the hydrolysates were monitored by HPAEC-PAD (High Pressure Anion-Exchange Chromatography coupled with Pulse Amperometric Detection) using a Dionex ICS-5000 platform (Dionex, Thermo Fischer Scientific, MA, USA) with an analytical column (Aminopack 10) and its corresponding guard column. An electrochemical detector with an AgCl reference electrode was used for detection. The analysis was performed using an isocratic flow of 4.8 mM KOH generated by the Eluent Generator technology (Dionex, Thermo Fischer Scientific, MA, USA) for 20 min. Then the column was washed with 100 mM KOH between each run and re-equilibrated with 4.8 mM KOH prior to injection. The column temperature was kept at 30 °C, and the flow rate was set to 0.25 mL min⁻¹. Calibration curves were produced for each sugar with internal standards. In this work we quantified rhamnose, arabinose, galactose, glucose, xylose, glucuronic acid, mannitol, fucose, and mannose.

Glucuronic acid content was monitored by using a program that involved three eluents (NaOH, ultrapure water and sodium acetate) — see Supplementary information Table S3. Two additional small peaks were observed but not analyzed in the area of glucuronic acid peak in all samples that were sensed as aldobiouronic acid and iduronic acid as stated in [49]. Each algal sample was hydrolyzed in duplicate before analysis. All samples were analyzed in duplicate. All data were reported as weight fraction of the specific monosaccharide biomass (μg of monosaccharide mg⁻¹ DW (dry weight) biomass).

2.5. Carbohydrate diversity determination

In this work we denote types as chemicals, monosaccharide, and dataset for the individual macroalgae specimens. Diversity is a quantitative measure that reflects how many different types (of monosaccharides, in our case) are in a dataset, taking into account how basic entities are distributed among those types [27]. In recent work [27], a family function describing the diversity of the biological system was described, Eq. (1):

\[
D^q(p_1, p_2, \ldots, p_n) = \left( \sum_{i=1}^{N} p_i^q \right)^{1/q} 
\]  

(1)

where \( D \) is the true system diversity, \( p \) is the system state (monosaccharide fraction from total monosaccharide content in the biomass), \( N \) is the number of states in the system (number of chemical species, in this work limited to the number of different monosaccharides, as the counter integer, and \( q \) is the diversity order [50]. \( D^q \) functions, also called “Hill numbers”, possess the doubling properties, expected for biological systems, and are equal unity when there is only one possible state of a system. Interestingly, the definition of true diversity as given in Eq. (1) combines the widely used diversity indices as richness, \( D^0 (q = 0) \) Eq. (2); the exponential of Shannon entropy \( D^1 \) (limit at \( q = 1 \), Eq. (3), and inverse Simpson concentration \( D^2 (q = 2) \) Eq. (4).

\[
D^0(p_1, p_2, \ldots, p_n) = \sum_{i=1}^{N} p_i^0 
\]  

(2)

\[
D^1(p_1, p_2, \ldots, p_n) = \exp \left( \sum_{i=1}^{N} -p_i \ln p_i \right) 
\]  

(3)

\[
D^2(p_1, p_2, \ldots, p_n) = 1/\sum_{i=1}^{N} p_i^2 
\]  

(4)

In this study we calculated diversities up to \( q = 7 \), as after this the changes between the consequent diversity levels are very small [27]. Examples of full calculations of diversities appear in the Supplementary Information.

2.6. Analysis of macroalgae market value based on the monosaccharides prices

We denote the market price for the monosaccharide \( i \) as \( c_i \) (S kg⁻¹). Then the total price (\( TP_b, $ kg^{-1} \)) for monosaccharides extracted from a specific biomass would be

\[
TP_b = \sum_{i=1}^{N} c_i p_i 
\]  

(5)

The market prices for individual monosaccharides vary according to purity, quantity and manufacturer. For this study, we used the price catalog from Sigma Aldrich (http://www.sigmaaldrich.com, 2017 price list) with lowest prices per gram of material (Table S4). We made the assumption that monosaccharides released during hydrolysis can be separated and purified to the level corresponding to the selected available product on Sigma Aldrich catalog (Table S4).

