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Contributed Paper

Hydrolysis of Konjac Flour under Subcritical Water Conditions

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ABSTRACT

Konjac glucomannan-derived oligosaccharides are a potential dietary fiber source. In this study, 5 g/L konjac flour solution was treated under subcritical water conditions at 160 °C, 180 °C, 200 °C, 220 °C, and 240 °C for 10 min in a batch-type reactor. The solutions showed an immediate decrease in viscosity (from 150 to less than 10 mPa·s) and increases in reducing sugar contents (from 0.1 to 2.3 g/L) after treatment, indicating that glucomannan polymers were hydrolyzed to shorter chains. The treatment at 220 °C resulted in carbohydrate yield of greater than 46% and considerably higher oligosaccharide contents than the other treatments. Subcritical water treatment was demonstrated to be a promising method for preparing oligosaccharides from konjac flour.

Keywords: *Amorphophallus konjac*, konjac glucomannan, superheated water, oligosaccharides, hydrolysis

1. INTRODUCTION

Konjac flour is a root flour from *Amorphophallus konjac* and is traditionally used as a food in Japan and some other Asian countries. The flour usually contains low starch content (2%) and the main component is glucomannan (70–90%) [1], which is composed of D-mannose and D-glucose residues linked via β-1,4 glycosidic bonds [2]. The molecular weight of konjac glucomannan ranges from 500–2,000 kDa. Konjac glucomannan is a water-soluble dietary fiber and is able to absorb high amounts of water [3].

Oligosaccharides, particularly those with dietary fiber functions, are of interest to researchers and consumers because they add various health benefits to food products. Many groups of oligosaccharides have been studied with respect to their preparation and functionality. Processes for producing konjac glucomannan-derived oligosaccharides have also been studied, particularly hydrolysis with the enzyme mannanase [4, 5]. Chen et. al. [5] reported the optimum conditions to give high yield of oligosaccharides as a reaction time of 3.4 h and an enzyme to substrate ratio of 0.49 which clearly demonstrated that the enzymatic process is a time consuming and high-cost process.

Subcritical water (superheated water), which is liquid hot water in the temperature range of 100–374 °C under pressurized conditions, has been studied as a medium for the hydrolysis of several macromolecules to value-added smaller molecules. Subcritical water treatment is one of "green chemistry" techniques because water is cheap, non-toxic, and non-explosive. Hydrolysis of carbohydrates into oligosaccharides by subcritical water treatment is gaining much interest [6]. We have reported that the hydrolysis of coconut meal, which consists of a mannan polymer, to produce manno-oligosaccharides using subcritical water can be accomplished within 15 min [7].

The objective of this study was to demonstrate the feasibility of using subcritical water treatment to hydrolyze konjac flour into oligosaccharides.

2. MATERIALS AND METHODS

2.1 Materials

Konjac flour was purchased from a local market in Japan. The neutral sugar composition of the konjac flour determined by high-pressure anion-exchange chromatography with pulse amperometric detection (HPAEC-PAD) included mannose (59.7%), glucose (39.7%), galactose (0.3%), fucose (0.2%), and fructose (0.1%). 3,5-Dinitrosalicylic acid (DNS), potassium sodium tartrate tetrahydrate, and ethanol (99.5%) were purchased from Wako Pure Chemical Industries (Osaka, Japan).

2.2 Subcritical Water Treatment

Konjac flour (5 g) was dispersed in distilled water (1000 mL) and stirred at room temperature for 1 h using a magnetic stirrer. The obtained viscous solution (80 mL) was poured into a stainless steel vessel with a net volume of 117 mL (Taiatsu Techno, Osaka, Japan), heated using a 200-W ribbon heater (Heater Engineer, Tokyo, Japan) controlled with a temperature controller (TXN 700B, As One, Osaka, Japan) to the desired temperature (160 °C, 180 °C, 200 °C, 220 °C, or 240 °C), and kept at the temperature for 10 min. The temperature inside the vessel was recorded every 1 min using a type K thermocouple. The heating times to reach each temperature were 23, 30, 32, 36, and 41 min, respectively.

2.3 Viscosity and pH

The hydrolysate was filtered with No. 2 Advantec filter paper (Tokyo, Japan) to remove dispersing solid particles, and the viscosity of the filtrate was determined at 25 °C with the sine-wave vibro viscometer (SV-10, A&D, Tokyo, Japan). Briefly, 35 mL of sample was poured into the sample cup and the two thin sensor plates were moved down into the sample. The instrument then measured the electric current required to resonate the sensor plates. The pH value of the filtered samples was measured by a pH meter (D-51, Horiba, Kyoto, Japan).

