Enhancing Pressurized Water Extraction of β -glucan from Barley Grain by Adding CO₂ under Hydrothermal Conditions

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Abstract

Subcritical water extraction is one of the green ways to isolate plants biomass components. In this work, it has been applied as a media to extract β -glucan from barley (*Hordeum vulgare* L.). The extractions were carried out at temperatures of 130–170 °C and a pressures of 10–40 MPa, using a semi-batch system. UV–vis spectra of liquid fraction products showed a high content of hemicelluloses, including glucan compounds. Results of FT–IR spectra analysis showed that β -glucan has been extracted from barley grains after subcritical water treatment. The elemental analysis showed that dehydration and decarboxylation occurred during subcritical water extraction of barley grains at these conditions. The gel permeation chromatography (GPC) analysis showed that the average molecular weight of water-soluble products were approximately 500 Da to 1167 kDa. To improve the extraction process, the CO₂ was added. At the same extraction time, the yield of β -glucan in the liquid fraction products increased clearly with the presence of CO₂.

Keywords: Barley; β -glucan; Extraction; Subcritical water; Autohydrolysis.

1 Introduction

Generally, cereal grains contain nutrients such as protein, fat, carbohydrate that are needed by humans for growth and survive. Barley (*Hordeum vulgare* L.) is one of grown cereal grains in the world, currently ranking fourth in world cultivation and utilization after maize, rice, and wheat [1,2]. As food, feed, and raw material for processing, the quality of the barley grains is determined by the structure and composition of the mature grain. Carbohydrates, as one of main components of barley grains, have been reported to account for 78% - 83% of the mature barley grain [3]. Hence, many studies of barley focus on the mature grain and within this on the major groups of components that determine end use properties: starch, proteins, and cell wall polysaccharides [3,4].

The main components of cell wall polysaccharides in barley grains were β -glucan, cellulose, and arabinoxylans [3]. β -glucan is linear homopolymers of D-glucopyranosyl residues linked mostly via two or three consecutive β -(1-4)-linkages that are separated by a single β -(1-3)-linkage. It is also the most abundant component of the soluble dietary fiber fraction in barley [5]. This has led to the wide acceptance of barley as human food with significant human health benefits [1]. β -glucan is considered a functional food, as it not only provides desirable quality attributes such as texture and mouthfeel, but it also confers specific health benefits to the consumer [6]. Furthermore, from a nutritional health benefit viewpoint, barley β -glucan has potential roles in reducing blood cholesterol and producing a flattened blood glucose response, and may also contribute to reduced incidences of colon cancer and obesity. Several researchers reported that β -glucan has been implicated in lowering plasma cholesterol, improving lipid metabolism, and reducing glycaemic index [7,8].

In this work, barley grains, as an excellent source of β -glucan, were used as a feed material to extract glucan compounds under subcritical water conditions (temperatures ranging from 130 to 170 °C, and a pressure of 10 MPa) in semi batch process. Extraction is

one of the step processes for the recovery and isolation of bioactive phytochemicals from plant materials, before analysis. The process is affected by their chemical nature, the extraction method employed, sample particle size, as well as the presence of interfering substances. As an extraction media, when the temperature of water increases above boiling point (between 100 and 374 °C) but pressure is adjusted to maintain water in its liquid form, the physical and chemical properties of water change quite dramatically. Under these conditions, known as subcritical water, water behaves like an organic solvent that can dissolve a wide range of analytes of medium to low polarity [9]. Subcritical water conditions are known as a "natural and green" way for product extraction via autohydrolysis, has received increased attention as an important alternative to conventional separation methods, such as hot water extraction conducted at boiling point temperature and atmospheric pressure. In this process, the addition and recovery of chemicals different from water were not needed; the drying of feedstock is not required and extraction can therefore be conducted at the high moisture content typical for biomass feedstock [10].

This technique has been applied to recover water-soluble compounds from plant biomass, including barley [11–14]. Leppanen et al. [15] reported that the most promising temperature range for isolating hemicelluloses with high molecular masses was 180–190 °C; within this range, a high yield of extracted polysaccharides could be obtained without extensive degradation. Vila et al. [12] demonstrated that autohydrolysis, at temperatures of 160–220 °C, led to hemicellulose degradation products (low molecular weight polysaccharides, oligosaccharides, monosaccharides, and furans). Following this process, low molecular weight polysaccharides and oligosaccharides can be hydrolyzed to sugars and acetic acid as final products. Benito-Roman et al. [16] extracted glucan compounds from different cereals in a fixed-bed extractor, using subcritical water as a solvent, a temperature range of 110–175 °C and at a pressure of 20 bar. In order to enhance the hydrolysis rate, the

properties of water at subcritical conditions were improved by adding carbon dioxide (CO₂) since it reacts to carbonic acid, which serves as a catalyst [17–24]. Hunter and Savage [17] demonstrated the feasibility of using CO₂–water mixtures at 300 °C as an environmentally benign reaction medium for acid catalysis to form cyclohexene from cyclohexanol via dehydration reaction. In addition, CO₂ can also easily be separated from the extraction system by depressurizing it to ambient conditions.

