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Purification of Glucomannan from Porang (*Amorphophallus oncophyllus*) Tuber Flour through Hydrolysis of Hydrochloric Acid

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Abstract

Porang (Amorphophallus oncophyllus) is a local perennial plant that commonly grown in Indonesian forest. Its tuber contains a lot of glucomannan that has many benefits as food ingredient or as functional food. Glucomannan has a lot of health benefits, such as anti-diabetic effect, low glycemic index, prebiotic effect, etc. Despite that, the extraction is still a problem because it is difficult to get pure glucomannan extract out of Porang flour. Thus, in this study we are going to use hydrocloric acid to extract the glucomannan from the porang flour through hydrolysis extraction. This research will test the effect of hydrochloric acid concentration on the purity of glucomannan extract obtained from porang flour, to study the effect of nonsolvent, ethanol and isopropyl alcohol on glucomannan concentration, and to obtain the optimal condition to get high concentration of glucomannan using extraction with pretreatment hydrochloric acid hydrolysis.

Keywords: catalytic hydrolysis; extraction; glucomannan; porang flour

Full length article **Corresponding Author*, e-mail: *kristinahharyani1@gmail.com* Doi # https://doi.org/10.62877/15-IJCBS-24-26-20-15

1. Introduction

Porang (Amorphophallus oncophyllus) is a local perennial plant that commonly grown in Indonesian forest. Its tuber contains a lot of glucomannan that has many benefits as food ingredient or as functional food [1]. This plant is a shrub that can be found growing in the area tropical and subtropical. Porang can grow in the shade, so it is suitable to be developed as an intercrop between types of woody plants or treesmanaged by an agroforestry system [2]. One kind of modification of porang that has been developed in society is in the form of porang flour. Porang tubers (not yet dried) contain water (83.3%), starch (7.65%), protein (0.92%), fat (0.02%), fiber (2.5%), glucomannan (3.58%), ash (1.22%), and Cu metal (0.09%), calcium oxalate (0.19%). One of the ways to make porang flour is by peeling, washing, and size reduction, drying, flouring and sifting [3]. This modified porang (porang flour) generally used as industrial raw material, chemical preparation raw material, food thickener, etc. Porang contains a lot of glucomannan which is very potential to food and health industry material [4], but it's still a problem to extract clean pure glucomannan in porang. We're going to discuss about how to extract glucomannan in porang through hydrolysis with hydrochloric acid. Glucomannan (GM) is a polysaccharide of mannan family, very abundant in nature, specifically in softwoods (hemicellulose), roots, tubers and many plant bulbs [5]. Glucomannan consists of a polysaccharide chain of β-D-glucose and β-D-mannose with attached acetyl groups in a molar ratio of 1:1, 6 with β-1, 4 linkages [6]. Glucomannan has a lot of health benefits, such as anti-diabetic effect, anti-hypercholesterolemia, low glycemic index, prebiotic effect, and laxative effect. And how to extract this from porang flour is by hydrolysis extraction process with hydrochloric acid. Porang is one of glucomannan potential source in Indonesia due to its high concentration of glucomannan content [4]. Porang was exported by an Indonesian farmer in great quantities, but at a low price because it is sold as chips. To increase the value, it is crucial to get high concentration of glucomannan from porang flour [1]. Glucomannan has some advantages such as its ability to overcome obesity, reduce the fat storage process through extra and intracellular mechanisms, and lower blood cholesterol levels [7]. Consequently, Porang cultivation has a very big potential in industry going forward especially if it's about the glucomannan which will be discussed in this study. Even though porang contains a lot of glucomannan, the extraction is still a problem because it is difficult to get pure glucomannan extract out of Porang flour.