2.4 Reducing Sugar Content

The reducing sugar content of the hydrolysate was measured using the DNS method [8]. DNS reagent (1% w/v) was prepared by dissolving 1 g DNS in 20 mL of 2 mol/L NaOH while warming on a hot-plate stirrer. Potassium sodium tartrate (30 g) was then added and the mixture was filled to 100 mL with distilled water. The sample (1 mL) was mixed with the DNS reagent (1 mL) and heated in boiling water for 15 min. After immediate cooling with ice water, the absorbance at 540 nm was measured using a spectrophotometer (UV-1600, Shimadzu, Kyoto, Japan). Glucose solutions (100–500 mg/L) were used to prepare a standard curve.

2.5 Fractionation of Saccharides with Ethanol

The hydrolysate (1 mL) was combined with ethanol (2 mL) and mixed well to precipitate large carbohydrate polymers. The mixture was filtered through No. 2 Advantec filter paper.

2.5.1 UV-Vis Absorption

The filtrate was analyzed for UV-Vis absorption after appropriate dilution to examine the formation of UV-absorbing compounds and color development during the treatment.

2.5.2 High-Pressure Gel Permeation Chromatography

The filtrate (0.5 mL) was dried using nitrogen blowing at 40 °C and 200 uL of distilled water was added to re-dissolve the residue. The solution was then subjected to high-pressure gel permeation chromatography (HP-GPC) using an LC-10Avp solvent delivery unit and a refractive index detector (RID-10A, Shimadzu, Kyoto, Japan). Chromatographic separation was performed on a YMC-Pack Diol 120 Column (8.0 mm I.D. × 500 mm; YMC, Kyoto, Japan) using distilled water at a flow rate of 1.0 mL/min as a mobile phase.

2.7 Statistical Analysis

The subcritical treatments were conducted in duplicate. Analysis of variance (ANOVA) with Tukey's HSD post-hoc test was performed using R [9] to indicate the difference of analysis results.

3. RESULTS AND DISCUSSION

3.1 Appearance, Viscosity, pH, and UV Absorption

Konjac flour formed a viscous solution via absorption of water at very high rate. The appearance of the samples obviously changed after the treatments at different temperatures (Figure 1, inset). The viscous konjac flour solution at 5 g/L obtained after stirring for 1 h (Untreated) appeared translucent and showed a very high viscosity of *ca.* 150 mPa·s. The translucent appearance of solution might be because konjac glucomannan molecule is very large (500–2,000 kDa) [3]. After the treatment under subcritical water conditions for 10 min, the viscosity rapidly decreased as the treatment temperature increased (Figure 1), and the solution became transparent yellowish at 160–200 °C and dark brown at 240 °C. The transparent appearance and decreased viscosity was the primary evidence for hydrolysis of carbohydrate polymers into short chains of oligomers and monomers. The hydrolysis of polysaccharides into oligosaccharides and monosaccharides has been demonstrated for many materials, such as lemon peel [10] and lignocellulosic materials (e.g., tobacco stalk, cotton stalk, sunflower stalk, and wheat straw) [11]. The pH values of the treated solutions also gradually decreased from 5.3 to 3.2, indicating the formation of acid products during the subcritical water treatment. The majority of acidic products might come from the degradation of monosaccharides, such as mannose and glucose, via a dehydration reaction [12].

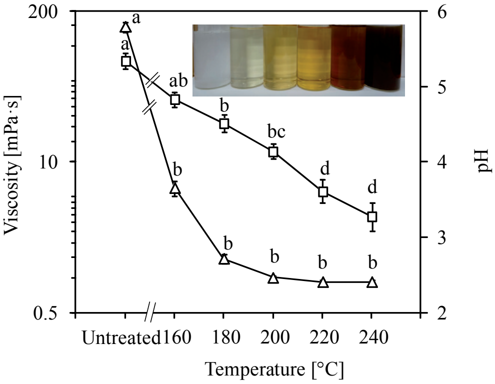

Figure 1. Viscosity (△) and pH (□) of hydrolysates obtained by subcritical water treatment of konjac flour. Inset photograph shows (from left to right) untreated sample and treated samples at 160 °C, 180 °C, 200 °C, 220 °C, and 240 °C. Different letters indicate significant difference in mean values (Tukey's HSD, $p < 0.05$).

During the degradation process of sugars in subcritical water, several intermediates are formed [12, 13]. The UV absorption spectra

(Figure 2) revealed that high treatment temperatures were associated with high absorbance values, around 280-285 nm, which might represent the degradation products from carbohydrates, especially furfural and 5-hydroxymethyl-2-furaldehyde [14, 15]. This also agreed with the observed color changes of the hydrolysates.

