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Subcritical Water Extraction of Polysaccharides Using a Semi-Batch Extractor

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Abstract

Subcritical water is an environmentally friendly method with a wide range of applications, such as extraction, hydrolysis, and wet oxidation of organic compounds. Here, water at subcritical conditions was applied to extract polysaccharides from *Ganoderma lucidum* (*G. lucidum*) and barley grains at 120 – 180 ^{o}C and 4.0 MPa using a semi-batch system. The liquid products were directly micronized and contacted with hot air to form microsphere particles. During extraction process, cell wall disruptions of *G. lucidum* and barley grains took place, allowing the removal of the polysaccharides isolating other constituents in *G. lucidum* and barley grains via autohydrolysis. Scanning electron microscope (SEM) images described that the particle products produced had sphere and wrinkled morphology of particles with diameters varying from 1 to 10 μm. The experimental result revealed that the particles formed from *G. lucidum* and barley grains extract contained 40-45% and 30-35% weight β-glucan, respectively.

Keywords: subcritical water, extraction, polysaccharides, micronization, *Ganoderma lucidum*, barley grains

1. Introduction

Hot water extraction is usually applied in traditional methods for isolation of polysaccharides or carbohydrates, however, this technique is related to lower recoveries and needs both higher extraction temperatures and longer fractionation time. Therefore, an advanced technique for extraction and isolation of carbohydrates able to avoid the hot water extraction disadvantages is desired. Subcritical water extraction or pressurized hot water extraction is one such alternative. Subcritical water extraction has been considered as a popular green isolation and extraction method for various classes of substances present in numerous kinds of materials like in environmental, food and botanical and biological samples. It is also applied in preparation of sample for organic contaminants extracted from foodstuff used in food safety analysis and soils/sediments used in environmental monitoring aims.

In this work, water under subcritical conditions, temperatures of 120–190 ^{o}C and pressure of 4.0 MPa, was employed to recover carbohydrates from mushroom of *G. lucidum* and barley grains (*Hordeum vulgare*). (1-3),(1-4)-β-D-glucans are carbohydrates that can be obtained in the fruit body of *G. lucidum* and in the subaleurone and the endosperm of the cell wall of different cereals, such as barley grains. The structure of this polymer is mainly linear: glucose is linked to other two or three units by means of β-(1-4)-O-glucosidic linkage (forming cellobiose or cellotriose blocks) which are separated by a β-(1-3)-O-glucosidic linkage. In addition, glucans, a kind of carbohydrates, from *G. lucidum* and barley grains are well recognized useful for anti-tumors, anti-inflammation, immunomodulation and against radiation.

Moreover, to concentrate the glucans extract contained in the liquid products, the micronization and the drying particle were immediately performed after extraction process. This method was expected could reach a small particle size and reduce the water content; hence, the stable products with various chemical composition and structure are obtained.

2. Method

2.1 Materials

Mushroom *G. lucidum* received from Refarmer Co., Ltd. (Kumamoto, Japan) and barley grains received from

Asahi Co., Ltd. (Tokyo, Japan) were employed as starting materials. They were crushed by a miller into a certain particle size and sieved through 16-mesh sieves; the samples were furthermore stored in refrigerator at < 5 ^{o}C. Distilled water was utilized as liquid solvent. Supporting materials including potassium hydroxide (KOH, 85.0%), sodium hydroxide (NaOH, 97.0%), hydrochloric acid (HCl,35.0% ~ 37.0%), acetic acid (CH_3COOH, 99.9%), 2,5-dihydroxybenzoic acid (DHB, 98.0%), and analytical reagent (methanol, 99.7%) were provided by Wako Pure Chemicals Industries Ltd., Japan.

2.2 Experiment

The schematic diagram of the experimental apparatus is shown in Figure 1. It included an extraction system and a micronization system. The system for extraction was composed of a high-pressure HPLC pump, preheating coil and an extractor with 10 mL volume. After the extractor loaded by 1.0 g of mushroom *G. lucidum* or 3.5 g of barley grains was placed in the extraction unit, liquid solvent without heating was delivered into the extractor equipped with preheating coil for several minutes in order to replace air and damp mushroom *G. lucidum* perfectly. Water as liquid solvent was then compressed into desired pressure by controlling the back pressurized regulator made in AKICO, Japan. Furthermore, heater with electric controller was employed to increase liquid solvent temperature after a certain pressure and a steady state was reached. Finally, the particle products were characterized using a scanning electron microscope (SEM; Hitachi S-4300, Japan), while the particle sizes were determined from the SEM images using image analyzer software (Image J 1.42).