2 Material and Methods

2.1 Materials

Dried barley grain was obtained from Asahi Group Holdings, Ltd. (Tokyo, Japan). Grain was milled (using a millser; IFM–800, Iwatani, Japan) to achieve a particle size of <2 mm, then passed through 16-mesh sieves before storage by refrigeration at <5 °C. Water was distilled, using a distillation apparatus (model PW–16, Shibata Co., Japan), prior to use as a solvent. The pH of water was around 6.8–7.1 (pH/ion meter F-53, Horiba, Ltd., Japan). The following chemicals were obtained from a commercial supplier (Wako Pure Chemicals Industries Ltd., Japan) and were used without further purification: potassium hydroxide (KOH; 85% purity); sodium hydroxide (NaOH; 97%); hydrochloric acid (HCl; 35–37%); acetic acid (CH₃COOH; 99.9%); ammonium acetate (CH₃COONH₄; 97.0%); and methanol (99.7%; for use as an analytic reagent). CO₂ was purchased from Sogo Kariya Sanso, Inc. Japan

2.2 Experimental Setup and Procedure

The experiment apparatus (Fig. 1) consisted of: two high-pressure pumps (LC–6AD Shimadzu, Japan; Isco syringe pump 260D, Teledyne Isco, USA); chiller (CCA-1111, EYELA, Japan); solvent mixer (Swagelok, USA); electric water heater (ESPEC ST-110, Japan); reactor (10 mL in volume; Thar Design Inc., USA); and back-pressure regulators (BPRs; AKICO,

Japan). The reactor was equipped on both sides with removable threaded covers; these included stainless steel filters (0.1–1.0 μm). The pre-heater was fabricated from $\frac{1}{2}$ -inch diameter stainless steel tubing (SUS316) with a volume of 5 mL. A 1/16-inch diameter stainless steel tube was used to introduce hot water from the pre-heater to the reactor (located in the heater). The reactor was loaded with 3.5 g of barley and installed in the system, following which, distilled water at room temperature (25–26 °C) was pumped through the reactor-inclusive pre-heater for a few minutes to purge air from the system and saturate the barley. The system was pressurized to 40 MPa through the BPRs and monitored using a pressure gauge (P, Migishita, Japan). When the system reached the desired pressure and a steady state was achieved, the electric heater was applied to heat the water. The reactor temperature was maintained at 130–170 °C. Temperatures of the pre-heater, reactor, and electric heater were measured using K-type thermocouples and monitored using a temperature controller (OMRON E5CJ, Japan). The time required to heat the reactor from room temperature to the desired temperature (the reactor temperature) was 5–8 min at which point, water was pumped into the system at a rate of 1.0 mL min⁻¹. At the same time, CO₂ was also applied into the system with the same flow rate. The duration of the experiment was 3 hours, which at the given water feed rate, produced a collected extract volume of 180 mL. Solid residues and liquid extracts were transferred into petri dishes and sealed bottles, respectively. Solid residues were dried in an oven at 60 °C for 24 hours then stored in a desiccator at room temperature. Extracted solution was collected every 30 minutes and they were directly stored in a refrigerator until further analysis. Each experiment was conducted in duplicates/triplicates and cited as mean $\pm$ standard deviation.

2.3 Analytical Methods

The water soluble compounds content in extracts was analyzed using UV–vis

spectrophotometry V-550 (Jasco Corporation, Japan). Spectra were recorded with a PC-driven scanning spectrophotometer, operating in fast scan mode, which detected spectra between 190 and 800 nm, at a rate of 10 nm min⁻¹ of bands. The molecular weights of the water soluble compounds extracted were determined by gel permeation chromatography (GPC), in combination a high-performance liquid chromatography apparatus (JASCO International, Co. Ltd., Japan) equipped with tosoh column (TSKgel G6000PWXL, 300 mm × 8 mm, Japan) and a model evaporative light scattering (ELS-2040) detector. The operation conditions were mobile phase: 0.05 M ammonium acetate; flow rate: 0.5 mL/min; column temperature: 50 °C; injection volume: 20 µL; running time: 60 min. The calibration curve for molecular weight determination was made using a series of β-glucan molecular weight standards (Megazyme International Ireland Ltd., Wicklow, Ireland). The temperature of the ELSD system was kept at 60 °C and the flow-rate of the nebulising nitrogen at 1.2 sLm (Standard Liter per Minute). The temperatures of nebulizer and exhaust were set at 85 and 90 °C, respectively. These temperatures setting were selected in order to evaporate small amounts of water in the mobile phase and possible droplets of non-retained (aqueous) stationary phase. GPC calculation program (JASCO ChromNAV) was used for the calculation of weight-average molecular. Solid residues collected at each operating temperature were analyzed using a Spectrum Two FT-IR spectrophotometer (Perkin-Elmer Ltd., England), in order to determine their structure following subcritical water treatment. Spectra were measured in attenuated total reflectance (ATR) mode (golden single reflection ATR system, P/N 10500 series, Specac) at 4 cm⁻¹ resolution. The scanning wave number ranged from 4000 to 650 cm⁻¹. Elemental analysis of solid residue was conducted using a CHN analyzer (Yanaco, CORDER MT-6). This type of analysis is especially useful for organic compounds; that is, those compounds containing carbon-carbon bonds. The morphologies of barley grains before and after treatment by subcritical water were observed by using a scanning electron

