Enhancing Saccharide Extraction from Konjac by Microwave-assisted Acid Hydrolysis

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Abstract

This article presents a study on the use of microwave-assisted acid hydrolysis to produce glucomannan oligosaccharides from konjac powder. Acid hydrolysis is a well-established method for the depolymerization of polysaccharides. This study aims to optimize the conditions of acid hydrolysis using microwave radiation to achieve high yields and specific saccharide sizes. Konjac powder was hydrolyzed using hydrochloric acid in a microwave digestion instrument. The optimal conditions for hydrolysis were determined based on previous research, and the resulting soluble oligosaccharides were purified by ethanol precipitation. The carbohydrate content and size distribution of the hydrolyzed products were analyzed by using phenol-sulfuric acid assay and size exclusion chromatography, respectively. This study showed that microwave-assisted acid hydrolysis is a promising method for the preparation of glucomannan oligosaccharides from konjac powder, offering advantages such as shorter reaction time and lower acid catalyst requirement. The results contribute to the understanding of konjac powder hydrolysis and provide insights for the development of efficient and sustainable production processes for glucomannan oligosaccharides.

Keywords: Acid hydrolysis, Microwave radiation, Ethanol extraction, Konjac

1. Introduction

Konjac (Amorphophallus konjac) has been utilized by humans in multiple ways, encompassing medical applications like detoxification, as well as in food additives and dietary supplements such as noodles and rubbery jelly [1]. The primary polysaccharide found in konjac is glucomannan, composed of β-1,4-linked D-glucose and D-mannose monomers with a ratio of 1.0 to 1.6 (w/w) [2]. Interestingly, glucomannan oligosaccharides have demonstrated superior prebiotic activity compared to glucomannan polysaccharides, as evidenced by both in vivo and in vitro studies. Glucomannans derived from konjac powder (KP) offer significant health benefits and are consumed for medicinal purposes, including selective stimulation of gut-friendly bacteria growth and their value as functional foods [3]. It is noteworthy that konjac contains approximately 19.2% glucomannan oligosaccharides [4].

Numerous extraction methods have been explored to extract glucomannans from konjac, including acid degradation, enzymatic hydrolysis, oxidative degradation, and physical methods [4]. However, these methods are often time-consuming. Furthermore, the enzymatic

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method presents limitations such as the complexity of the preparation process, high cost, and the requirement for specific temperature ranges for enzyme activity.

Acid hydrolysis is a relatively simple, inexpensive, and easy-to-control process [5]. Sulfuric, hydrochloric, and trifluoroacetic acids are commonly used for polysaccharide depolymerization. Generally, the process is optimized at higher temperatures, above 60 °C, and the time often varies, averaging 2-6 hours [6]. The regulation of acid hydrolysis involves meticulous monitoring of the acid concentration or pH in the medium where polysaccharides are dispersed. For instance, Avila-Fernandez *et al.* employed hydrochloric acid at a concentration of 0.54 N to facilitate the production of fructooligosaccharides (FOS) from agave fructans [7]. In a similar vein, Hu *et al.* utilized trifluoroacetic acid with a molarity of 0.2 M to hydrolyze polysaccharides derived from N. indicum, yielding a diverse range of oligosaccharides with distinct molecular weights [8]. Remarkably, the original polysaccharides, initially ranging from 670 to 5 kiloDaltons (kDa), were successfully reduced to a remarkable range of 5,000 to 324 Daltons (Da) through the application of 1.38 M sulfuric acid, as reported by Du *et al.* [9]. However, acid hydrolysis does have drawbacks, including the degradation of monosaccharides, leading to the sequential formation of toxic compounds such as furfural and 5-hydroxymethylfurfural, as well as a reduced yield of oligosaccharides. Additionally, the oligosaccharides obtained from the process exhibit a wide range of chain lengths [5], [10].

In a study by Warrand & Janssen, amylose was hydrolyzed using 0.45 M hydrochloric acid at 90°C with both microwave radiation heating and conventional heating methods [5]. Microwave radiation heating proved to be more efficient. Microwave radiation is widely employed for generating heat in various industries such as food processing, drying, polymers, and organic synthesis [1]. Hence, microwave radiation is utilized to hydrolyze konjac powder (KP) and obtain smaller saccharides. When compared to conventional heating for complete digestion of KP into various saccharide sizes, microwave radiation requires less time for complete hydrolysis. Furthermore, the amount of acid catalyst necessary is reduced by twenty-fold. Importantly, this heating method does not compromise the structure or quality of the products. In the present study, the conditions for acid hydrolysis with microwave radiation are investigated to achieve high yield and specific saccharide sizes [1]. A combination of microwave radiation and acid hydrolysis is employed to extract glucomannans from konjac powder.