Thus, in this study we are going to use hydrocloric acid to extract glucomannan from the Porang flour through hydrolysis extraction. In this experiment, eter bond in starch from porang flour would be broken using hydrochloric acid

(hydrolysis) as acid catalyst. It has claimed that hydrochloric acid has higher catalytic activity and selectivity during hydrolysis of starch than sulfuric acid and phosphoric acid. Hydrochloric acid has some advantages as a catalyst in acid-catalysed hydrochloric acid, such as high catalytic activity, non-disturbing residue after neutralization (NaCl), and its simplicity. But the acid-catalysed is slow compared than enzymatic hydrolysis [8]. Polysaccharides are broken down into monosaccharides, fats into glycerol and fatty acids, and proteins into amino acids [9]. So, starch in porang flour will be broken down into glucose. Glucose is a hydroxyaldehyde that exists mainly as a six-membered cyclic hemiacetal (>99% of glucose is cyclic in solution) [10]. The objective of this study was to examine at effect of hydrochloric acid concentration and extraction time on glucomannan content produced by extraction process. Functional groups (FTIR) and morphology (SEM) are also discussed in this paper.

2. Materials and methods

2.1. Materials

Porang flour purchased from "KUSUKA" UBIKU, along with distilled water, ethanol, isopropanol, phenol, sodium bisulfite, sodium hydroxide, sodium potassium tartrate, formic acid, hydrochloric acid, and sulfuric acid, were the raw materials used in this investigation. All chemicals used for extraction and analysis are obtained from Indrasari Chemical Store in Semarang-Indonesia.

2.2. Extraction of Glucomannan using Acid-Catalyzed

2.2.1. Hydrolysis Pretreatment

At room temperature, 1000 ml of hydrochloric acid solution with predetermined concentrations (0,1; 0,3; 0,5; 0,7; 0,9) M and 10 gr of crude porang flour were mixed together in a 2000 ml three-neck round bottom flask. The mixture was then heated in a water bath heater while being continuously stirred to reach the necessary extraction temperature (70°C) according to the time variables (60; 90; 120; 150; 180) minutes. Then, to precipitate the glucomannan, non-solvents ethanol and isopropyl alcohol were added to the solution. After settling, then centrifuged at 2500 rpm for 15 minutes at a control temperature of 25 ºC. The solution was precipitated overnight and followed by filtering using filter paper.

2.2.2. Drying, Milling, and Sifting of Extracted Glucomannan

After that, the glucomannan precipitate was dried for 24 hours at 55°C. After the samples had cooled, a mortar was used to grind them, and a 100-grit screen was used to sift them. Until any leftover material could pass through the sieve, the material was ground into powder one more time. The study evaluated the glucomannan content, specific functional groups, glucomannan granule shape, phase structure, and degree of crystallinity in the porang flour samples.

2.2.3. Analysis of Glucomannan Content in Porang Flour Using the 3, 5-Dinitro Salicylic Acid (DNS) Method

The 2, 5-Dinitro Salicylic Acid (DNS solution) consists of two combined solutions, A and B. Five milliliters of distilled water, 0.7 grams of sodium bisulfite, 1.5 milliliters of sodium hydroxide (10%), and 0.7 grams of phenol were mixed to create solution A. 22.5 grams of sodium potassium tartrate, 30 milliliters of sodium hydroxide (10%), and 88 milliliters of Dinitro Salicylic Acid (1%) were mixed to create solution B. After that, solutions A and B were combined and stored at room temperature in a brown reagent bottle. A buffer solution (formic acid and 0.1 M NaOH) was prepared by mixing 1 ml of formic acid with 60 ml of distilled water into a 250 ml volumetric flask, then weighing 0.2 grams of sodium hydroxide and dissolving it in 50 ml of distilled water. A standard glucose solution (1 mg/ml) was prepared by weighing 0.1 gram of glucose and then diluting it in 100 ml of distilled water. Ten-milliliter volumetric flasks were filled with equal parts of the standard glucose solutions (0.4; 0.44; 0.48; 0.64; and 0.8) and 0.8 milliliters of pure water (as a blank). After adding Aquadest until each volume reached 0.8 ml, each volumetric flask was homogenized and filled with 0.6 ml of 3.5-Dinitro Salicylic Acid solution. Following five minutes of heating in a water bath, the mixture was allowed to cool before ten milliliters of distilled water were added. The absorbance was measured using a spectrophotometer at a wavelength of 540 nm.