microscope (SEM; JEOL JSM–6390LV).

3 Results and Discussion

3.1 Products identification

Under subcritical water conditions, the cellular structure of plants could be disrupted, releasing their components, which allowed to dissolve in the hot liquid water. The releasing their components may react during the extraction process, producing other compounds with different structures and chemical properties compared to the native compounds. Fig. 2 showed the liquid products collected at the end of each experiment which carried out at 130 and 170 °C with and without adding CO₂. At 0-30 min, the color of liquid products were slightly yellow. With increasing extraction time (30-60 min), they changed to dark yellow. After 90 min extraction time, the color of liquid products gradually became transparent. The change of liquid products color indicated that the content of extract components in liquid products change as a function extraction time and temperatures. Physical appearance, the increasing pressure and the presence of CO₂ in extraction system did not affect significantly on the color of liquid products. Changes in the color of the extracts might be due to the fragmentation of barley grains components during the extraction process via the Maillard reaction or caramelization reaction [18,25,26]. Moreschi et al. [18] conducted ginger bagasse extraction via hydrolysis, using subcritical water at approximately 170 °C, in order to reduce sugars and other low molecular mass substances; they reported that reaction products obtained for the static period or reaction time of 15 min had a yellow-brown color with a mixed caramel and coffee aroma; this may describe the Maillard reaction. Plaza et al. [26] informed that chemical reactions such as Maillard and caramelization reactions involving major components found in plants might be favored under pressurized hot water extraction at elevated temperatures. Toor et al. [25] proved that during hydrolysis of biomass, the Maillard reaction may occur between

nitrogen compounds and sugars, which are formed simultaneously.

To understand the barley grains components which extracted by subcritical water treatment with and without CO₂ addition, the liquid products were analyzed by using UV-vis spectrophotometry at 280 nm. Fig. 3 showed UV-vis spectra of water-soluble extraction products when the extraction processes were carried out at 170 °C with and without CO₂ addition. UV-vis spectrophotometry commonly was used to analyze the solution and hydrolysis of all other plant biomass constituents. The water-soluble polysaccharides strongly absorb at 280 nm. At the same wavelength, aldehyde groups (such as furfural and hydroxymethylfurfural) and lignin and its derived compounds were also detected well [27,28], therefore, the potential interference from these compounds might be occurred during analysis. Such as general plants biomass, the most abundant carbohydrates in barley grains are the polysaccharides cellulose and hemicellulose. Cellulose, the major cell wall structural component in plants, is an unbranched linear chain of several thousand glucose units with β -D-(1 $\rightarrow$ 4)-glucosidic linkages. Hemicelluloses are polysaccharides in plant cell walls that have β -(1 $\rightarrow$ 4)-linked backbones with an equatorial configuration. Hemicelluloses include xyloglucans, xylans, mannans and glucomannans, and β -(1 $\rightarrow$ 3,1 $\rightarrow$ 4)-glucans [29]. Under subcritical water conditions, carbohydrates undergo rapid hydrolysis to form glucose and other saccharides, which are then further degraded. The rate of hydrolysis varies between different carbohydrates. Hemicellulose is the most thermochemically sensitive than the other components, consequently, it was hydrolyzed much faster than cellulose and could be fractionated easily using subcritical water treatment [11,30]. The side groups of hemicellulose are first to react followed by the hemicellulose backbone. Next, it could be said that hemicellulose and its derived compounds have been extracted from barley grains at this temperature by subcritical water. Leppanen et al. [15] performed Norway spruce saw meal extraction with subcritical water at 120–240 °C, using a flow-through system. Only small

amounts of hemicelluloses were extracted at 120–160 °C, but dissolution was significantly enhanced when higher extraction temperatures were applied. Benito-Roman et al. [16] extracted hemicellulose from different cereals in a fixed-bed extractor using subcritical water as a solvent, at temperatures of 110–175 °C, and concluded that subcritical water at 155–160 °C was a suitable solvent for the extraction of glucan compounds from barley. Matsunaga et al. [14] also reported that small amounts of hemicellulose were extracted in water at 140 °C, then the solubilization of hemicellulose in water increased significantly at 190 °C.