2.7. Statistical analysis

All samples were analyzed with 2 biological replicates and 2 technical repetitions at least. Results were reported as average ± SD. Clustering was operated with hierarchical clustering algorithm, Ward method (compact spherical clusters, minimizing variance) implemented in reference [51].
3. Results and discussion

3.1. Diversity of monosaccharides in species collected at the Rosh-HaNikra reef

To study interspecific differences, we quantified content and diversity of monosaccharides from macroalgae collected from the same location at Rosh-HaNikra reef. The quantification of 9 monosaccharides derived from the macroalgal biomass by acid hydrolysis is reported in Fig. 2a and Table 1. Interestingly, the monosaccharide with the highest abundance was different in 5 out of the 8 species collected from this site.

Ulva sp. and C. pellucida had the highest content of glucose (49.18 ± 1.37 and 34.90 ± 2.38 μg mg⁻¹ respectively). However, in the red algae, the most abundant monosaccharide in G. rugosa was xylose (103.33 ± 0.01 μg mg⁻¹), in N. helminthoides it was mannose (88.94 ± 9.36 μg mg⁻¹) and in Gracilaria sp. it was galactose (55.49 ± 0.41 μg mg⁻¹). In the brown algae, fucose was observed as the most abundant monosaccharide in P. pavonia (31.30 ± 7.00 μg mg⁻¹), and mannitol in S. vulgar (49.2 ± 6.10 μg mg⁻¹). Even though glucose was the most abundant monosaccharide in Ulva sp. and C. pellucida, the content of other monosaccharides differed significantly between the two green algae as rhamnose was noted as the second most abundant monosaccharide in Ulva (39.69 ± 1.32 μg mg⁻¹), and observed in much lower concentration in C. pellucida (4.97 ± 0.60 μg mg⁻¹). Arabinose was the second most abundant monosaccharide in C. pellucida (20.95 ± 0.40 μg mg⁻¹), but was observed in very low concentration in Ulva sp. (1.02 ± 0.04 μg mg⁻¹). In addition, compared to Ulva, C. pellucida showed higher content of galactose (20.41 ± 0.67 μg mg⁻¹ vs 7.74 ± 0.34 μg mg⁻¹); but lower levels of xylose (10.94 ± 0.64 μg mg⁻¹ vs 18.02 ± 0.72 μg mg⁻¹) and glucuronic acid (3.00 ± 0.33 μg mg⁻¹ vs 9.64 ± 1.71 μg mg⁻¹). Mannitol, fucose and mannose were completely absent in both Ulva sp. and C. pellucida.

The red algae species showed the largest variation in monosaccharide diversity (Figs. 1b, 2a) and in the total released monosaccharides content (161.91 ± 9.40 μg mg⁻¹ for N. helminthoides and 64.01 ± 0.41 μg mg⁻¹ for Gracilaria sp.). Interestingly, in G. rugosa the most abundant monosaccharide was xylose (103.33 ± 0.01 μg mg⁻¹); while in other tested species (including Chlorophyta and Ochrophyta) hexoses were found as the most abundant. In all other tested species, the major released monosaccharides were hexoses. Xylose content in N. helminthoides was 32.43 ± 0.01 μg mg⁻¹, but only 3.66 ± 0.03 μg mg⁻¹ of this monosaccharide was found in Gracilaria sp. The most abundant monosaccharide in N. helminthoides was mannose (88.94 ± 9.36 μg mg⁻¹), which showed only 7.83 ± 3.00 μg mg⁻¹ in G. rugosa, and 0.74 ± 0.05 μg mg⁻¹ in Gracilaria sp. In addition, less abundant monosaccharides, but still released by acid hydrolysis in considerable amounts, were: galactose for G. rugosa (14.36 ± 2.09 μg mg⁻¹), glucose (20.02 ± 0.01 μg mg⁻¹), and galactose (16.43 ± 0.94 μg mg⁻¹) for N. helminthoides, and finally glucose (3.16 ± 0.05 μg mg⁻¹) for Gracilaria sp. Other monosaccharides occurred in lower quantities (Fig. 2a, Table 1).

The two species of Ochrophyta showed high diversities (Fig. 2b) with fucose (31.30 ± 7.00 μg mg⁻¹) as the most dominant monosaccharides in P. pavonia, and mannitol (49.20 ± 6.10 μg mg⁻¹) for S. vulgar. Fucose was the second most abundant monosaccharide for S. vulgar (34.30 ± 3.67 μg mg⁻¹), which was the highest content of this sugar in all tested species. Less abundant, but still released in considerable amounts by acid hydrolysis in P. pavonia and S. vulgar, were the monosaccharides glucose (14.11 ± 0.56 μg mg⁻¹) and 13.37 ± 0.55 μg mg⁻¹), and xylose (13.37 ± 0.55 μg mg⁻¹ and 8.49 ± 0.94 μg mg⁻¹). Other monosaccharides occurred with lower contents (Fig. 2a, Table 1).