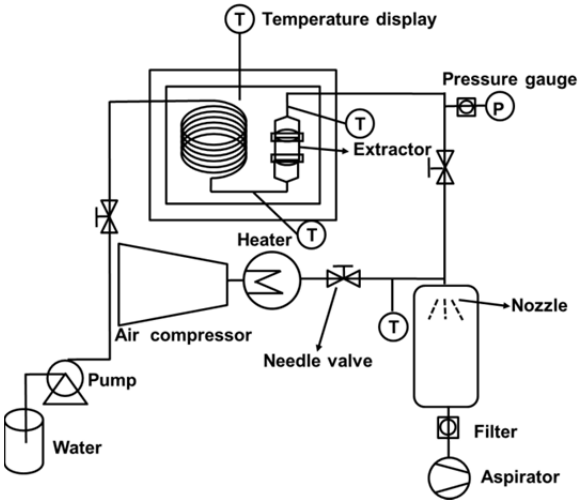

Figure 1. Subcritical water extraction and micronization apparatus

3. Results

To evaluate the extract of polysaccharides from barley grains after treatment by subcritical water extraction, the water soluble extracts were analyzed using UV–vis spectrophotometer. Figure 2 showed the UV-vis chromatogram of liquid product from barley grains after subcritical water extraction at 0.5 ml/min with 60 min extraction time.

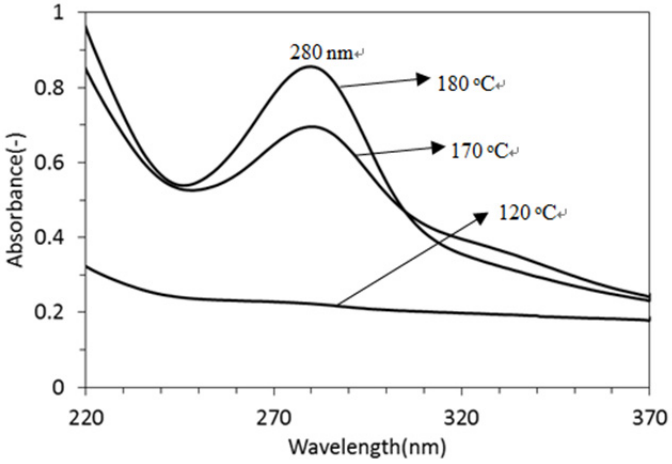

Figure 2. UV-vis chromatogram of liquid extracts from barley grains

When plant biomass is treated to elevated temperatures, changes can occur in its chemical structure that affects its performance. This phenomenon was also occurred in subcritical water extraction of hemicelluloses from barley grains, the chemical structure of barley grains was also change. So that, to observe the chemical bond of the solid materials remained in subcritical water treatment, the solid residues remained at each extraction temperature were examined using a Spectrum Two Fourier transform infrared (FT-IR) spectrophotometer (Perkin-Elmer, Ltd., England). This analytical technique may identifies the unknown materials and the forms of chemical structures of the substances in the materials contained. The FT-IR chromatogram of barley grains and its solid materials remained in subcritical water extraction at 140 ^{o}C is shown in Figure 3.

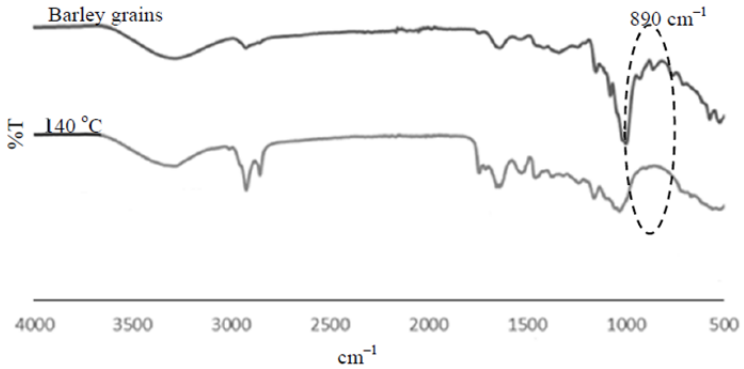

Figure 3. FT-IR chromatogram of barley grains and its solid material remained in subcritical water extraction at 140^{o}C

Figure 4 shows the morphology of particles formed from *G. lucidum* (a and b) and barley grains (c and d) with air temperature of 200 ^{o}C, respectively. The extraction temperatures of *G. lucidum* and barley grains were 160 and 170 ^{o}C, respectively. In fact, the contact temperatures between hot air and an extract solution in the nozzle inside was around 67–69 ^{o}C. The contact time between of them was not investigated.

