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Production of monosaccharides and bio-active compounds derived from marine polysaccharides using subcritical water hydrolysis

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ABSTRACT

Polysaccharides are the major components of brown seaweed, accounting for approximately 40–65% of the total mass. The majority of the brown seaweed polysaccharides consists of alginate (40% of dry matter), a linear hetero-polysaccharides commonly developed in fields. However, depolymerisation of alginate is required to recover high-value compounds. In this report, depolymerisation was performed using subcritical water hydrolysis (SWH) at 180–260 °C, with a ratio of material to water of 1:25 (w/v) and 1% formic acid as a catalyst. Sugar recovery was higher at low temperatures in the presence of catalyst. The antioxidant properties of *Saccharina japonica* showed the best activity at 180 °C in the presence of a catalyst. The mass spectra produced using MALDI-TOF showed that polysaccharides and oligosaccharides were produced during hydrothermal treatment. Hydrolysis treatment at 180 °C in the presence of a catalyst may be useful for modifying the structure of *S. japonica* and purified alginate.

1. Introduction

Seaweed (also known as marine macroalgae) is a heterogeneous assemblage of organisms with a long fossil history, contributing to 85% of the total global production of aquatic plants. Seaweed can be classified into three groups based on pigmentation: green seaweed (Chlorophyta), red seaweed (Rhodophyta), and brown seaweed (Phaeophyta). Seaweeds, multicellular algae rich in minerals and vitamins, are found in marine waters. Seaweed is an important component of food, feed, and medicine, and is considered a high-value marine plant because of its biotechnological properties. The seaweed species most commonly used for its bioactive compounds is brown seaweed. The major constituents of brown seaweed include carbohydrates (45–55%), proteins (10–20%) and lipids (1–10%) (Kumar, Ganesan, Suresh, & Bhaskar, 2008).

Brown seaweed (Phaeophyta) is one of the most abundant seaweed groups of economic importance. The major constituent of brown seaweed is polysaccharide. Alginate or alginic acid is an unbranched hetero-polysaccharide consisting of two hexuronic acids, β-D-mannuronic acid (ManA or M) and α-L-guluronic acid (GulA or G), which are linked by 1–4 bonds. The physical, chemical, and structural properties of alginic acid (e.g., viscosity, solubility, interaction with metal cations) are directly attributable to the molecular weight and guluronic/mannuronic (G/M) ratio, both of which show important variations among species (Clementi, Crudele, Parente, Mancini, & Moresi, 1999). The biochemical and biophysical properties of alginate are also dependent on the molecular weight and G/M ratio. Alginate has been used in the food industry as gelatinisers and thickeners, as wound coatings and antifouling agents, for gastric parietal protection in various industries (e.g., food and pharmaceuticals), and for the biosorption of heavy metals (Lodeiro et al., 2005; Renn, 1997). Alginate has a molecular weight between 32 and 200 kDa, and the G/M ratio varies by species, age, portion of the plant, and distance from the shore. For applications using alginate, depolymerisation to adjust the molecular weight is required.

The decomposition of carbohydrate into a reducing sugar, monosaccharides, and polysaccharides offers economic value. Many studies have been performed to convert carbohydrates from marine macroalgae into monosaccharides, polysaccharides, and other compounds. Alnaief, Alzaitoun, García-González, and Smirnova (2011) proposed using alginate as a nanoporous biodegradable material, and Fitton, Irhimeh, and Falk (2007) produced marine cosmetics using fucoidan fractions and phloroglucinol. Glucose, mannose, and galactose in brown seaweed belong to the

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reducing sugar group, which contain essential compounds that can be converted into valuable intermediate products.

Alginate depolymerisation methods have been widely investigated. Several methods have been used to adjust the molecular weight and G/M ratio, including acid hydrolysis (Haug, Myklestad, Larsen, & Smidsrod, 1967), base hydrolysis (Rigouin, Delbarre, Sinquin, Colliec-Jouault, & Dion, 2009), enzymatic depolymerisation (Kim et al., 2012), photolytic depolymerisation (Burana-Osot et al., 2009), and hydrothermal conditions (Aida, Yamagata, Watanabe, & Smith, 2010). Traditional methods use organic solvents such as ethanol, methanol, or hexane during the extraction process (Demirel, Yilmaz-Koz, Karabay-Yavagoslu, Ozdemir, & Sukatar, 2009; Osman, Abushady, & Elshobary, 2010). Although these methods are useful, they have several disadvantages, including the use of harsh chemicals, the need for stable environmental conditions during enzymatic depolymerisation, long reaction times, and high experimental costs. To address these disadvantages, hydrothermal conditions with subcritical water are being used to break down the complex polysaccharide alginate in brown seaweed. Subcritical water is liquid water under pressure at temperatures between the usual boiling point (100 °C) and the critical temperature (374 °C), also known as superheated water and pressurised hot water.

