Effect of catalyst concentration and reaction time on the extraction of glucomannan from porang (*Amorphophallus oncophyllus*) flour via acid hydrolysis

A C Kumoro¹,², T H A Yuganta², R Ratnawati¹ and D S Retnowati¹

¹ Department of Chemical Engineering, Faculty of Engineering, Diponegoro University. Prof. H. Soedarto, SH Road-Tembalang, Semarang-INDONESIA 50275
² Master of Chemical Engineering Program, Department of Chemical Engineering, Faculty of Engineering, Diponegoro University. Prof. H. Soedarto, SH Road-Tembalang, Semarang-INDONESIA 50275

E-mail: andrewkomoro@undip.ac.id

Abstract. High demand of glucomannan for various applications has attracted the attention of researchers to look for efficient extraction method from its botanical sources. The aim of this study is to investigate the effect of catalyst concentration and reaction time on the yield and purity of glucomannan, and profile of reducing sugar during glucomannan extraction from crude porang flour via acid hydrolysis. The effect of catalyst concentration was found to be more pronounced over the effect of reaction time. When catalyst concentration was varied from 0.03125 to 1 M, extraction of glucomannan from porang flour for 1 hour at 60°C yielded 40.5 to 70% glucomannan with purity of 47.35 to 90.18% (w/w). The yield and purity of glucomannan obtained from extraction using catalyst concentration of 1 M for 0.25 to 3 hour ranged between 49 to 66.67% and 58.32 to 90.18% (w/w), respectively. Reduction in glucomannan yield and purity observed at high catalyst concentration and prolong reaction time was likely to be due to over-decomposition. Glucomannan with highest purity (90.18% w/w) was obtained at 66.67% yield from acid hydrolysis of porang flour using 0.5 M hydrochloric acid solution with flour:water ratio of 1:50 at 60°C for 1 hour.

1. Introduction

Glucomannan is a neutral polysaccharide composed mainly of β, 1-4 linked D-mannose and D-glucose with molar ratio of 1.6:1. The backbone is lightly branched, which short side branches at the C-3 position of the mannoses. Some acetyl groups randomly present at the C-6 position of the sugar units [1]. This acetyl groups, which is believed to contribute to the solubility and gelling properties are located every 9 to 19 sugar units. Those properties are related to high water absorbance (100 g of water per g of glucomannan), and its high molecular weight, $10^5 – 10^6$ g/mol. Glucomannan has been used as traditional Chinese medicine for thousands of years for treatment of asthma, cough, hernia, breast pain, burns and skin disorders [2]. Recently, clinical studies have proven the ability of glucomannan to lower blood sugar, lower blood cholesterol, helping weight loss and promote healthy micro flora in intestinal [3]. With regard to biodegradability and good gel-forming ability, glucomannan is also used in pharmaceutical field for drug delivery system [4]. In addition, due to unique rheological and gelling properties glucomannan is also used in food industries as emulsifier and stabilizer for foods, drinks, and cosmetics products [2].

Glucomannan can be obtained from various botanical sources. However, the tuber of porang (*Amorphophallus oncophyllus*), which belongs to the family of Araceae, has been reported to be the most potential source due to highest glucomannan content and largest availability [5]. Because porang plant is sensitive to high temperatures, dry atmosphere and high wind [6], it is cultivated in Indonesia as secondary crops under shady environment such as the teak, mahogany or sonokeling plantation [7]. Generally, the glucomannan content in porang flour is about 50% to 70% [8]. The impurities trapped in
glucomannan molecules are starch, protein, and insoluble sugar, which are usually derived from tissue space in the tuber [2]. Starch is the main impurity that seriously affects the purity and quality of glucomannan, especially reducing viscosity and increasing turbidity [9]. In addition, starch molecules usually interact with glucomannan and make them even more difficult to be separated for its low solubility under mild condition. However, gelatinization behaviour efficiently boosts its solubility. In fact, glucomannan molecules possess better swelling ability and become insoluble in aqueous ethanol, whereas the impurities do not. Gelatinization temperature of porang flour is between 68.5-77.6 °C [10]. These properties are beneficial for extraction of glucomannan from porang flour by removal of the starch via hydrolysis [11] and followed by precipitation of glucomannan by addition of alcohol [12]. Based on the swelling property in alcohol solution, ethanol precipitation is the most common and practical method to purify glucomannan and to remove the impurities on the surface or trapped inside of porang flour particles [13]. Glocumannan has lowest solubility when ethanol concentration in the solution of about 45% v/v by which its separation from the solution may occur via precipitation [14]. Alcohol contributes to unfold glucomannan molecular chain and expose the impurities trapped in the macromolecules to the solution for easier removal.