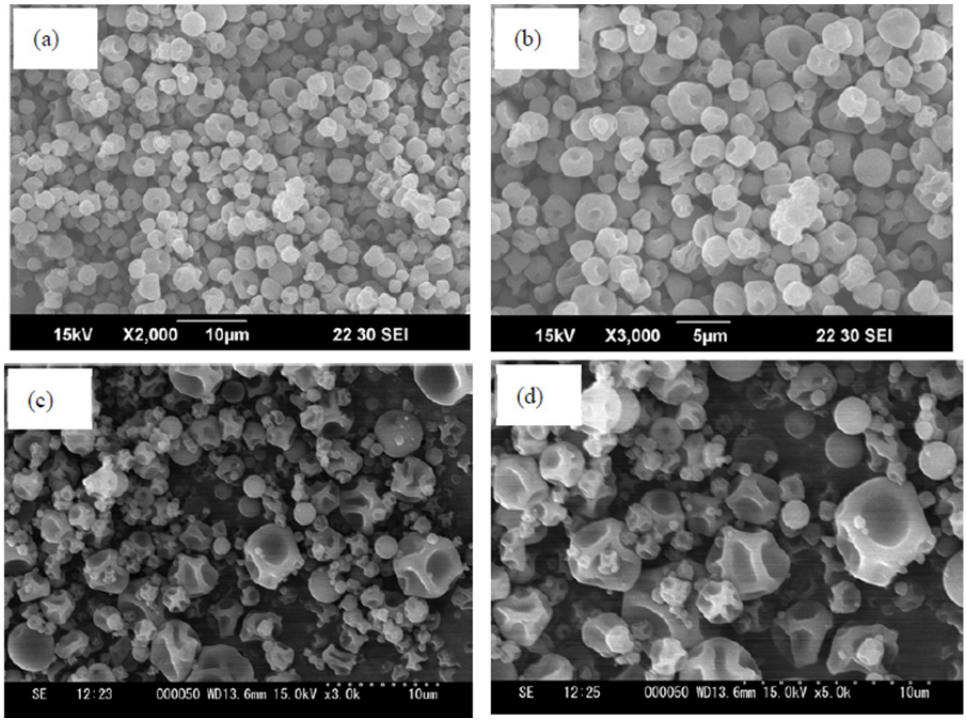

Figure 4. SEM images of particles formed from *G. lucidum* (a and b) and barley grains (c and d)

4. Discussion

UV–vis spectrophotometer is a simple technique and a useful tool for studying polysaccharides extraction and its derivatives from plants biomass. Unfortunately, this determination technique includes a drawback: polysaccharides decomposition compounds absorb spectrum at the wavelength range of 280 nm, while lignin, furfural, and hydroxymethylfurfural are also capable to absorb spectrum at the same wavelength. Figure 2 displays a prominent spectrum in a wavelength range of 280 nm, where peak spectra near 280 nm corresponds to aldehyde groups which generated from polysaccharides hydrolysis during the extraction process by subcritical water. Lignin and its derived substances also exhibited high peak spectra at the same wavelength of 280 nm (Machmudah et al., 2015). Nevertheless, hemicelluloses, as one of the main compounds in plant biomass, are the most thermochemically sensible compound. Under subcritical water extraction, initially, the side structures of hemicellulose react, and then the main structure of hemicellulose decomposes; hence, the compounds are separated directly in subcritical water extraction. As depicted in Figure 2, the UV chromatogram became sharp with the elevating extraction temperatures. It indicates that hemicellulose and its derived substances started to solubilize in water at these extraction temperatures. At 180 ^{o}C, due to high content of hemicellulose and its derived substances in the extract, the UV spectra became sharper than the others. It can be explained that hemicellulose underwent hydrolysis reactions in the existence of the H^+ ions produced from auto-ionization of water as catalyst, which resulted in dissolve hemicellulose substances in water at temperature of 150 ^{o}C (Garrote et al., 1999). Firstly, the heterocyclic ether groups of hemicelluloses reacts to promote both formation of oligosaccharides and splitting of the acetyl bonds from the hemicellulosic group contained in the starting materials. In the next reaction steps, the production of H^+ ions by auto-ionization of acetic acid, which is naturally exist in the hemicelluloses, also serve as catalysts for carbohydrates decomposition that resulted in the increasing reaction rates. Based on these results, it indicated that hemicelluloses were fractionated from barley grains by subcritical water method at the experimental temperatures, even though it was not easy to recover hemicellulose in high yield.