Interestingly, the analyzed species showed a specific class of diversity patterns (Fig. 1c), especially at D² and D³ (corresponding to Shannon entropy and Simpson concentration, which are the most informative levels of diversity [27]) (Fig. 2b). D² (number of types of monosaccharides) depends on the detection level of the device. Ochrophyta showed the highest first (D¹) and second (D²) order diversities, followed by Chlorophyta and then by Rhodophyta. S. vulgar showed the highest D³ (5.51), followed by P. pavonia (5.45), C. pellucida (4.67), Ulva sp. (4.01), N. helminthoides (3.55), G. rugosa (2.23) and Gracilaria sp. (1.38), (Fig. 2b). P. pavonia showed the highest D² (4.34), followed by S. vulgar (4.20), C. pellucida (4.06), Ulva sp. (3.33), N. helminthoides (2.72), G. rugosa (1.61) and Gracilaria sp. (1.31) (Fig. 2b).

The monosaccharide diversity plot and its hierarchical clustering analysis (Figs. 1b and 2b) showed that C. pellucida and Ulva sp. have similar diversity trends, but different from Rhodophyta and Ochrophyta. Further analysis suggests that although both C. pellucida and Ulva sp. shared common taxonomic status (both being within the class Ulvophyceae), their structural carbohydrates, cladophoran [52,53] and ulvan [54–56] fibers, which include arabinose (cladophoran) and rhamnose/glucuronic acid (ulvan) evolved separately. This suggestion is supported by placement of C. pellucida in the order Cladophorales and Ulva to the order Ulvales, a separation that is well established based on morphology and molecular phylogenetic data [57].

This is also supported by hierarchical clustering analysis of monosaccharides of all tested algae (Fig. 1b), which showed that C. pellucida is different from Ulva sp. Future evolutionary genetic studies are needed.
Table 1. Monosaccharide content (based on biomass measured after drying at 40 °C) of macroalgae collected at Rosh Hanikra rocky beach. Average contents of rhamnose (Rha), arabinose (Ara), glucose (Glc), galactose (Gal), xylose (Xyl), fucose (Fuc), mannose (Man), and total (Tot) shown ± SD. * = date of collection.

<table>
<thead>
<tr>
<th>Species</th>
<th>Algae type</th>
<th>Rha μg mg⁻¹</th>
<th>Ara μg mg⁻¹</th>
<th>Glc μg mg⁻¹</th>
<th>Gal μg mg⁻¹</th>
<th>Xyl μg mg⁻¹</th>
<th>Fuc μg mg⁻¹</th>
<th>Man μg mg⁻¹</th>
<th>Tot μg mg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galaxaura rugosa</td>
<td>Rhodophyta</td>
<td>0.36 ± 0.08</td>
<td>0.20 ± 0.03</td>
<td>14.36 ± 2.09</td>
<td>6.04 ± 1.18</td>
<td>103.33 ± 0.01</td>
<td>0.98 ± 0.41</td>
<td>0.00</td>
<td>7.83 ± 3.00</td>
</tr>
<tr>
<td>Nemalion helminthoides</td>
<td>Rhodophyta</td>
<td>0.00</td>
<td>0.00</td>
<td>55.49 ± 0.41</td>
<td>3.16 ± 0.05</td>
<td>3.66 ± 0.03</td>
<td>0.95 ± 0.01</td>
<td>0.00</td>
<td>0.74 ± 0.05</td>
</tr>
<tr>
<td>Chlorophyta</td>
<td>4.97 ± 0.60</td>
<td>20.95 ± 0.40</td>
<td>20.41 ± 0.67</td>
<td>34.90 ± 2.38</td>
<td>10.94 ± 0.64</td>
<td>3.00 ± 0.33</td>
<td>0.00</td>
<td>0.00</td>
<td>95.17 ± 2.67</td>
</tr>
<tr>
<td>Cladophora pellucida</td>
<td>Chlorophyta</td>
<td>39.69 ± 1.32</td>
<td>1.02 ± 0.04</td>
<td>7.74 ± 0.34</td>
<td>49.18 ± 1.37</td>
<td>18.02 ± 0.72</td>
<td>9.64 ± 1.71</td>
<td>0.00</td>
<td>125.29 ± 2.68</td>
</tr>
<tr>
<td>Ulva Padina pavonia</td>
<td>Ochrophyta</td>
<td>2.78 ± 0.26</td>
<td>1.73 ± 0.20</td>
<td>9.92 ± 0.73</td>
<td>8.78 ± 0.65</td>
<td>8.49 ± 0.94</td>
<td>13.22 ± 3.02</td>
<td>49.2 ± 6.10</td>
<td>34.30 ± 3.67</td>
</tr>
</tbody>
</table>
| Sargassum vulgare         | Phaeophyceae       | 122.12 ± 6.12 | 7.91 ± 0.42 | 24.50 ± 0.22 | 318% for galactose | (7.91 ± 0.42 μg mg⁻¹ for the algae collected in Haifa), 318% for galactose | (7.91 ± 0.42 μg mg⁻¹ for the algae collected in Haifa); 242% for glucose (55.47 ± 2.27 μg mg⁻¹ for the algae collected in Mikhmoret vs 68.10 ± 1.40 μg mg⁻¹ for the specimen collected in Haifa) (Fig. 3a and Table 2). This is a considerable variation for a species that is abundant macroalgae along the Israeli Mediterranean coast. Ulva sp. monosaccharides content released by acid hydrolysis varied by 68% (134.23 ± 3.72 μg mg⁻¹ for the algae collected in Mikhmoret and 68.10 ± 1.40 μg mg⁻¹ for the specimen collected in Haifa) (Fig. 3a and Table 2). This is a considerable variation for a species that is considered an eligible feedstock for biorefineries, especially for fermentation [60,61] where the final output depends on the total sugar content [62].