The traditional processing methods to obtain purified porang flour are by dry or wet processing which can separate starch granules from larger glucomannan granules. The dry processing method includes milling of dried porang tuber chips into crude porang flour, which is subsequently purified via wind-sifting [15]. Porang flour obtained using such method is of low purity and is usually marketed as food commodity at a low price [14]. Wet methods, which include enzymatic hydrolysis, ethanol washing, centrifugation may results high purity of glucomannan, but at higher operating cost [2]. Therefore, it is clear that more efficient technologies are required to be developed to replace the existing porang flour glucomannan purification technology. In the case of glucomannan extraction via starch hydrolysis, the use acid hydrolysis has never been broadly explored. Operating parameters such as temperature, reaction time, acid concentration and solid-to-liquid ratio play critical roles in obtaining optimum acid hydrolysis of starch [16]. In general, the catalytic activity of hydrochloric acid during hydrolysis of starch is higher than that of sulfuric acid [17] and phosphoric acid [18]. This high catalytic activity, non disturbing residue after neutralization (sodium chloride) on glucomannan precipitation using alcohol and its simplicity are the advantages of using hydrochloric acid as catalyst. The drawback of acid hydrolysis is merely slower reaction rate as compared to enzymatic hydrolysis [2]. The governing factor of the homogeneous acid catalysis turned out to be the acidity (pH) of the solution, which is directly proportional to the concentrations of the hydroxonium ions (H₃O⁺) exist in the solution [19]. On the other hand, the temperature and the reaction time have less influence on the hydrolysis reaction [20].

The aim of this study is to investigate the effect of catalyst concentration and reaction time on the yield and purity of glucomannan, and profile of reducing sugar during glucomannan extraction from porang flour via acid hydrolysis.

2. Materials and Method
2.1. Materials
The crude porang flour used in this research was purchased from PT. Prima Agung Sejahtera-Surabaya. Other chemicals used for extraction and analysis were of analytical grade (purity ≥ 98% w/w) and purchased from Sigma Aldrich via authorized chemicals distributor in Semarang-Indonesia. All the chemicals were used directly without further treatments.

2.2. Method
The glucomannan extraction from porang flour was performed using acid hydrolysis employing hydrochloric acid as catalyst. The details of the experiments are as follows:
2.2.1. Glucomannan extraction

Ten grams of crude porang flour was suspended in 500 mL hydrochloric acid solution of predetermined concentration in a 1000 mL three-neck round bottom flask at room temperature. The mixture was heated in a water bath heater to achieve reaction temperature at 60°C under continuous stirring. Reaction temperature was kept at 60°C and continuous stirring was performed to allow acid hydrolysis of starch. When a desired reaction time (0.25, 0.5, 1, 2 and 3 hours) was achieved, the reaction was terminated by quenching of the reaction mixture to room temperature using chilled water. Fifty millilitres sample was withdrawn for reducing sugar analysis. The rest of the reaction mixture was then diluted to 1000 mL with demineralised water, prior to centrifugation (9000 ×g, 30 min, 25°C) to remove the insoluble materials. Subsequently, rotary evaporation is performed to reduce the volume of the filtrate to ∼1/3 the original volume. Glucomannan presents in the solution is precipitated overnight with addition of 95% (v/v) ethanol at 4°C, followed by centrifugation (9000 ×g, 40 min, 25°C). The resultant pellet was washed twice with anhydrous ethanol and subsequently isolated by vacuum filtration, before being freeze-dried for 48 h. The dried material was ground and sieved to produce purified glucomannan and was then subjected to yield and purity analysis.

