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Production of Reducing Sugars from *Laminaria japonica* by Subcritical Water Hydrolysis

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Abstract

This study was to investigate the production of reducing sugars in hydrolysates from raw and deoiled *Laminaria japonica* produced by subcritical water hydrolysis. Deoiled *Laminaria japonica* was collected by supercritical carbon dioxide (SCO_2) extraction process. Experiments were performed in a batch-type reactor with stirring. It investigated that the effects of reaction temperature and acetic acid as catalyst on content of reducing sugar production. The addition of acetic acid led to an increase in content of reducing sugar. But Removal of oil in *Laminaria japonica* by SCO_2 and increasing of temperature led to decrease in content of reducing sugar production. The highest content of reducing sugar was 814.10 mg/100 g raw dried sample at 200°C, adding of 1% acetic acid as catalyst

Keywords: Subcritical water hydrolysis; *Laminaria japonica*; reducing sugar; Superctitical carbon dioxide; HPLC

1. Introduction

Seaweeds have been used as human foods, cosmetics, fertilizers and a source of chemicals for medicine and industry. Seaweeds are also gaining increasing interest as a feedstock for sustainable bio-fuels production [1].

Brown seaweeds are the most common edible seaweeds in Korea and Japan. Their carbohydrates vary

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strongly between algae species. Typical carbohydrates in brown algae varieties consist of fucoidan, laminaran (β-1.3-glucan), cellulose, mannitol and alginates. *Laminaria japonica* that has many nutritional value and many health functions is a sort of brown seaweeds [2].

Main reserve polysaccharides of brown seaweed such as laminaran are built up by long chains of sugar unit. After pretreatment and hydrolysis, they can be converted into intermediate products which can be transformed into bio-fuels or other industrially important products [3].

Because of the importance of brown seaweeds as feedstock for industrially important products, there is great interest in developing methods for the production of fuel and chemicals that offer economic, environmental and strategic advantages [4].

Subcritical water has been gaining attention as both an environmentally friendly solvent and attractive reaction medium for a variety of applications. It is cheap, non-toxic, non-flammable, and non-explosive and offers essential advantages compared to other substances, partially in the field of "green chemistry". Its distinctly different behavior compared to water at ambient conditions is due to the dramatic changes in physical properties, namely dielectric strength and ionic product, which in turn can easily be altered by changing temperature and pressure [5]. The ion product or dissociation constant is about three orders magnitude higher near critical point than it is for ambient liquid water. This fact makes subcritical water an ideal reaction medium for the hydrolysis of organic compounds [6] and different polymers [7].

In the present study, subcritical water hydrolysis was employed as a method for producing reducing sugars as valuable substances from *Laminaria japonica*. The objectives of this study were to examine the effects of hydrolysis conditions such as temperature, addition of acetic acid on contents of total reducing sugar production. The effect according to existence of oil in sample for reducing sugars production was also investigated. The results of this study would lead to the development of a value-added product from *Laminaria japonica*.

2. Materials and methods

2.1. Materials

Laminaria japonica, used as substrate, was collected from Guemil-eup, Wando-gun, Jeonnam, South Korea. The carbon dioxide (99.99%) was supplied by KOSEM, Korea. All solvents and reagents were of analytical grade. Before the experiments, fresh *Laminaria japonica* samples were thoroughly washed with water. The cleaned samples were frozen and then dried in a freeze-drier for 3 days. The dried samples were ground in a mill to pass through a mesh for sieve (700 μm) and then stored at -20^{o}C.

2.2. Subcritical water hydrolysis

The subcritical water hydrolysis was carried out in 200 cm^3 of a batch reactor made of 276 Hastelloy with temperature control. Fig 1 shows a schematic diagram of the hydrolysis apparatus. 3 g of freeze dried samples were suspended separately in 150 ml of distilled water and charged into reactor. The reactor was then closed and heated by an electric heater to the desired temperature. The temperature and pressure in reactor of each experiment were measured by temperature controller and pressure gauge, respectively. The sample was stirred by stirrer at 150 rpm. In the experiment, reaction temperatures ranged from 200 to 280^{o}C. The reaction pressures ranged from 1.3 to 6.0 MPa. Reactions were carried out from 28 to 42 min. 1% acetic acid as catalyst was used. After rapid cooling, the hydrolyzed samples from reactor were collected and filtered.