3.2 Reducing Sugar Content and HP-GPC

Subcritical water increases its ability to promote hydrolysis reaction as temperature increases [6]. The reducing sugar content of the hydrolysates indicated the number of reducing ends of the hydrolyzed polysaccharide chains and could be used for estimating the degree of polymerization [5]. As shown in Figure 3, the reducing sugar content increased slightly at 160 °C and increased drastically at 180 °C, but there were no significant differences in content between the samples treated at 220 °C and 240 °C. This confirmed the results in 3.1 that at 240 °C the degradation of carbohydrates might extensively occurred. We estimated the carbohydrate yield from the reducing sugar content. The reducing sugar contents of the samples treated at 220 °C and 240 °C were both approximately 2.3 g/L, indicating that the yield was greater than 46%, since the starting konjac flour solution was 5 g/L.

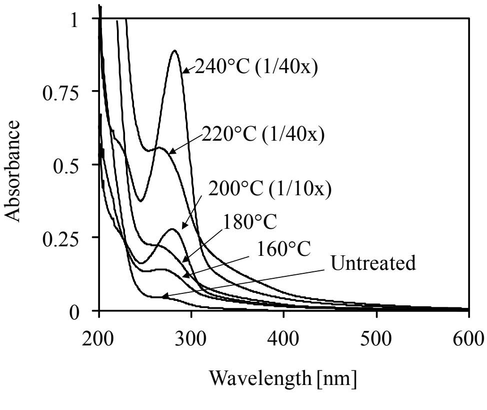

Figure 2. UV-Vis absorption of hydrolysates obtained by subcritical water treatment of konjac flour with various dilution factors.

As shown in Figure 4, HP-GPC clearly showed that the hydrolysis degrees were different among samples treated with different temperatures. Note that the samples used for GPC were fractionated using ethanol and therefore only certain sizes of polymers, oligomers, and monomers were contained in the samples. The peak at about 15 min represented the peak of monomers and low-molecular mass oligomers.

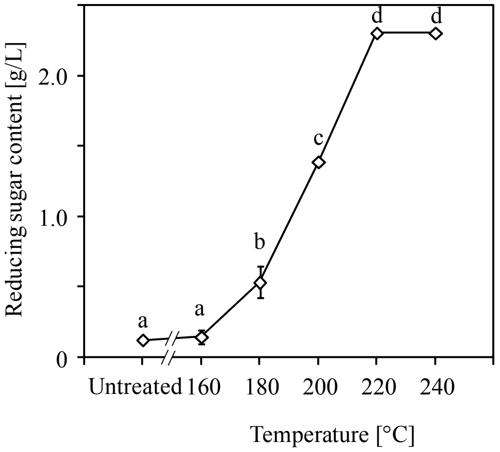

Figure 3. Reducing sugar content of hydrolysates obtained by subcritical water treatment of konjac flour. Different letters indicate significant difference in mean values (Tukey's HSD, $p < 0.05$).

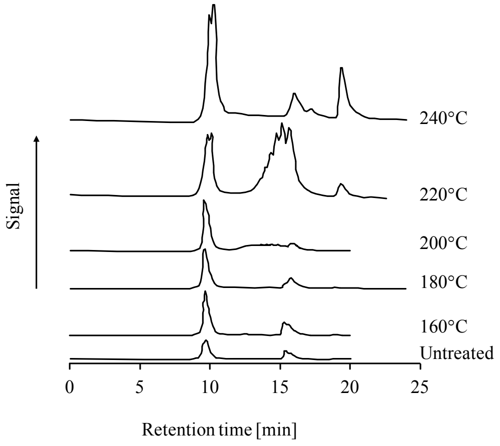

Figure 4. HP-GPC chromatograms of hydrolysates obtained by subcritical water treatment of konjac flour at various temperatures.

The chromatograms showed that the samples without treatment and those from the subcritical water treatments at 160–200 °C contained only high-molecular mass polymers and small amounts of monomers or low-molecular mass oligomers. This corroborated that only a small amount of glucomannan polymer was converted to shorter oligomers at these treatment temperatures. However, the sample obtained at 220 °C had a distinctive chromatogram in which the high-molecular mass polymer peaks were somehow smaller, but the band of peaks for several sizes of oligomers appeared at a relatively greater amount. At 240 °C, the chromatogram showed a different pattern. At this treatment temperature, a group of oligosaccharide peaks decreased and a peak at longer retention time (*ca*. 20 min) was observed; this might represent smaller molecules such as degradation products [12, 13], which were discussed above.

4. CONCLUSIONS

In this study, we found that subcritical water treatment could be used as an alternative method for hydrolyzing konjac glucomannan in konjac flour. The treatment at 220 °C can be considered the optimal condition for obtaining konjac glucomannan-derived oligosaccharides with several degrees of polymerization and a yield that exceeds 46%.

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