The content of individual monosaccharides showed higher variation between specimens: 132% for rhamnose (24.30 ± 0.43 μg mg⁻¹ for the algae collected in Mikhmoret vs 68.10 ± 1.40 μg mg⁻¹ for the specimen collected in Haifa), 318% for galactose (7.91 ± 0.42 μg mg⁻¹ for the algae collected in Mikhmoret vs 1.89 ± 0.03 μg mg⁻¹ for the algae collected in Haifa); 242% for glucose (55.47 ± 2.27 μg mg⁻¹ for the algae collected in Mikhmoret vs 24.50 ± 0.22 μg mg⁻¹ for the algae collected in Haifa), 219% for xylose (18.02 ± 0.72 μg mg⁻¹ for the algae collected in Rosh-Hanikra...
vs 5.64 ± 0.15 μg mg⁻¹ for the algae collected in Haifa) and 155% for glucuronic acid (24.59 ± 0.16 μg mg⁻¹ for the algae collected in Tel Baruh vs 9.64 ± 1.71 μg mg⁻¹ for the algae collected in Rosh-HaNikra). Such variations between macroalgae individual monosaccharides amounts are considerable, when specific organisms are needed to convert the numerous non-glucose monosaccharides to fermentation products [62]. Each batch of algae requires specific development adjusting its chemical composition. However, hierarchical clustering analysis showed that all of the Ulva species collected have similar carbohydrate content, unlike with the other species (Fig. 1b).

Indeed, diversity profile analysis showed a tiny difference (up to 18%) in D¹ between the tested specimens; yet, higher levels of diversity increased the difference up to 48% (D³) (Fig. 3b). Hierarchical clustering analysis found all tested samples closer to each other than Rhodophyta and Phaeophyceae sub-groups (Fig. 1c).

### 3.3. Diversity of monosaccharides in Ulva sp. collected at Mikhmoret sampling site

We noticed that monosaccharides content and diversity profile of Ulva sp. changed with time at Mikhmoret sampling site (Fig. 3c, d,