2. Materials and methods

2.1. Materials

The brown seaweed *Saccharina japonica* was collected from Guemil-eup, Wando-gun, Jeollanam-do, South Korea. Purified alginate from brown algae was provided by Sigma Aldrich (United Kingdom). High-purity nitrogen gas (99.99%) was supplied by KOSEM (Yangsan, Republic of Korea). Standards of 2,2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid, catechin, D-gulose, and L-mannose were purchased from Sigma Aldrich Chemical Co. (St. Louis, Mo. USA). Distilled water was used in these experiments. All reagents used in this study were ofanalytical or high performance liquid chromatography (HPLC) grade and obtained from Sigma Aldrich Chemical Co.

2.2. Sample preparation

After washing fresh *S. japonica* samples with fresh water, unused materials, attached salt, and minerals were removed, and the samples were cut into small pieces. The pieces were dried at −80 °C for 3 days in a freeze dryer (Eyela FDU-2100, Tokyo Rikakikai Co., LTD, Japan) equipped with a square-type drying chamber (Eyela DRC-1000, Tokyo Rikakikai Co., LTD, Japan). The dried samples were collected into sealed plastic bags. The samples were then finely ground using a mechanical blender (PN SMKA-4000 mixer) and sieved through a 710-μm stainless steel sieving mesh.

2.3. Subcritical water hydrolysis

Subcritical water hydrolysis was performed in a 200-cm^3 batch reactor made of 276 Hastelloy with temperature control (Meillisa, Chun, & Woo, 2012). A total of 6 g of material samples were loaded into the reactor. Formic acid (1%), which is used as a catalyst, was suspended separately in 150 ml of distilled water. The reactor was then closed and heated using an electric heater to the required temperature (180–260 °C). Pressures were estimated based on saturated steam to be between 15 and 65 bar for the temperature range studied. The temperature and pressure in the reactor were controlled using a temperature controller and pressure gauge, respectively. The sample was stirred using a four-blade stirrer at 140 rpm. The time to reach the desired temperature was 30–75 min. The hydrolysate samples from the reactor were collected and filtered using Whatman nylon membrane filter (0.45 μm) and stored at 4 °C.

2.4. Total glucose

Total glucose measurements were performed using the phenol sulphuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) with minor modifications. Briefly, hydrolysate samples (0.75 ml) were mixed with 2.25 ml of concentrated sulphuric acid. Then, 0.45 ml of 40% phenol was added and the mixture heated in a water bath. The mixture was cooled at room temperature and its absorbance at 490 nm measured using a spectrophotometer (Shimadzu 1240 UV–Vis spectrophotometer). D-Glucose was used as a standard to generate a calibration curve. Each hydrolysate was analysed in triplicate, and the results are expressed in milligrams per litre (mg/l).

2.5. Reducing sugar

Reducing sugar analysis was conducted using the 3,5-dinitrosalicylic (DNS) acid method (Miller, 1959). The reagent solution was prepared by mixing 10 g of sodium hydroxide and 700 ml of water until the mixture completely dissolved. Then, 300 g of potassium sodium tartrate was added to the mixture followed by 10 g of 3,5-dinitrosalicylic (DNS) acid. After all of the components were fully dissolved, 0.5 g sodium sulfite was added followed by 2 g of phenol. The volume of the mixture was adjusted to 1 L and protected from light. Reducing sugar analysis was performed by mixing 0.5 ml of hydrolysate water and 0.5 ml of the reagent solution, heating the mixture for 10 min, and immediately adding 5 ml of cold water. Finally, the absorbance at 540 nm was measured.

2.6. HPLC analysis

Gulose and mannose were quantified using high performance liquid chromatography (HPLC) with an evaporative light scattering detector (ELSD). HPLC analysis was performed using a Jasco HPLC (Easton, USA) model 400 equipped with ChromNav analysis software. High-purity nitrogen (99.99%) from KOSEM Co. was used as a carrier gas. A Shodex (Japan) SUGAR column (SP0810) of 300 mm with 8 mm i.d., thermostated to 80 °C, was used to analyse gulose and mannose compounds. Hydrolysate samples were diluted fourfold using filtered and sonicated water (HPLC grade). The water used for elution was filtered using a Whatman nylon membrane filter (0.45 μm) and sonicated. The flow rate of the eluent was maintained at 0.6 ml/min. Gulose and mannose standards (purity > 98%) were purchased from Sigma Aldrich (United Kingdom).

2.7. Matrix assisted laser desorption/ionisation-time of flight (MALDI-TOF) analysis

The mass spectra of the hydrolysate solutions were obtained using the MALDI-TOF Bruker Autoflex-III, as described by Aida et al. (2010) with minor modifications. Briefly, the device was equipped with a smart beam laser and the spectra were collected with an accelerating voltage of ±19.8 kV, according to the polarity of the recording mode. The measurement was performed using 2,5 dihydroxybenzoic acid (DHBA) as a matrix and acetonitrile:water (75:25) as a solvent. Samples were prepared by mixing 0.5 μl of the product and 0.5 μl of the matrix solution. The matrix solution was prepared by mixing a norharmane–acetonitrile solution and trifluoroacetic acid at a ratio of 7:3. The norharmane–acetonitrile solution was prepared by dissolving norharmane (10 mg) in acetonitrile (1 ml).