Absorbance measurements were carried out at each concentration of glucose solution and then a standard curve plot was made with glucose content (mg) as the abscissa (x) and absorbance as the ordinate (y). To prepare the extract, 0.2 grams of the sample (crude and hydrolyzed porang flour) were weighed and placed in a beaker with 50 milliliters of the formic acid-sodium hydroxide buffer solution. The mixture was stirred magnetically for four hours at 30°C, and then diluted to a volume of 100 milliliters using the buffer solution. To extract the glucomannan, the mixture was centrifuged for 15 minutes at 2500 rpm. The purpose of adding the buffer is to dissolve the glucomannan, which has lower solubility than starch due to the presence of acetyl groups. Meanwhile, stirring for four hours with a magnetic stirrer can increase glucomannan's solubility and remove insoluble materials like cellulose and starch [11]. The process of making the hydrolysate was by homogenizing 2 milliliters of glucomannan extract with 1 milliliter of 3M sulfuric acid in a 10-milliliter volumetric flask. After 1.5 hours of heating in a boiling water bath, the mixture was cooled.

After homogenizing the mixture with 1 ml of 6M NaOH, 10 ml of distilled water was added. Sulfuric acid is used which functions as a catalyst which helps the hydrolysis process of glucomannan into its constituent monomers, namely glucose and mannose. Apart from that, NaOH was also added which aims to create an alkaline atmosphere so that the redox reaction process between the Dinitro Salicylic Acid (DNS) reagent and glucose is more optimal. Next, 0.6 ml of 3, 5-Dinitro Salicylic Acid (DNS) was added to a 10-milliliter volumetric flask that contained 0.8 milliliters of glucomannan extract, 0.8 milliliters of glucomannan hydrolysate, and distilled water. The flask was then submerged in a water bath for five minutes. After the solution had cooled to room temperature, ten milliliters of distilled water were added. The absorbance value at a wavelength of 540 nm was calculated. By substituting the absorbance value into the straight line equation of the glucose standard curve regression, the amount of glucose present in glucomannan extract and hydrolysate calculated. Equation (1) was used to calculate the glucomannan (GM) content.

$$\text{Glucomannan Content (\%)} = \frac{5000f(5T - T_0)}{m} \quad (1)$$

Where "m" is the mass of the extracted porang flour, "T" is the amount of glucose in the glucomannan hydrolysate,

and "To" is the amount of glucose in the extracted glucomannan.

2.2.4. Analysis of Water Content and Ash Content of Porang Flour

The AOAC 1995 method was used when analyzing the moisture and ash contents. The dry cup for the water content analysis was weighed empty before samples of extracted porang flour and crude porang flour with the highest glucomannan content (3 grams) were added to the cup and weighed again. The cup containing the sample was put into the oven with a temperature of 105℃ for 1.5 hours, the oven was confirmed to be hot and ready to dry the sample. After drying, the cup containing the sample was put into the desiccator, cooled to a constant temperature until the weight of the sample and cup remained constant. In the analysis of ash content, the porcelain cup was heated first in the oven, then cooled in a desiccator until it reached room temperature. Next, 3 grams of the sample is weighed and then burned in a porcelain cup until it is not smoking and turned into ash in a furnace at 550℃ until the sample turns to ash or reaches a constant weight. Then it is cooled in a desiccator until it reaches room temperature constantly and weighed. Equations (2) and (3) were used to determine the water and ash content.

$$\%\text{Water} = \frac{(\text{wt wet sample+porcelain}) - (\text{wt dry sample+porcelain})}{(\text{wt wet sample+porcelain}) - (\text{wt porcelain})} \times 100 \quad (2)$$

$$\%\text{Ash} = \frac{\text{Ash weight}}{\text{Sample weight}} \times 100 \quad (3)$$

2.2.5. Fourer Transform Infra-Red Analysis (FT-IR)

Using Fourier Transform Infrared Spectroscopy (FTIR, Thermo Scientific Diamond Nicolet IS 5, US), specific functional groups from glucomannan granules in porang flour were identified.