<table>
<thead>
<tr>
<th>Date of collection 2016</th>
<th>Location</th>
<th>Rha μg mg⁻¹</th>
<th>Arb μg mg⁻¹</th>
<th>Glc μg mg⁻¹</th>
<th>GluA μg mg⁻¹</th>
<th>Tot μg mg⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/3</td>
<td>Mikhmoret_1</td>
<td>41.58 ± 2.69</td>
<td>0.11 ± 0.10</td>
<td>7.91 ± 0.42</td>
<td>55.47 ± 2.27</td>
<td>159.29 ± 8.22</td>
</tr>
<tr>
<td>16/5</td>
<td>Mikhmoret_2</td>
<td>27.53 ± 0.11</td>
<td>3.34 ± 0.09</td>
<td>50.45 ± 1.28</td>
<td>54.07 ± 1.87</td>
<td>152.10 ± 4.72</td>
</tr>
<tr>
<td>16/5</td>
<td>Mikhmoret_3</td>
<td>31.10 ± 0.45</td>
<td>0.53 ± 0.53</td>
<td>7.19 ± 0.29</td>
<td>49.51 ± 1.93</td>
<td>136.82 ± 4.94</td>
</tr>
<tr>
<td>1/3</td>
<td>Rosh-HaNikra</td>
<td>39.69 ± 1.32</td>
<td>1.02 ± 0.23</td>
<td>7.74 ± 0.34</td>
<td>49.18 ± 1.37</td>
<td>125.29 ± 2.68</td>
</tr>
<tr>
<td>5/5</td>
<td>Tel Baruh</td>
<td>56.41 ± 1.26</td>
<td>0.36 ± 0.01</td>
<td>1.79 ± 0.29</td>
<td>16.18 ± 0.38</td>
<td>24.59 ± 0.16</td>
</tr>
<tr>
<td>16/3</td>
<td>Haifa</td>
<td>24.30 ± 0.43</td>
<td>0.27 ± 0.01</td>
<td>1.89 ± 0.03</td>
<td>24.50 ± 0.22</td>
<td>5.64 ± 0.15</td>
</tr>
</tbody>
</table>

Table 3. We sampled at 3 different sites of Mikhmoret (1 March 2016). Total monosaccharide content released with acid hydrolysis varied by up to 79%, with the highest yields of 159.29 ± 8.22 μg mg⁻¹ observed on 15 May 2016, and lowest yield of 88.98 ± 1.33 μg mg⁻¹ observed on 5 May 2016. The content of individual monosaccharides varied from 63% for glucose (58.97 ± 5.56 μg mg⁻¹ observed on 15 May 2016 to 36.21 ± 0.18 μg mg⁻¹ observed on 5 May 2016) to 270% variation observed for xylose (21.14 ± 1.60 μg mg⁻¹ observed on 14 April 2016 to 57.0 ± 0.18 μg mg⁻¹ observed on 1 March 2016 on site 2). The content of arabinose measured was negligible.

Results showed 32% variation in the total monosaccharide content (100.88 ± 1.30 μg mg⁻¹ at site 2 to 134.23 ± 3.72 μg mg⁻¹ at site 1). The rhamnose content varied by 51%, galactose by 14%, xylose by 20% and glucuronic acid by 16% (Fig. 3c, Table 3). These results are important for the potential use of Ulva sp. as biorefinery feedstock for sugar production and fermentation substrate, as it shows large variations of critical carbohydrates that affect the final product content [13,63,64].

Similar to the geographic location study, diversity profile analysis showed a slight difference (up to 18%) in D¹, of the tested specimens;

![Fig. 3. Spatial and seasonal changes in monosaccharide diversity of Ulva sp. a. Content of monosaccharides in species sampled at 4 different sites about 130 km apart on the Eastern Mediterranean shores of Israel. Three sites were samples at Mikhmoret. b. Diversity profile of monosaccharides in species sampled at 4 different sites about 130 km apart on the Eastern Mediterranean shores of Israel. c. Content of monosaccharides in algae sampled at different months at Mikhmoret site. d. Diversity profile of monosaccharides of species sampled at different months at Mikhmoret site. Error bars show ± standard deviation.](image-url)

Table 2

Monosaccharide content of Ulva sp. collected from various locations along the Israeli Mediterranean coast (spring 2016). Average content of rhamnose (Rha), arabinose (Arb), galactose (Glc), glucose (Glu), xylose (Xyl), glucuronic acid (GluA), mannitol, fucose (Fuc), and mannose (Man), total (Tot) are shown ± SD. (Measures are based on biomass dry weight measured after drying at 40°C).
yet, higher levels of diversity increased the variance up to 35% ($D^6$) (Fig. 3d). Hierarchical clustering analysis of diversity profiles clusters all tested Ulva sp. samples closer to each other than to any of the Rhodophyta and Phaeophyceae (Fig. 1c).

3.4. Ulva sp. monosaccharide content changes with time during cultivation in the photobioreactor

The algae collected in Haifa were cultivated for 4 months in a photobioreactor [47] under natural illumination and natural sea water fortified with nitrogen and phosphors. Total content of monosaccharides released by acid hydrolysis increased in comparison to the biomass collected in the field before cultivation in the photobioreactor [47] under natural illumination and natural sea water all tested Ulva sp. samples closer to each other than to any of the Rhodophyta and Phaeophyceae (Fig. 1c).

