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Seasonal and depth variations in the chemical composition of cultivated *Saccharina latissima*

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ABSTRACT

Sugar kelp (*Saccharina latissima*) is an abundantly available macroalgae species along the Norwegian coast, and there is currently emerging an industry based on seaweed cultivation. In this study, the biomass growth of cultivated *S. latissima* deployed in February was studied at cultivation depths of 3 and 8 meters (m) and monitored over the period of May, June, and August. The highest biomass production was observed in June at the depth of 3 m (38.3 kg wet weight m^{-2}). Furthermore, all seaweed samples underwent a detailed chemical characterization including analysis of carbohydrates (glucose, mannitol, fucose, xylose, uronic acids), amino acids and minerals. The macroalgae deployed in February at 3 m depth and sampled in June had the highest proportion of total sugars (534.5 g kg^{-1} of DM) and the lowest content of ash (252.7 g kg^{-1} of DM). Thus, cultivation at 3 m and harvesting in June are suitable when the feedstock is used for biochemical production of fuels and chemicals. Macroalgae deployed at 8 m depth and harvested in August had the highest proportion of total amino acids (242.4 g kg^{-1} DM) and ash content (411.5 g kg^{-1} DM). This biomass may be suitable as a nitrogen and mineral source in microbial growth media. Overall, the choice of cultivation depth and harvesting time depends on the intended use of the seaweed biomass.

1. Introduction

Macroalgae have gained attention globally as a potential feedstock for production of biopharmaceuticals, food and feed ingredients, biofuels, and bio-fertilizers [1–5]. In 2014, the global production of farmed macroalgae was 27.3 million tons wet weight, of which 99.3% was produced in Asian countries, pre-dominantly China and Indonesia [6]. Macroalgae can exhibit higher growth rates and production yields than terrestrial biomass [1,2]. A relatively small number of macroalgae genera constitute almost 98% of the global production of cultivated seaweed, i.e., *Saccharina/Laminaria* and *Undaria* (brown macroalgae), and *Eucheuma/Kappaphycus*, *Porphyra/Pyropia* and *Gracilaria* (red macroalgae). *Laminaria* spp. are widely distributed in the surface water environments on both sides of the Atlantic Ocean and off the coasts of China and Japan [7].

Saccharina latissima belongs to the Laminariaceae family (Phaeophyceae), and is a perennial species that can grow in sheltered waters attached to the seabed. The main groups of carbohydrates found in *S. latissima* are laminarin, alginate, cellulose, fucoidan, and the sugar alcohol mannitol. Laminarin and mannitol are storage carbohydrates, which accumulate in the seaweed during the light season, while alginate is a structural component with little annual variation [1,4,8,9]. Structurally, alginate is a linear polysaccharide of mannuronic and guluronic acids, which due to its physiological and rheological properties is used as a thickening agent for drinks, ice cream and cosmetic products [10]. Cellulose and laminarin may be hydrolyzed to glucose which, together with mannitol, could be used as a carbon source in fermentation to bioethanol or other valuable products [1,2,11]. Fucoidan is a sulphated polysaccharide composed of L-fucose units and possesses biological activities such as anticoagulant, antioxidant and antibacterial [12].

S. latissima is also known as a source of amino acids, minerals, and phenolic compounds [4,13,14]. *S. latissima* has a nitrogen reservoir that can sustain the growth in the periods of the summer when the available nitrogen (nitrates) in the sea are low [13,15]. The protein fraction of *S. latissima* contains all essential amino acids (EAAs) and non-essential amino acids (NEAAs) and their amount varies over the season [5]. *S. latissima* has a relatively high ash content, where the most important

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cations are sodium, potassium, calcium and magnesium, associated with the anions, chlorine, bromine, iodine, phosphorus and sulfur. The polyphenolic compounds in brown seaweed possess biological activities and are responsible for their inhibitory action towards microorganisms [13]. The growth rate of macroalgae varies according to the season, from hardly any growth during the dark winter time to rapid growth during spring and summer. Generally, macroalgal composition and growth rate vary considerably according to a range of environmental factors such as the salinity of seawater, pH, sunlight, mineral availability, waves, and water current [4,13,14].

In this work, we present a thorough study of the chemical composition of cultivated *S. latissima*. The objective was to investigate the effect of different cultivation depths and harvesting times on seaweed growth and chemical compositions, and how this affected potential applications of the feedstock.

2. Materials and methods

2.1. Seedling preparation, cultivation and measurements

Sorus induction of *S. latissima* was carried out from individuals of wild populations of *S. latissima* near the island of Frøya (63°42′15″N, 8°52′40″E), by Seaweed Energy Solutions AS (Trondheim, Norway). The induced sori of *S. latissima* were used for the release of zoospores. Young sporophytes were seeded onto ‘spools’ (plastic tubes covered with 60 m of polyester silk string), and incubated for 8 weeks in a 200 liter tank with continuous water flow, under a 16:8 light:dark (L:D) photoperiod. The culture was maintained at 8–9 °C and the light intensity was 30–60 μmol/m^2/s [16]. The young seedlings were transported to Frøya and placed temporarily in the sea at a pier for 2–3 days before being deployed. Frøya has a mild maritime climate with the driest season in May–June and coldest season from January–March. On average the highest tide at Frøya region is 3 m and lowest is 0.5 m. According to a study by Tønder et al. [16], the water salinity and temperature at Frøya over the period of June–August 2013 was 32–34 ppt and 12–14 °C, respectively. On February 16th 2015, the seedlings of *S. latissima* were deployed 3 and 8 m below sea level on 0.5 m^2 plastic frames (see supplementary data Fig. S1), and monitored after 84, 134 and 183 days (May, June and August 2015). Cultivation was carried out in triplicates with a total of 18 frames. In each frame, 20 plants were attached with a distance of 5 cm apart and suspended from a horizontal rope at the surface and down to 3 or 8 m and anchored on the same line. Possible shading of the 3 m frame on the 8 m frame was limited due to the relatively low angle of sun at the cultivation site and scattering of light in the water.