2. Materials and methods

Konjac was purchased from Kanchanaburi province, Thailand. Hydrochloric acid for the hydrolysis reactions, ethanol for the precipitation process, and phenol and sulfuric acid (95-97%) for total carbohydrate determination were purchased from Merck (Germany). Glucose and mannose used for standard calibration were purchased from Ajax Finechem.

The outer layer of konjac was carefully removed prior to slicing it into thin, small pieces. These slices were subsequently washed with water and arranged meticulously on a sturdy metal tray. To achieve a consistent weight, the samples were then subjected to drying in an oven set at 60°C. Once the biomass reached a constant weight, a household blender was employed to finely blend the dried biomass. The resulting konjac powder was sieved through a mesh size of 0.25 mm and then securely stored in an airtight container, ensuring its preservation for subsequent analysis.

The experimental setup involved the utilization of a microwave digestion instrument (CEM, USA, Discover SP 909155) for the purpose of hydrolyzing konjac dried powder.

Konjac powder was hydrolyzed using microwave radiation in combination with hydrochloric acid. The konjac powder, along with a magnetic bar, was combined with diluted HCl in a closed vessel with a capacity of 35 mL. The experimental parameters were carefully calibrated to achieve optimal results, with a maximum power of 100 watts, a maximum pressure of 200 psi, a ramping time of 10 minutes, a microwave time of 15 minutes, and a cooling time of 20 minutes. These conditions were chosen based on the insightful optimization research conducted by Cuong Viet Bui, which highlighted that a combination of 2 M HCl, a temperature of 110°C, and a duration of 15 minutes selectively hydrolyzes konjac powder into glucomannan oligosaccharides [1].

Following the completion of the hydrolysis reaction, the samples were carefully cooled to room temperature for 20 minutes and subsequently stirred for a duration of 1 hour, facilitating the separation of the soluble and insoluble fractions. To accomplish this, the mixture was subjected to filtration employing a vacuum pump. The resulting liquid portion was neutralized by utilizing a sodium hydroxide solution (10 M) and then stored in a small container for further analysis. Meanwhile, the filter paper containing the solid residue was placed in an oven to undergo drying (60°C, overnight reaching a constant weight). The solid residue remaining on the filter paper was utilized to determine the extent of solid loss. Solid loss serves as a pivotal parameter for assessing the extent of hydrolysis within a given system. A higher magnitude of solid loss corresponds to a greater magnitude of hydrolysis that has transpired.

The solid loss was calculated by the equation (1).

$$Solid\ loss\ (\%) = \frac{IS-RS}{IS} \times 100 \quad (1)$$

IS: Initial dried solid (g), *RS*: Residual dried solid (g).

2.1 Purification of soluble oligosaccharides by precipitation in ethanol

The soluble oligosaccharides obtained from the hydrolyzed konjac powder were then purified through ethanol precipitation. The liquid portion obtained was carefully combined with ethanol in a 50 mL centrifuge tube. Two different ratios of sample to ethanol were employed, namely 1:5 and 1:9. Subsequently, the samples were subjected to agitation in a shaking incubator under specific conditions, including a temperature of 4°C, a duration of 18 hours, and a shaking speed of 150 rpm. Afterwards, the samples were centrifuged using a SCILOGEX D3024R centrifuge from the USA. A predefined condition was established with a rotation speed of 3,000 rpm and a duration of 20 minutes. This centrifugation step effectively separated the solid components from the supernatant. The solid portion was then carefully transferred to an oven for drying. The solid precipitate was determined for total carbohydrate content and subsequent molecular size by a high-performance liquid chromatography (HPLC) equipped with a size exclusion column.

2.2 Total carbohydrate determination

Total carbohydrates were measured by the phenol-sulfuric acid assay [1]. Five milligrams of the solid precipitate were mixed with 5 mL of RO water and then further diluted by adding 1 mL of the sample to 9 mL of RO water. After dilution, the samples were transferred to test tubes with a volume of 1 mL. To each test tube, 1 mL of 5% aqueous phenol solution and 5 mL of 95-97% sulfuric acid were added. The resulting mixture was thoroughly mixed and incubated in a water bath at 25°C for 20 minutes. UV-Vis spectrophotometry (Thermo Fisher Scientific, G10S UV-VIS, USA) was utilized to measure the absorbance of the samples at 490 nm under all conditions. A blank solution consisting of a mixture of RO water, 5% aqueous phenol solution, and sulfuric acid (95-97%) in a ratio of 1:1:5 (v/v/v) was used for reference.