2.2.2. Analysis

Starch content of porang flour was determined by acid hydrolysis method [21]. Total nitrogen analysis was conducted using the Kjeldahl method for protein determination [22]. The protein content was then calculated by applying the nitrogen conversion factor of 5.7, as proposed by the U.S. Food Chemicals Codex (FCC) and European Commission [15]. The reducing sugar of the sample was determined using phenol-sulfuric acid colorimetry [23]. While the glucomannan purity was analysed based on the reducing sugar content of the hydrolysate and porang flour sample solution as previously used by Chua et al. [24].

3. Results and Discussion

As the reaction proceeded, it was obvious that the dispersed porang flour granules absorbed water and swelled. As the heat was continuously supplied to the reaction mixture, the starch in the flour underwent gelatinization. Therefore, the shearing effect of stirring is expected to intensively get rid of the gelatinized outer part of the starch molecules and dissolves into the solution [25]. Thus, hydrolysis will soon take place.

3.1.1. Chemical composition of crude porang flour.

Prior to extraction study, porang flour was subjected to composition analysis. The results are presented in Table 1.

| Component      | This work | Literature [26] |
|----------------|-----------|-----------------|
| Glucomannan    | 57.56     | 59-60           |
| Starch         | 24.64     | 10-30           |
| Protein        | 7.43      | 5-14            |
| Soluble sugar  | 4.86      | 3-5             |
| Ash            | 5.51      | 3.4-5.3         |
| Inorganics     | -         | 2.6-7           |

It is clear in Table 1 that glucomannan and soluble sugar contents of the porang flour used in this study were lower than those of porang flour obtained from the corm of porang plant grown in China [26], but still within the range of glucomannan content of porang flour produced in Thailand [8]. The protein content fell within the range of that mentioned in the literature. However, the starch content was slightly on the upper level of that given in the literature [26]. This suggests more extensive starch removal via acid hydrolysis is required to obtain high purity glucomannan. The differences in
chemical composition of porang flour may be affected by ecological environment, growing condition and harvest time [27]. In addition, the utilization of fertilizers, shades, planting densities and flour manufacturing process also may significantly influence the chemical composition of porang flour [13, 28, 29].

3.1.2. Effect of catalyst concentration
The effect of catalyst concentration ranging from 0.03125 M to 1 M was studied by hydrolysis at 60°C using flour: water ratio of 1:50 for 1 hour. The results are shown in Figure 1 and Figure 2.

![Figure 1](image1.png)

![Figure 2](image2.png)

**Figure 1.** Yield and purity of glucomannan at various catalyst concentrations

**Figure 2.** Reducing sugar at various catalyst concentrations

As seen in Figure 1, glucomannan with highest purity (90.18% w/w) was obtained from hydrolysis of porang flour using 0.5 M hydrochloric acid solution. Since homogeneous acid hydrolysis of starch is governed by the acidity (pH) of the solution, which is directly proportional to the concentrations of the hydroxonium ions (H$_3$O$^+$) exist in the solution [19], then the concentration of hydroxonium ions at that condition is 0.5 M. Based on this principle, similar result was reported by Tanaka et al. for recovery of glucomannan from porang flour using sulphuric acid [30]. The highest recovery was achieved using 0.25 M sulphuric acid, which corresponded to 0.5 M hydroxonium ions.