Fig. 3 also showed effect of CO₂ addition on the extraction system. UV-vis spectra depicts that the presence of CO₂ had influence on the extraction rate process of barley grains components mainly hemicellulose. When the extraction was conducted at ~60 min, it was clearly that the peak at 40 MPa with CO₂ addition was sharper than the others at the same extraction temperature. The UV-vis spectra of liquid products with the same intensity were found when the extraction times were expanded to 180 min. This indicates that the addition of CO₂ in the extraction system did not affect significantly on the chemical composition of the final products. As informed above that the addition of CO₂ to subcritical water extraction system can accelerate the rates of acid-catalyzed reactions due to the formation of carbonic acid [17–24]. Walsum and Shi [19] conducted experiments for carbonic acid and liquid hot water pretreatments to corn stover. The CO₂ was introduced into water to form carbonic acid. Comparing the results with and without CO₂, the addition of CO₂ could enhance the hydrolysis and increased the accumulation of small chain oligomers at all temperatures investigated. Rogalinski et al. [20] informed that the addition of carbon dioxide leads to an acid hydrolyzed catalysis due to the formation of carbonic acid according to Eq. (1) and the connected decrease in pH of the system water/CO₂. In this work, the pH was not measured during extraction process. The final pH of liquid products (4.8–5.6) were measured at the end of all experiments.

3.2 Characterization of solid residues

In principle, lignocellulosic fibers from different lignocellulosic plants biomass seem quite different, but the chemical composition is almost similar although with different magnitudes of constituents. Lignocellulosic plants biomass usually composed of cellulose, hemicellulose and lignin as major compositions. In these plants biomass, the polymers of carbohydrates (celluloses and hemicelluloses) are strongly bounded to the lignin parts by covalent and hydrogen bonds. When the subcritical water treatment was applied in the plants biomass, the transformations of the chemical structure of them are caused by autohydrolysis reactions of the cell-wall constituents occurred. Here, the chemical structure of water-insoluble products portion (solid residue) from barley grains after subcritical water treatment were investigated by FT-IR. Fig. 4 showed the FT-IR spectra of barley grains and barley grains solid residues after subcritical water treatment with and without CO₂ at 130 and 170 °C. The typical functional groups and the infrared signal with the possible compounds are summarized in Table 1 for a reference [14]. As a part of plants biomass, barley grain is also composed primarily of cellulose, hemicellulose, and lignin is most likely composed of alkene, esters, aromatics, ketone, and alcohol, with different oxygen containing functional groups observed. These chemical bonds are slightly elastic: they can stretch, bend, or vibrate. Essentially, the FT-IR spectra of barley grains and their solid residues are similar and they differ only in the intensity of some peaks. It indicated that the functional group compositions of them were not changed after subcritical water extraction at these range temperatures (130–170 °C). Absorbance intensity due to hydrogen bonded O–H stretching (3329.86–3285.09 cm⁻¹) could be found in each spectrum with the similar strong intensity. After subcritical water treatment, the sharper peak spectra in the region 2924.45–2852.01 cm⁻¹ relation with C–H stretching

was found in all sample solid residues. This suggested that the solid residues have an aromatic-rich structure. Bands in the following regions; 1456.99–1337.24; 1161.47–1149.67; and 1030.22–996.36 cm^{-1} are ascribed to O–CH₃ (methoxyl–O–CH₃ groups); C–O–C stretching vibration; and aromatic skeletal vibrations. The intensity of these bands have been obtained in each spectrum and they had the same strong intensity. Similar to CH group asymmetric and symmetric stretching, the peak spectra in all sample solid residues at the region 1744.17–1742.56 cm^{-1} assigned to stretching of aromatic C=O groups was also obtained. It showed that solid residues also contained of ketone and carbonyl groups prominently. The absorbance intensity at 1642.71–1633.43 cm^{-1} , which was due to stretching of CN and NH groups, was found in each spectrum. This indicated that amide linkages and amino groups remained intact in barley grains following treatment by subcritical water [5]. The original barley grains exhibited absorption peaks at 1076.34 cm^{-1} , which is the absorption peak of aromatic CO group deformation. After subcritical water treatment, the intensity of this absorbance disappeared because of the loss of CO group. This indicated that they have been consumed during the application of subcritical water treatment on the barley grains. The absorption peak of the β -glucan groups were detected at 928.42–860.69 cm^{-1} [5]. Following subcritical water treatment, this absorbance intensity also disappeared, indicating that subcritical water treatment resulted in the extraction of β -glucan from barley grains within the temperature range studied. As shown in Fig. 4 (4g and 4h), it was clearly that the addition CO₂ in the subcritical water extraction system had high influence on the extraction processes. The peak spectra of solid residues obtained from water-CO₂ system were almost sharper the peak spectra of solid residues obtained water system only. Rogalinski et al. [22] studied the catalytic influence of CO₂ on hydrolysis of polysaccharides in subcritical water. They reported that glucose yields from corn starch could be significantly increased by acidification when the CO₂ was added. They compared the course of glucose yields in water-CO₂ and pure