To construct a standard curve, aqueous solutions of mannose and glucose were prepared at different concentrations with a ratio of 1.6:1 (w/w), considering the predominance of glucomannan in konjac powder. The absorbance values of these standard solutions were also measured. The total carbohydrate content in the hydrolyzed product was reported as a percentage. This assay provides a reliable method for quantifying the carbohydrates present in the hydrolyzed product, allowing for accurate assessment and comparison of carbohydrate content in different samples.

2.3 Size determination by size exclusion chromatography

The solid precipitate was diluted before being injected into the HPLC column. Five milligrams of the solid precipitate were mixed with 5 mL of RO water, and then further diluted by adding 1 mL of the sample to 9 mL of DI water. Following dilution, the samples underwent filtration through a 0.2 µm membrane, and 100 µL of the resulting filtrate was injected into the high-performance liquid chromatography (HPLC) system. In this study, an Agilent 1260 Infinity HPLC system (G1329B, Germany) was employed to separate the sample based on size using Size Exclusion Chromatography (SEC). For size exclusion, a PL aquagel - OH column (PL aquagel - OH 20 SEC columns, 5 µm, 7.5 x 300 mm) with a guard column (PL aquagel).

3. Results and discussion

Konjac powder was hydrolyzed using microwave radiation in combination with hydrochloric acid. The intriguing outcome of this process revealed a remarkable solid loss percentage of 88.3±0.5%, indicating a highly pronounced degree of hydrolysis. To further investigate the system, a subsequent step involved the precipitation of the hydrolyzed product using ethanol under two distinct conditions, namely a 1:5 and a 1:9 ratio of aqueous sample solution to ethanol. After the precipitation step, the resulting solution was meticulously separated into a supernatant and a solid portion. Notably, the solid yield obtained from the 1:5 ratio precipitation exhibited a substantial increase compared to that obtained from the 1:9 ratio. The observed precipitated yields showcased their typical values at 7.8±0.3% for the 1:5 ratio and 6.9±0.2% for the 1:9 ratio, highlighting the significant impact of the precipitation conditions on the final solid yield.

Both precipitated solids (at 1:5 and 1:9 ratio) were further analyzed for the contents of soluble saccharides. It should be noted that after the precipitation process, some solids may become tightly packed and insoluble. Therefore, this protocol was implemented to assess the proportion of soluble saccharides present in the precipitated solid fraction. The solids were redissolved in water, and the solutions were subjected to total carbohydrate determination by the phenol assay. Total carbohydrate determination is used to determine the amount of total sugars present by breaking all the bonds with sulfuric acid and measuring the amount of fructose, mannose, and glucose released. The percentage of soluble saccharides is 49.4±2.2% and 35.8±1.8% for 1:5 and 1:9 precipitation ratios, respectively (Fig. 1). From the results of the total carbohydrate of soluble polysaccharides of the precipitated products, the precipitation with a ratio of 1:5 gave higher soluble saccharides than the ones with the 1:9 ratio. The contents of the soluble carbohydrates are rather low because the solid samples are not totally dissolved. However, this method did not determine the exact value of total carbohydrates since the solid does not totally dissolve after precipitation.

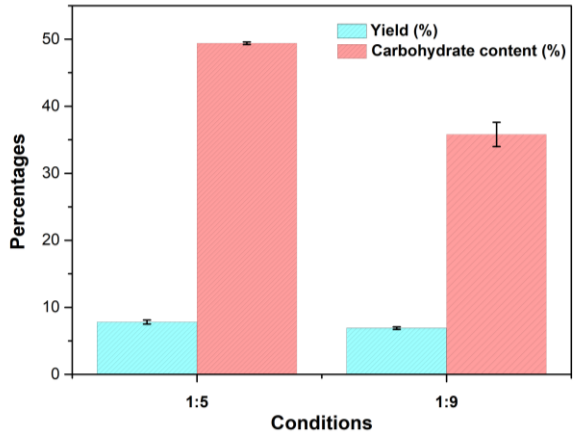

Fig. 1. Yields and carbohydrate contents of precipitated solids from two different precipitation conditions

a)

b)