Glucomannan yields varied slightly with catalyst concentrations used. However, the average glucomannan yield was 54%. Since glucomannan was obtained from a series of processing step including hydrolysis, filtration, centrifugation, vaporization, drying, grinding and sieving, then loss of glucomannan within this process was inevitable consequence.

Generally, the rate of hydrolysis and the reducing sugar formation rise with increasing acid concentration (Figure 2), possibly due to the increase in the activity of hydrogen ions taking part in the reaction as catalyst [17]. Theoretically, purity of glucomannan should increase steadily as more starch as the main impurity being hydrolyzed into reducing sugar. However, purity of glucomannan decreased when the catalyst concentration was higher than 0.5 M. Usually, the treatment with concentrated acid and/or high temperature causes the degradation of polysaccharides to form byproducts such as furfural and hydroxymethylfurfural, and thus reducing glucomannan purity [31]. Over decomposition of glucomannan into other products may have been the cause of this phenomenon [30].

3.1.3. Effect of reaction time.
The effect of reaction time ranging from 0.25 hour to 3 hour was studied by hydrolysis at 60°C using flour: water ratio of 1:50 employing 0.5 M hydrochloric acid solution as catalyst. The results are depicted in Figure 3 and Figure 4.
Figure 3 shows that yield and purity of glucomannan increase as hydrolysis goes by to 1 hour, but level off afterward. Highest yield (66.60%) and purity (90.18% w/w) of glucomannan was achieved at 1 hour reaction. Acid hydrolysis leads to partial degradation of polysaccharides into soluble lower molecular weight polymers, oligosaccharides and monosaccharides. The proportion of the soluble compounds depends on the operation conditions. Temperature, acid concentration, and reaction time are the most crucial parameters in the hydrolysis of hemicelluloses, since they affect hydrolysis rate and selectivity [32]. Degradation of polysaccharide, such as ι-carrageenan under mild acid conditions required a longer time (3 hour) than that for κ-carrageenan (1.5 hour) [33].

It is observed in Figure 4 that reducing sugar concentration increased proportionally to the reaction time in the beginning of the hydrolysis process, but level off after 1 hour. Theoretically, hydrolysis of starch will produce 60% glucose [34]. Prolong reaction time decreased the reducing sugar concentration indicating possible decomposition of glucose to degradation products. In the acid hydrolysis of polysaccharides, the acid catalyzes not only the conversion of polysaccharides into monosaccharides, but also there are further degradation reactions of monosaccharides into furan and carboxylic acids [35]. Decreased of monosaccharides from acid hydrolysis of polysaccharides due to degradation under prolong time and increased acid concentrations was also reported previously [36].

This proposed extraction method resulted in a comparable purity of glucomannan with that previously reported by Xu et al. [37] and Chua et al. [38], which is about 90% (w/w). However, the method proposed in this work is simpler, lower temperature and requires shorter extraction time. The ethanol washing technique used by Xu et al. [37] employed 40% (v/v) ethanol solution, feed:liquid ratio of 1:6, temperature 68°C for 4 hour to obtain 90% (w/w) purity. Chua et al. [38] also used ethanol washing to purify glucomannan from porang flour utilizing 50% (v/v) ethanol solution for multi stage washing at room temperature to achieve 92% (w/w) purity. The differences between the method of Xu et al. [37] and Chua et al. [38] were the temperature, ethanol concentration and the number of stages. Unfortunately, both research groups did not report the yield obtained from their experiments.

4. Conclusion
Based on the experimental results, it can be concluded that acid hydrolysis offers attractive potential as an efficient method for extraction of glucomannan from crude porang flour. Both catalyst concentration and reaction time affect the yield and purity of glucomannan significantly. Possible degradation of glucomannan and reducing sugar under high catalyst concentration and prolong...
reaction time was observed. Glucomannan with highest purity (90.18% w/w) was obtained from acid hydrolysis of porang flour using 0.5 M hydrochloric acid at 60°C for 1 hour.

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