water, the onset of glucose formation in water-CO₂ is shifted to lower residence times due to the faster cleavage of glycosidic bonds. Glucose is also formed at higher yields. Hunter and Savage [23] also conducted an experimental survey to examine the effect of CO₂ addition on dibenzyl ether hydrolysis in high-temperature liquid water. The results showed that dibenzyl ether hydrolysis increased in product yield in each experiment with added CO₂. They also explained that the increasing rates of acid-catalyzed reactions can be achieved via increased temperature as well as by added CO₂. In high-temperature liquid water, an increase in operating pressure was also required.

It was well known that subcritical water extraction technique is one of the methods used for the isolation of valuable components from plants. Under subcritical water conditions, the cellular structure of plant tissues can be cleaved and consequently result in releasing several chemical compounds to the extra-cellular medium that are dissolved in liquid water. Some of the plant biomass components underwent reactions such as hydrolysis and/or degradation into their derived compounds. In this process, a substantial part of the oxygen in the biomass is removed by dehydration or decarboxylation (stated by the molecular O/C and H/C ratio), mainly by dehydration and decarboxylation [13,30,31]. Table 2 shows the ultimate analysis of barley grains solid residue content in weight percent dry and ash-free material after subcritical water treatment with and without CO₂ addition. The concentration of carbon increased gradually with increasing temperature of subcritical water extraction in each solid residue. This showed that the applied temperatures were sufficient to cleave biomass components in barley grains mainly ester and ether bonds via hydrolysis. Sevilla and Fuertes [32] reported that at 170 °C, sucrose underwent hydrolysis and forms individual monosaccharides (glucose and fructose). Simultaneously, dehydration and fragmentation (i.e., ring opening and C–C bond breaking) of glucose and fructose was observed, following which, dehydrated monomers and their decomposition products underwent polymerization and condensation

forming carbon microspheres. Demirbas [33] also reported that biomass components produced by hydrolysis are degraded to smaller compounds via dehydration, dehydrogenation, deoxygenation and decarboxylation and that following this, compounds rearrange through condensation, cyclization and polymerization to lead to new compounds. When the CO₂ was applied in 10 and 40 MPa at the same temperature, the concentration of carbon in the solid residues are almost the same. These confirmed that the addition of CO₂ in the subcritical water extraction system did not affect significantly on the chemical composition of the final products. McWilliams and Walsum [34] conducted experiment for aspen wood hydrolysates produced by pretreatment with liquid hot water and carbonic acid. The carbonic acid obtained by applying CO₂ in liquid hot water. They reported that under most conditions tested, it was found that the presence of CO₂ had no discernible effect on the amount of xylose released or concentration of furan compounds at the end of experiments.

3.3 Molecular weight distribution

To determine the molecular weights of the water-soluble compounds extracted from barley grains, the water soluble extracts were directly injected to HPLC system with ELS detector. Like other plant biomass water-soluble compounds, liquid fraction products from barley grains also have molecules covering a fairly wide range of molecular weights, a molecular weight gives an average of this distribution. Fig. 5 showed the molecular weight distribution of water-soluble compounds from barley grains after subcritical water extraction at 130, 150, and 170 °C with and without CO₂ addition, respectively. The GPC analysis showed that the average molecular weight of water-soluble compounds were approximately 500 Da to 1167 kDa. In this study, the compound in monomer units extracted by subcritical water was not observed. As reported above that the most of water-soluble compounds are hemicellulose and its derived compounds which extracted from barley grains at these temperature ranges, hence,

this molecular weight region might correspond to the existence of hemicellulose groups involving linkages at the glycosidic bonds. At 130 °C, the higher molecular weight of water-soluble compounds extracted obtained at 90 min extraction time. After that, they gradually decrease with expanding of extraction time. At 150 and 170 °C, the higher molecular weight of water-soluble compounds extracted obtained in the beginning of extraction process. With extension of extraction time, they decreased significantly. It showed that the long heating time during the extraction process caused the compounds degradation. Fig. 5 also showed that the higher molecular weight of water-soluble compounds extracted obtained when the lower extraction temperatures were applied. The relationship between temperature-time strongly affects the physical-chemical characteristics of plants biomass in hydrothermal processing. Ruiz et al. [10] reported an increase of hemicelluloses degradation at higher temperatures (>200 °C) and residence times, concluding that at more severe operational conditions there are more losses of them. Therefore, the strict control was required for high temperature reactions due to thermal degradation. Song et al. [35] suggested that the yield of hemicellulose from spruce wood reached a plateau after 40 min extraction at 160 to 180 °C. At these temperature ranges, the degradation of saccharides, including depolymerisation and deacetylation of monosaccharides, become dominant. Benito-Roman et al. [16] also said the longer the extraction time, the lower the molecular weight obtained when they conducted subcritical water extraction of barley grains at 155 °C. They explained that short extraction times are useful for extracting small amounts of high molecular weight β -glucan. Longer times serve to dissolve β -glucan, which, once dissolved, begin to be degraded, and hence lose part of the molecular weight. Generally, varying the pressure did not result in any changes and often has little effect on the subcritical water extraction efficiency. The elevated pressures under subcritical water were applied to keep the water from boiling and maintaining its liquid phase [14]. Here, the addition of CO₂ in the extraction system