2.2.6. Scanning Electron Microscope (SEM) Analysis

A scanning electron microscope (SEM, Philips XL Series 30, and Netherlands) was used to assess the shape of the glucomannan granules in porang flour.

3. Results and Discussions

Determining the water and ash content serves the objective of observing the properties of porang flour. According to tests, 0.7 M of hydrochloric acid and 120 min of hydrolysis produced the maximum glucomannan content. The water and ash content of crude and hydrolyzed porang flour are shown in Table 1. The data in Table 1 clearly show that compared to hydrolyzed porang flour, crude porang flour contains more water and ash. The water and ash content standards for porang flour are governed by SNI 7939:2013 and must be less than 13% and 4%, respectively. It was also shown that the hydrolyzed porang flour with the highest glucomannan content had met the standard for ash content.

3.1. Glucomannan Content

The glucomannan content was determined by analyzing both the extract and hydrolysate. The analysis commenced with the creation of a glucose standard calibration curve using a spectrophotometric method. Glucose, a glucomannan monomer, was selected for its higher accuracy and precision in measurement compared to mannose. Previous studies have shown that glucose provides greater sensitivity and a more linear correlation coefficient than mannose. A wavelength of 540 nm was used to measure absorbance. With an R^2 value of 0.98866, the linear regression equation y = 0.97511x + 0.05221 was generated using the glucose standard curve shown in Figure 1. Using this equation, the amount of glucomannan in the extract and hydrolysate was determined. The α-1, 4 glycosidic linkages within starch polymers can be hydrolyzed by HCl, an acidic hydrolysis catalyst. When concentration and extraction time are properly adjusted, HCl will not damage the β-1, 4-glycosidic linkages between D-glucopyranose and D-manopyranose, building blocks of glucomannan. This results in a higher amount of glucomannan being produced after hydrolysis. Increasing the concentration of HCl can increase glucomannan concentration because HCl that works to degrade starch into simpler compound bonds becomes more abundant, which results in more glucomannan products being produced. However, if HCl exceeds 0.7 M, the glucomannan content will be decreased to 77.96% as shown in Figure 2.

According to [8], exposing polysaccharides to concentrated acid and/or high temperature leads to their deterioration, generating byproducts like monosaccharides, furfural, and hydroxymethylfurfural, consequently diminishing the purity of glucomannan. As seen in Figure 3. The amount of glucomannan in hydrolyzed porang flour may vary depending on the extraction process duration. The glucomannan content in the flour increases with the length of the hydrolysis time. This is due to the fact that a longer hydrolysis period increases the chances of HCl interacting with the substrate, facilitating the breakdown of starch covering the glucomannan and extracting more glucomannan [13]. But If the extraction time exceeds the ideal limit, HCl could break the β-1, 4-glycosidic bonds. Nonsolvent ratio is also affect the glucomannan content and glucomannan yield as shown in Figure 4 and Figure 5. Figure 4 shows that the composition of nonsolvent can affect the value of the glucomannan concentration of porang flour resulting from hydrolysis, the more isopropyl content, the higher the glucomannan concentration. Optimal glucomannan concentration were obtained at 0:1 of ethanol:IPA, namely 86.03%. Isopropanol works excellently to remove impurities, while ethanol is also effective for obtaining glucomannan but does not dissolve impurities thoroughly [14].