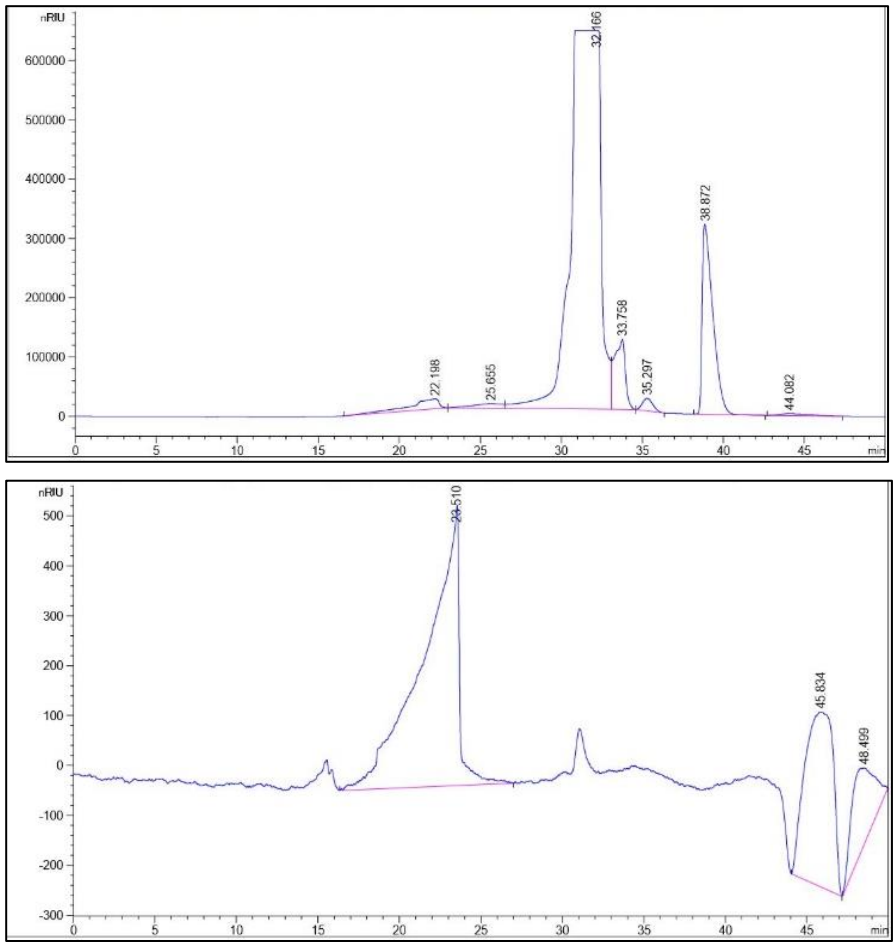

Fig. 2. HPLC chromatogram of a) supernatant and b) precipitated solid

Table 1. Size of carbohydrates after precipitation

No. of sugar unit	Molecular Weight (g/mol)	Retention time (min)	Percent Content (%)	
			Sample : Ethanol	
			1:5	1:9
1 – 2	180 – 342	31.1 – 38.9	–	–
2 – 10	342 – 1,638	29.4 – 26.1	–	–
11– 20	1,800 – 3,258	25.9 – 24.6	–	–
21 – 50	3,420 – 8,118	24.5 – 22.7	–	–
51 – 100	8,280 – 6,218	22.6 – 21.2	–	–
101 – 1,000	16,380 – 162,018	21.2 – 16.3	66.3 ± 0.7	78.5 ± 1.6
> 1,001	> 162,180	< 16.3	33.7 ± 0.7	21.5 ± 1.6

The size of the saccharides in the precipitated solid was determined by size exclusion chromatography. The HPLC chromatograms are shown in Fig. 2. The number of repeating units in the samples may have effects on the biological activities of the saccharides. The sizes of the soluble portion of the precipitated solid, after redissolving in water, are reported in Table 1. From size exclusion chromatography analysis for the degree of polymerization performed on the soluble saccharides from the solids precipitated in both conditions. Two ranges of size were observed: 101-1,000 units and more than 1,000 units. Meanwhile, the supernatant of both precipitated conditions shows the same degree of polymerization of 1-2.

The observation that the 1:5 ratio precipitation resulted in a higher carbohydrate yield and bigger saccharide size can be attributed to the greater ethanol content in the 1:9 ratio precipitation, which facilitated the gelation of konjac glucomannan. Zhou *et al.* have demonstrated that konjac glucomannan exhibits gelation properties when exposed to a solvent system containing ethanol [11]. The presence of ethanol promotes the formation of gel among konjac glucomannan molecules. As a result, less solid was precipitated in the 1:9 ratio conditions, as some konjac glucomannan remained in the solution in the form of a gel.

4. Conclusion

In conclusion, acid hydrolysis, particularly when combined with microwave radiation heating, offers a simple and cost-effective method for depolymerizing polysaccharides such as glucomannan from konjac powder. This approach allows for the selective production of glucomannan oligosaccharides with improved prebiotic activity by reducing polymer size. Furthermore, the purification of soluble oligosaccharides through ethanol precipitation provides a means to obtain specific saccharide sizes, which can have applications in the food and medical industries. These findings contribute to the exploration of efficient extraction methods for valuable functional ingredients from natural sources like konjac.

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