seems has high influence on the molecular weight of water-soluble compounds extracted by subcritical water at 40 MPa due to the density and solubility of CO₂. Several literatures reported that the CO₂ solubility in water decreases with temperature and increases with pressure [36-38]. Hence, the experiments could be done at as a low temperature as possible and at relatively high CO₂ pressures to obtain a high solubility of CO₂ in water. This is, of course, a beneficial effect in terms of extracting β -glucan from the barley grains. At these conditions, the addition of CO₂ favored acid hydrolyzed catalysis, possibly due to a decrease in pH. McWilliams and Walsum [34] reported that aspen wood chips processing with the liquid hot water-CO₂ produces a hydrolysate with a higher pH in comparison to the liquid hot water-only experiments. Rogalinski et al. [22] also explained that when CO₂ was added into water under hydrothermal conditions, the pH will drop sharply and lowers progressively slower with increasing carbon dioxide addition. The decrease in the pH value could sufficiently promote dissolution and hydrolysis of barley grain active components into their derived compounds. Hence, it was possible to break the active sides of barley grains components in an acid media without the addition of further acids [20,22,39-40]. Miyazawa and Funazukuri [39] conducted experiments for polysaccharide hydrolysis accelerated by adding CO₂ under hydrothermal conditions. In the presence of CO₂, a significant yield improvement was achieved, next, they concluded that during hydrothermal degradation of polysaccharides, the yields of monosaccharides increased with the amount of CO₂ added. They explained that the molecular weight distributions of the polysaccharide hydrolyzates shifted to lower molecular weights by increasing the CO₂ load, corresponding to higher pressures of CO₂. Gurgel et al. [41] studied to investigate the effectiveness of liquid hot water associated with high pressure CO₂ on the releasing of xylo-oligosaccharides and xylose from depithed sugarcane bagasse at temperatures of 93.8 - 136.2 °C and pressures of 8.62-12.96 MPa. They reported that liquid hot water associated with high pressure CO₂ promoted a

higher degree of deacetylation in comparison with liquid hot water alone, which increased the acidity and allowed a higher hemicelluloses extraction. Therefore, it could be said that the high pressure of CO₂ promotes hydrolysis of water-soluble compounds into low molecular weight compounds.

3.4 Hydrolysis products

In order to determine the β -glucan contents in the water-soluble products, (1-3)(1-4) β -D-glucan assay kit (McCleary Method, Megazyme International Ireland Ltd., Wicklow, Ireland) was used following to the manufacturer's instruction. The absorbance of the solution samples was measured by using UV-vis spectrophotometry at 510 nm. Fig. 6 showed the level of β -glucan content in liquid fraction products at 130–170 °C and 10–40 MPa with and without CO₂ addition. It is already known that the subcritical water extraction utilizes the application of water at temperatures higher than its boiling point under high pressure to maintain its liquid state. The temperature applied during extraction process has an important factor on the extraction efficiency and selectivity. As shown in this figure, the amounts of β -glucan increased greatly with increasing extraction temperature at the same extraction conditions. The increasing extraction temperature allows to reduce the strength of the atomic bonds in barley grains which led to the more efficient extraction process. At 60 min extraction time, the addition of CO₂ in the subcritical water extraction system seemed to give a rise clearly effects on the amount of β -glucan in each liquid fraction product. It can be seen, the accumulation of β -glucan in the liquid fraction product at 170 °C was around 0.019 g/g sample with water as an extraction media. When the CO₂ was added in the subcritical water extraction system at the same extraction time, the accumulation of β -glucan in the liquid fraction product could approach to 0.024 g/g sample at 170 °C and 40 MPa. The results might indicate that subcritical water extraction with the addition of CO₂ could produce acid catalysts