In this work, the characterization of solid material was only conducted for solid residue produced at temperature of 140 ^{o}C. The solid residue characterization was conducted in the wavenumber range of 4000-650 cm^{-1}. From Figure 3, it could be known that barley grains is mostly composed of cellulose, hemicellulose, and lignin as main compounds of plant biomass. While the plant biomass is well known consisted of alkene, esters, aromatics, ketone, and alcohol, with various oxygen-bonds structures examined. Based on Figure 3, the peak spectrum of infrared bands are mainly observed at wavenumber ranges of 3600-3000, 2860-2970, 1730-1510, 1470-1430, 1440-1400, 1060, and 900-700 cm^{-1} corresponding to O-H stretching (acid, methanol), C–H_n stretching (alkyl, aliphatic, aromatic), C = O stretching (ketone, carbonyl), O–CH_3 (methoxyl–O–CH_3), O–H bending (acid), C–O stretching and C–O deformation (C–OH (ethanol)), and C–H (aromatic hydrogen), respectively. The spectral features of barley grains and their solid materials remained in subcritical water extraction are substantially the same, showing that the solid materials remained after subcritical water treatment are having a similar structure as barley grains. In Figure 3, the original barley grains showed absorption peak of 890 cm^{-1} that correspond to the spectrum of β-glucan compounds. The solid residues remained after subcritical water treatment had smaller peak spectra at 890 cm^{-1} and almost disappeared because β-glucan compounds have been exhausted. It showed that β-glucan compounds in barley grains have been successfully extracted by subcritical water.

In this work, the liquid products were immediately micronized by hot air to instantaneously produce a particle because heat and mass transport from liquid solvent into hot dry air were occurred. Since the removing most of liquid solvent in the extract and reducing activity of the liquid solvent, the micronization and drying process allow to generate a microbiological stable powders, eliminate the risk of chemical or biological decompositions, decrease the storage and transportation charges, and provide a particle with special characteristic. Gharsallaoui et al. (Gharsallaoui et al., 2007) informed that the optimum temperature of dry air exit from the chamber for the micronization of foodstuffs is 50 to 80 ^{o}C; hence, in this research, temperature of dry air insert does not affect the formation of particles due to the freshness of powder surface restraints. During micronization process, balances of temperature and the vapor pressure are constructed around the water and air. According to Elversson & Millqvist - Fureby (Elversson & Millqvist - Fureby, 2005), temperature gradient resulted in heat transport from the dry air into the powder, the difference in vapor pressure caused the transfer of water from the powder to the air. In principle, most of the particle morphologies in Figure 4 were uniform with sphere shapes and had diameters less than 10 μm. It could be described due to high solubility of particles in liquid solvent that affected on the typical sphere form and powder geometry. The existence of carbohydrates in the liquid solvent promoted to the sphere powder generation. Asada et al. informed that spray-drying processing yielded sphere powders with the presence of chitosan; nevertheless, wrinkled particles and indentations on the surfaces of particles took place

(Asada et al., 2004). Twu et al. (Twu et al., 2003) explained that polysaccharide sphere produced by spray-drying shows sphere geometry and more fine surface structure. As shown in Figure 4, the size of sphere particles is less than 3 μm. In order to understand the content of *β*-glucans in the powders generated, *β*-glucan assay kit for mushroom and yeast, and (1-3)(1-4)*β*–D–glucan assay kit (Megazyme International Ireland Ltd., Wicklow, Ireland) was applied for analysis. In detail, procedure of *β*-glucan contents examination has been demonstrated extensively elsewhere. In brief, a several amount of particles formed dissolved in water. These solutions were then treated according to the manufacturer's instruction. The absorption spectra of the aliquot was determined by UV–vis spectrophotometry at wavelength of 510 nm. The content of *β*-glucans was calculated from subtraction of *α*-glucan in the total glucan contents. The analytical result revealed that the content of *β*-glucans in the powders extracted from *G. lucidum* and barley grains are 40-45% and 30-35% weight, respectively.

In conclusion, under subcritical water conditions, *G. lucidum* and barley grains underwent thermal degradation, resulting the releasing of the carbohydrates isolating other components in mushroom *G. lucidum* and barley grains through dehydrogenation and deoxygenation reactions. Based on the SEM analysis, the powders generated has sphere and wrinkled geometry with range diameters from 1 to 10 μm. The result revealed that the content of *β*-glucans in the particles from *G. lucidum* and barley grains are 40-45% and 30-35% weight, respectively.

Acknowledgments

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