The growth of cultivated *S. latissima* was recorded on every monitoring date. This was done by measuring the total length of 5 random individuals from each replicate. Thallus length was measured from tips of blade to holdfast. Biomass measurement was carried out by weighing the total weight of each frame with cultivated *S. latissima* and subtracting the weight of an unseeded control frame. The weight of each frame was measured with a spring scale and a digital fish scale [16]. For chemical composition analysis of *S. latissima*, 5 random individuals from each replicate frames were arbitrarily sampled.

2.2. *S. latissima* sample preparation

Samples collected in the field on each monitoring date were immediately frozen once back on the land. The samples constituted the whole plant including blade, stipe and holdfast. The frozen unwashed *S. latissima* samples were later thawed and oven-dried at 50 °C until they reached equilibrium moisture. Samples were homogenized using a MF 10 basic micro-fine electric grinder (IKA, USA), shipped to the Norwegian University of Life Science (Ås, Norway) and stored in a desiccator until chemical analyses were performed. Epibionts were not removed from the samples.

2.3. Carbohydrate analyses

To determine the sugar composition of *S. latissima* samples, monomeric sugars were released by a modified two step acid hydrolysis. Dried ground samples and sugar recovery standards (SRS) were subjected to 72% (w/w) H_2SO_4 at 30 °C for exactly 60 min and then 4% (w/w) H_2SO_4 at 121 °C in an autoclave for 40 min [1]. After hydrolysis, the monosaccharide hydrolysates were filtered through ROBU glassfilter (16–40 μm, ROBU, Germany) and diluted with deionized water as per their standards' concentration range for HPLC and total uronic acid analysis. The released monomeric sugars and SRS were analyzed by a HPLC system equipped with refractive index detector. The separation column was a 300 × 7.8 mm Rezex ROA-Organic Acid H+ fitted with cation-H cartridge guard column. The column temperature was 65 °C with 5 mM H_2SO_4 as the mobile phase at a flow rate of 0.6 ml min^{-1}. Identification and quantification of sugars were carried out against external sugar standard curves (glucose, mannitol, fucose, and xylose), which were of analytical grade procured from Sigma Aldrich (USA). The same hydrolysates were used for quantification of total uronic acids (guluronic and mannuronic acids) by a spectrophotometrical method using absorbance at 550 nm and with carbazole as an indicator. The samples were filtered and diluted prior to the spectrophotometric analysis. The total content of uronic acids was measured as a galacturonic acid (GalA) equivalents. GalA was also used as recovery standard. All necessary chemicals (sulfuric acid, galacturonic acid, sodium borate, and carbazole) were procured from Merck (Germany).

2.4. Proximate, element and mineral analyses

The dry matter content of the samples was determined by a Metrohm Karl Fischer titrator (Florida, USA). Ash content was determined by incineration of the samples in a muffle furnace at 550 °C for 8 h. The content of C, H, and N was determined by elemental analysis (LECO, CHN-1000, USA). For metal analysis (cations) the samples were hydrolyzed with concentrated 65% HNO_3 in a high performance microwave reactor (UltraClave, MLS Milestone, Italy). For halides (anions) analysis the samples were digested with concentrated TMAH (Tetramethylammonium hydroxide). Both cations and anions were analyzed by inductively coupled plasma spectrometry coupled to a mass spectrometric detector (ICP-MS) (Perkin-Elmer, USA).

2.5. Amino acid composition analysis

Amino acid (AA) analysis of the *S. latissima* samples was performed according to Commission dir. No 152/2009/EC on a Biochrom 30 Amino Acid Analyzer (Oxidised Protein Hydrolysate System; Biochrom Ltd., UK) [17]. Tryptophan was analyzed on a Dionex UltiMate 3000 HPLC system (Dionex Softron GmbH, Germering, Germany) connected to a Shimadzu RF-535 fluorescence detector (Shimadzu Corporation, Kyoto, Japan). Both amino acids and tryptophan data were analyzed against external standards curves (amino acid standard solutions; Sigma Chemical, St. Louis, Mo., U.S.A.) using the Chromeleon® Chromatography Management Software (Dionex Ltd., Surrey, UK).

2.6. Total phenolic content

Total phenolic content of the *S. latissima* samples were determined using the Folin-Ciocalteu assay [18]. 100 mg of dried *S. latissima* samples were first extracted with 1 ml of 50% methanol (*v*/v) in the dark at ambient temperature for 15 h. Then the reaction mixture containing 100 μl of extracted sample, 500 μl of Folin-Ciocalteu reagent, 1500 μl of 20% Na_2CO_3 and 6000 μl of deionized water was kept for 2 h in the dark. The absorbance at 765 nm was measured using gallic acid as an external standard, and the results are represented as g GAE kg^{-1}(gallic acid equivalents).

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