and under acid catalyzed reactions, hydrolysis is more often occurred. It was studied that the hydrolysis rate increased drastically if the subcritical water was acidified with CO₂ [17,22]. Ruiz et al. [10] also explained that the formation of hydronium ions from water as an acid catalyst will be accelerated by the increasing temperatures and pressures. The formation of hydronium ions from water is an important factor during hydrothermal processing, since the lignocellulose material and water mixture will reach high temperatures and pressures during the process. Garrote et al. [11] conducted hydrothermal processing of lignocellulosic materials under subcritical conditions. Under these conditions (<230 °C), the lignocellulose material underwent hydrolysis reactions in the presence of the hydronium ions generated by water autoionization, which act as catalysts. They explained that the heterocyclic ether bonds of hemicelluloses are the most susceptible to this type of reaction, leading to both generation of oligosaccharides and splitting of the acetyl groups from the hemicellulosic fraction of the raw materials. However, the amount of the water-soluble β -glucan was almost the same, except at 130 °C, when the extraction time increases to 180 min at the same extraction temperature. This effect has been mentioned before that the addition of CO₂ in the subcritical water extraction system did not affect significantly on the chemical composition of the final products. Judging from the results, it could be said that the hydrolysis of barley grains hemicelluloses catalyzed by acid catalyst from CO₂ addition under subcritical water could break faster the β -(1 $\rightarrow$ 3,1 $\rightarrow$ 4)-glucan bonds in their structures. Since the water-soluble β -glucan from barley grains was around 1.2 to 3.5 % [3,42], this technique may extract all amount of water-soluble β -glucan from barley grains. In this work, the standard deviation is defined as [43]:

$$\text{Standard deviation} = S_x = \sqrt{\frac{\sum_{i=1}^n (X_i - \bar{X})^2}{n-1}}$$

where S is standard deviation, X_i is given value from the sample, $\bar{X}$ is mean-average of the

n -values, n is number of values x , and i is number of experimental data (1, 2, 3,...), respectively. The standard deviation range of our experimental data were 0.16-0.28.

3.5 Extraction mechanism

Fig. 7 showed SEM images of barley grains and their solid residues after treatment by subcritical water with and without CO₂ addition at 170 °C. Before subcritical water treatment, the surface morphology of barley grains were marked by some boundary edges clearly and did not exhibit the presence of any pores or surface cracks. After treatment by subcritical water, the physical structures disruption of barley grains were found and clearly observed at each condition. Apparently, this treatment has disrupted the structure of barley grains that contains polysaccharides, lignin, protein, and lipids as main components like plants biomass. The pores and cracks could be clearly seen on the morphology of solid residue frameworks and they are very prominent in all images of each solid residue. These showed the cell wall of barley grains was destroyed, indicating the removal of their components to dissolve in subcritical water. Similarly, the morphology of solid residues with pores and cracks were obtained when the CO₂ was added during subcritical water extraction process. The increasing pressure to 40 MPa seems to an increase of CO₂ diffusivity into barley grains and allows a more powerful swelling action to open up pores in the barley grains [44]. Nevertheless, based on these SEM images, it could be conclude that the physical changes of the barley grains surface occurred after application of subcritical water treatment with and without CO₂ addition. Compared to the degradation of plants biomass model compounds, the degradation of real plants biomass such as barley grains is more complex due the existence of their various components. The major components of plants biomass are mixed in a variety of ratios and determine the nature of each individual biomass species. When the plants biomass were treated by hydrothermal process, their components will break down into their backbone units.

Toor et al. [25] reported that the degradation products of plants biomass components in subcritical water differ; however, the basic reaction mechanisms can be summarized as follows: first, depolymerization of the plants biomass following by decomposition of plants biomass monomers by cleavage, dehydration, decarboxylation and deamination. Then the recombination of reactive fragments. Although it was known that hemicelluloses are the most thermochemically sensitive [14,26], their degradation process was still complex. Several researchers suggested that studying the conversion of plant biomass model compounds is very useful to clarify the mechanism of subcritical water extraction [10,14,25,29]. They described the hydrolysis of cellulose to glucose in subcritical water. As a major constituents of extraction system, initially the water molecules underwent auto-dissociation to produce hydrogen ion (H^+) and hydroxide ion (OH^-). Under subcritical water conditions, the auto-dissociation of water molecules is approximately 3 orders of magnitude larger than that at ambient conditions. This means that the increased amount of ions from the water will make acid and base catalyzed reactions far more likely to occur at these conditions than at ambient [28]. The generated H^+ cation interacts the oxygen atom in the β -1,4-glycosidic bond to form conjugate acid followed by the glycosidic bond cleavage and leads to the two glucose units. The OH^- anion from the dissociation of water molecules attacks at the anomeric carbon atom, renders the cleavage of the O bridge and again yields the two glucose units. Water molecules and the glycosidic bond split simultaneously and form two glucoses again. By extension of these reactions, glucose monomers were formed.

4 Conclusions

Water- CO_2 has been used as a medium for β -glucan extraction from barley grains. The extraction was carried out at 130–170 °C and 10–40 MPa, using a semi-batch system. Under these conditions, thermal softening of barley grains occurred, allowing extraction of β -glucan

via autohydrolysis. UV-vis spectra of liquid fraction products showed a high content of hemicellulose and its derived compounds, including β -glucan with an average molecular weight around 500 Da to 1167 kDa. FT-IR spectra showed that β -glucan have been extracted from barley grains at 130–170 °C. The β -glucan content in the liquid fraction products could be improved with the acidification of CO₂ compared with that in pure water. Finally, this technique could be proposed as an applicable technique to fractionate bioactive compounds from other types of biomass.