2.3. Removal of oil in Laminaria japonica *by* SCO_2 *extraction*

In order to investigate the effect according to existence of oil in sample for reducing sugars production, a laboratory scale of SCO_2 extraction unit was used to remove oil in sample. Fig 2 shows a flow diagram of the SCO_2 extraction apparatus. *Laminaria japonica* sample (250 g) was packed into a stainless steel extraction vessel which was 500 ml in volume. Before plugging with a cap, another layer of cotton was used at bottom of the extraction vessel. CO_2 was pumped at constant pressure into the extraction vessel by high pressure pump up to the desired pressure which was regulated by a back pressure regulator. The experiment was performed at temperature ranges of 35 to 55^{o}C and pressures from 15 to 25 MPa for 2 h. The flow rate of CO_2 was kept constant at 26.81 g/min for all extraction conditions and CO_2 volume passing through the apparatus were measured using a dry gas meter.

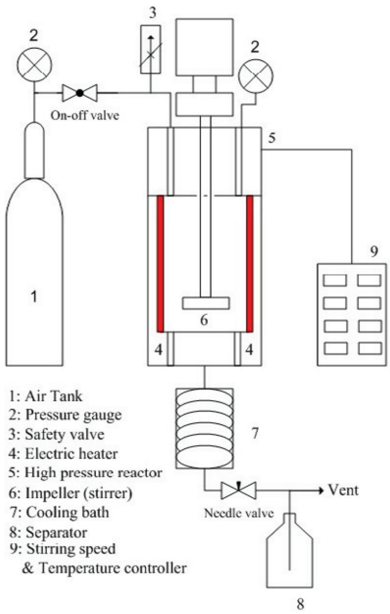

Fig. 1. Schematic diagram of subcritical water hydrolysis experimental apparatus

2.4. HPLC analysis for reducing sugars in hydrolysate

Measurement of reducing sugars content was carried out using a High performance liquid chromatography (HPLC). The HPLC equipment consisted of a Shimadzu Model LC-20AD HPLC pump, a Shimadzu Model Sil-20AC auto-sampler, a Shimadzu Model CTO-20AC Oven and CBM-20A system controller. The columns used for analysis were an ion exchange Shim-pack ISA-07 analytical column (4.0 mm I.D. x 250 mm L.) and Shim-pack ISA guard column (4.0 mm I.D. x 50 mm). The HPLC was operated at an oven temperature of 65^{o}C with 0.6 ml/min flow of solution A (potassium borate, pH 8) and solution B (potassium borate, pH 9) for 90 min as liner gradient elution method. The 20 μl of hydrolysate filtered by membrane filter (0.45 μm) was injected. After separation of sacchariedes by the column, arginine/boric acid reagent solution is continuously added to the column eluent to convert the saccharides to fluorescent derivatives for detection [8]. The detector used was RF-10Axl fluorescence detector set at 320 nm (Ex) and 430 nm (Em). The content of total reducing sugars was calculated based on a standard curve with reducing sugar standard material.

3. Results and Discussions

3.1. Removal of oil in sample by SCO_2

The highest removal yield of oil by SCO_2 extraction process from *Laminaria japonica* was 2.59 g/250 g at temperature, 55^{o}C and pressure, 25 MPa. At constant temperature, the amount of oil extracted from *Laminaria japonica* was increased with the pressure. Deoiled sample obtained by this experimental condition was used for experiment of subcritical water hydrolysis. The effect of pressure can be attributed to the increase in solvent power and by the rise of intermolecular physical interactions [9]. Similar results were found in the extraction of oil from green coffee [10] and boiled anchovy [11].

3.2. Total reducing sugar content in hydrolysate

The nine of reducing sugars were determined in hydrolysate by HPLC. Carbohydrate which reacts with hydronium and hydroxide ions produces reducing sugars. Total reducing sugars contents in each hydrolysates were shown Fig. 3 and 4. From Fig. 3 and 4, the amount of reducing sugar in both raw and deoiled *Laminaria japonica* tend to decreased with increasing temperature. Due to elevated temperature and a relatively long reaction time (28-42 min), it was considered that the reducing sugars produced may further decompose to other products with extension of reaction time. Similar results on effect of reaction time were found in subcritical water hydrolysis of bean dregs waste [4]. The addition of 1% acetic acid led to increase the content of reducing sugar in both raw and deoiled *Laminaria japonica*. The reducing sugar content in hydrolysate of raw *Laminaria japonica* was higher than that of deoiled *Laminaria japonica* hydrolysate. It can be observed that the highest content of total reducing sugar in hydrolysate was 814.10 mg/ 100 g in raw sample at 200^{o}C, addition of 1% acetic acid.

4. Conclusion

This study focused on the production of reducing sugars from *Laminaria japonica* using subcritical water hydrolysis. The raw and deoiled *Laminaria japonica* was treated in a bath reactor under different reaction conditions. It is feasible to produce reducing sugars from *Laminaria japonica* using subcritical water hydrolysis. It was observed that the production of reducing sugars and decomposition of reducing sugar depends primarily on reaction temperature and reaction time. The addition of 1% acetic acid led to increase in reducing sugar content of hydrolysate. But removal of oil in *Laminaria japonica* led to decrease in reducing sugar content in it. The highest content of reducing sugar in *Laminaria japonica* was 814.10 mg/100g in raw sample at 200^{o}C, addition of 1% acetic acid. This method may provide a practice and economical solution for the development of a value-added product from *Laminaria japonica*.