3.5. Monosaccharide diversity profile comparison between macroalgae, microalgae and higher plants

We compared the diversity of the monosaccharides from the tested macroalgae with the diversity of monosaccharides of some microalgae and from cell walls of a model plant Arabidopsis thaliana [70] (Fig. 5). The comparison is based on data from the literature [41]. Besides the diatom Phaeodactylum tricornutum, all other tested microalgae species showed lower diversity at all levels in comparison with the tested macroalgae and Arabidopsis (Table 4). This observation suggests that diversity of monosaccharides could be related to the development of extracellular matrix, as observed in macroalgae and high terrestrial plants.

We noticed that two commercially cultivated species, Dunaliella salina (microalgae) and Gracilaria sp. (macroalgae), have the lowest diversity at $D^1$ in comparison with all other species ($D^1$ 1.09 and 1.38 respectively). Indeed, D. salina showed the lowest monosaccharide diversity at all ranks. Gracilaria sp. had the lowest diversity of monosaccharides at all ranks among the analyzed species, with Scenedesmus ovaterrus and D. salina. These data suggest that our diversity profile methodology could be used as relevant criteria for screening projects to identify wild algae species fit for domestication. Nevertheless, we need further investigation based on a broader scope of knowledge and wider database comparing larger groups of domesticated algae, grown at different environmental conditions, to assert our suggestion.

Comparison with Arabidopsis carbohydrates cell wall showed a very similar diversity between Arabidopsis and Ulva sp. at all levels of diversity besides $D^6$ ($D^6$ 4.89 vs 4.13; $D^5$ 3.61 vs 3.5; $D^4$ 3.08 vs 3.22; $D^3$ 2.82 vs 3.07; $D^2$ 2.67 vs 2.98; $D^1$ 2.58 vs 2.92). This similarity between Arabidopsis and C. pellucida at $D^5$ (4.89 vs 4.67) was the largest among all tested algae. These results could be expected as vascular plants descended from green algae that moved to live on land.

3.6. Monosaccharide diversity and economic value

Acid hydrolysis has already been proposed as a method for monosaccharide production in biorefineries [71–74]. We established the potential income of macroalgae monosaccharides released by acid hydrolysis, compared to the potential income of monosaccharides from
These results suggest that marine macroalgae are a potential valuable source of monosaccharides. The variation of potential income (Ulva sp.) depends highly on the biomass composition. Therefore, we assert that controlled cultivation could mitigate these fluctuations, suggesting that this method is likely preferable to in situ sampling, especially for downstream processing (Fig. 4).

4. Conclusions

In this work, we quantified the content and the diversity of significant monosaccharides released by acid hydrolysis from various types of macroalgae collected along the Israeli Mediterranean Sea shore. Five of the seven investigated macroalgae species showed different monosaccharides with the highest comparable content. The analyzed species showed specific patterns of monosaccharide diversity profile, especially at $D^0$, which corresponds to Shannon entropy and $D^2$, which corresponds to Simpson concentration. Ulva sp. showed up to 79% variance in the total monosaccharide content released by acid hydrolysis, with up to 270% variance in the content of individual monosaccharides. Ulva sp. cultivation in the photobioreactor took up to 4 months, reducing the variance in the total carbohydrate content to 2%, and the variance in the individual monosaccharide content to 51%, as well. In addition, cultivation in the photobioreactor reduced the diversities 4 months after cultivation by 5% for $D^0$ to 17% reduction for $D^2$. This significant reduction of diversity profile indicates the fixing of specific monosaccharide content. The economic potential of monosaccharides, derived from tested macroalgae, microalgae and terrestrial plants species, are significant. Our work shows that Ulva sp. has the highest economic potential from investigated species with potential income of $1733$ kg$^{-1}$–$3140$ kg$^{-1}$ based on extractable and purified monosaccharides prices. However, new technologies are needed for faster separation and purification of multiple monosaccharides at the same time.

Author contributions

AR performed experiments, data analysis and drafted the manuscript, PC did ecosystem service and economic analysis and drafted the manuscript, AC collected and cultivated the algae, AI identified the species and edited the manuscript, and AG conceived the study, did data analysis and wrote the manuscript.
Conflict of interest

The authors declare no conflict of interest.

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Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

Appendix A. Supplementary data

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