Acknowledgements

A part of this research was supported by a grant from the Precursory Research for Embryonic Science and Technology Program of Japan Science and Technology Agency.

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Table Captions

Table 1. Main functional groups of plants biomass [14].

Table 2. Elemental analysis of barley grain and its solid residues using an CHN analyzer.

Figure Captions

- Fig. 1.** Hydrothermal extraction apparatus schematic.
- Fig. 2.** Typical photographs of water-soluble products obtained at 170 and 130 °C.
Extraction times: (1) 0~30 min; (2) 30~60 min; (3) 90~120 min; (4) 150~180 min.
- Fig. 3.** UV–vis spectra of water-soluble products at 170 °C with and without CO₂ addition: (a) water, 10 MPa, 60 min; (b) water-CO₂, 10 MPa, 60 min; (c) water-CO₂, 40 MPa, 60 min, (d) water, 10 MPa, 180 min; (e) water-CO₂, 10 MPa, 180 min; (f) water-CO₂, 40 MPa, 180 min, respectively.
- Fig. 4.** FT-IR spectrum of barley grains before and after subcritical water treatment with and without CO₂ at 170 and 130 °C: (a) Barley grains; (b) Water, 170 °C, 10 MPa; (c) Water-CO₂, 170 °C, 10 MPa; (d) Water-CO₂, 170 °C, 40 MPa; (e) Barley grains; (f) Water, 130 °C, 10 MPa; (g) Water-CO₂, 130 °C, 10 MPa; (h) Water-CO₂, 130 °C, 40 MPa.
- Fig. 5.** Molecular weight distribution of water-soluble products at 130, 150, and 170 °C, respectively.
- Fig. 6.** β -glucan extracted at various extraction conditions.
- Fig. 7.** SEM images of barley grains before and after treatment by subcritical water at 170 °C.

Table 1. Main functional groups of plants biomass [14].

Wave number [cm ⁻¹]	Functional groups	Compounds
3600 – 3000	O–H stretching	Acid, methanol
2860 – 2970	C–H _n stretching	Alkyl, aliphatic, aromatic
1700–1730, 1510–1560	C = O stretching	Ketone and carbonyl
1632	C = C	Benzene stretching ring
1613, 1450	C = C stretching	Aromatic skeletal mode
1470–1430	O–CH ₃	Methoxyl–O–CH ₃
1440–1400	O–H bending	Acid
1402	C–H bending	
1232	C–O–C stretching	Aryl-alkyl ether linkage
1215	C–O stretching	Phenol
1170, 1082	C–O–C stretching vibration	Pyranose ring skeletal
1108	O–H association	C–OH
1060	C–O stretching and C–O deformation	C–OH (ethanol)
700–900	C–H	Aromatic hydrogen
700–650	C–C stretching	

Table 2. Elemental analysis of barley grain and its solid residues using an CHN analyzer.

Operating condition	Ultimate analysis (wt. %, DAF [☆])				Atomic ratio (–)	
	H	C	N	O*	H/C	O/C
Room ⁺	6.63	42.48	1.16	49.74	0.16	1.17
Water, 130 °C, 10 MPa	6.01	47.73	2.36	43.89	0.13	0.92
Water-CO ₂ , 130 °C, 10 MPa	6.37	47.09	2.82	43.72	0.14	0.93
Water-CO ₂ , 130 °C, 40 MPa	7.19	48.23	2.56	42.02	0.15	0.87
Water-CO ₂ , 170 °C, 10 MPa	8.04	54.33	2.45	35.18	0.15	0.65
Water-CO ₂ , 170 °C, 40 MPa	8.41	54.49	2.36	34.74	0.15	0.64

[☆]Dry ash free.

*Oxygen content was determined by difference.

⁺Original sample.

Fig. 1. Hydrothermal extraction apparatus schematic.

Fig. 2. Typical photographs of water-soluble products obtained at 170 and 130 °C.
Extraction times: (1) 0~30 min; (2) 30~60 min; (3) 90~120 min; (4) 150~180 min.

d. Water, 130 °C, 10 MPa.

e. Water-CO₂, 130 °C, 10 MPa.

f. Water-CO₂, 130 °C, 40 MPa.

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Fig. 5. Molecular weight distribution of water-soluble products at 130, 150, and 170 °C, respectively.

Fig. 6. β -glucan extracted at various extraction conditions.

Fig. 7. SEM images of barley grains before and after treatment by subcritical water at 170 °C.

Graphical Abstract

β -glucan extracted at various extraction conditions with and without CO₂ addition.