Acknowledgements

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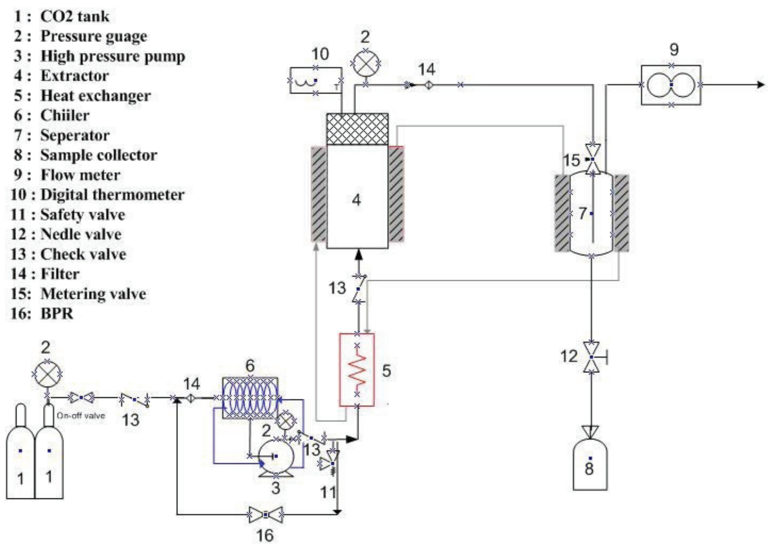

Fig. 2. Flow diagram of supercritical carbon dioxide extraction apparatus

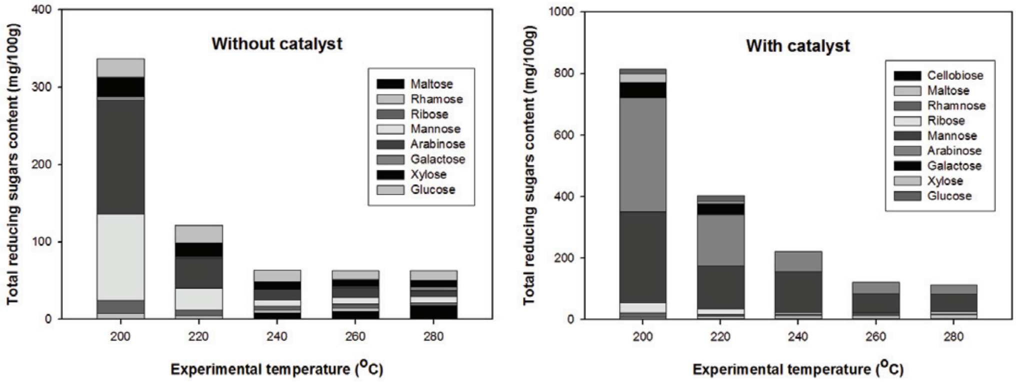

Fig. 3. Total reducing sugar content by subcritical water hydrolysis of raw *Laminaria japonica* at different temperatures (a) Without catalyst (b) with catalyst.

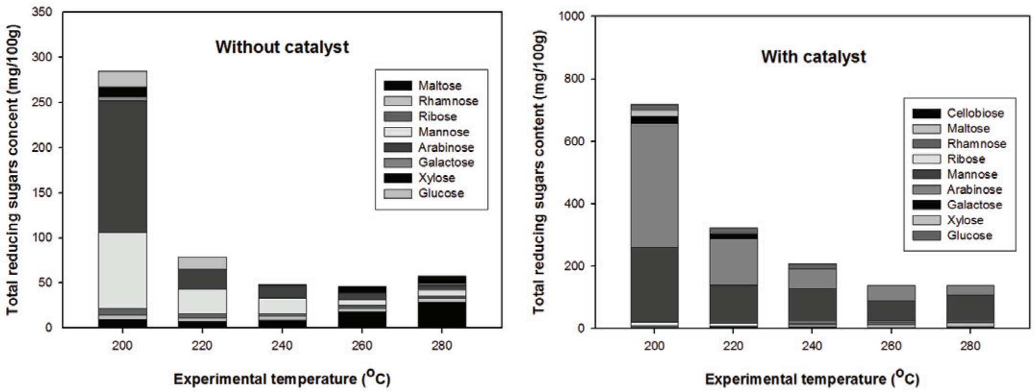

Fig. 4. Total reducing sugar content by subcritical water hydrolysis of deoiled *Laminaria japonica* at different temperatures (a) Without catalyst (b) with catalyst.