According to [15], due to isopropanol's low dielectric constant, substantially less of it was needed to produce the same quantity of precipitation as ethanol during the glucomannan extraction and get a higher the glucomannan concentration. So, isopropanol is the better choice when precipitating the glucomannan. Figure 5. Shows an increasing trend in the yield of the glucomannan as the proportion of the IPA in the nonsolvent mixture increases. Starting from around 9.81 % yield at a 0% IPA ratio, the yield steadily rises, reaching approximately 12.22% at a 100% the IPA ratio. This positive correlation suggests that a higher concentration of the isopropyl alcohol in the mixture leads to an enhanced yield of glucomannan. The increase in the glucomannan yield with higher IPA content could be attributed to the differences in solubility and precipitation characteristics of the glucomannan in IPA compared to ethanol. Isopropyl alcohol might create a more favorable environment for the extraction or recovery of glucomannan, possibly due to better solubility properties or less degradation during the extraction process. This finding is significant for the optimizing the industrial or the laboratory processes aiming to maximize the glucomannan yield. Adjusting the IPA ratio could be a crucial

parameter in achieving the higher efficiency in the glucomannan extraction.

3.2. Fourier Transform Infra-Red (FT-IR) Analysis

In the wavelength region of 4000-400 cm^{-1}, Figure 6. Shows the Fourier Transform Infrared (FT-IR) spectra of hydrolyzed porang flour at highest glucomannan content as well as crude porang flour glucomannan granules. According to [15], samples of crude porang flour, samples with highest glucomannan content, and optimized samples revealed a prominent peak for the OH group of glucomannan located at 3000-3700 cm^{-1}. Meanwhile, methyl group located at ~2900 cm^{-1} is directed at –CH stretching vibrations [16]. The β-1, 4 D-mannose and D-glucose bonds of glucomannan are indicated by presence of carbonyl stretching vibrations (C=O) which located at ~1650 cm^{-1} [17]. Wave numbers 1413 cm^{-1} and 1377 cm^{-1} represent the C–H angular deformation. The C–O bond of ether shown to stretch at ~1150 cm^{-1} while the C–O bond of alcohol is shown to stretch at 1079 cm^{-1} and 1022 cm^{-1}. Characteristic peak observed at 808–900 cm^{-1} is directed to β-pyranose between mannose and glucose units. An absorption band at 3277.66 cm^{-1} (O-H), 2924.00 cm^{-1} (C-H), 1636.38 cm^{-1} (C=O), 1373.55 cm^{-1} (C-H), 1149.41 cm^{-1} (ether), 1014.68 cm^{-1} (C-O primary alcohol), and 810.00 cm^{-1} corresponding to β-pyranose between mannose and glucose units can be seen in Fourier Transform Infrared (FT-IR) spectrum of crude porang flour. FT-IR spectrum of porang flour obtained after hydrolyzing it at highest glucomannan content, showing an absorption band at 3281.50 cm^{-1} (O-H), 2921.90 cm^{-1} (C-H), 1630.71 cm^{-1} (C=O), 1367.90 cm^{-1} (C-H), 1148.54 cm^{-1} (C-O ether), 1014.30 cm^{-1} (C-O primary alcohol), and 808.69 cm^{-1} corresponding to β-pyranose b/w mannose and glucose units.

The results of the Fourier Transform Infrared (FT-IR) spectrum analysis show that all distinct glucomannan peaks were present in this study. The absorption band pattern of the FT-IR spectrum of the glucomannan granules in crude porang flour and porang flour hydrolyzed at highest glucomannan content is almost identical, but peak intensity differs. This is because catalytic hydrolysis process increases intensity of entire FT-IR spectrum of hydrolyzed porang flour at highest glucomannan content. According to [18], this indicates that most common contaminants are typically Infrared (IR) inactive, meaning that impurities like starch and calcium oxalate are present at low concentrations. For comparison to commercial glucomannan and pure glucomannan was reported, glucomannan spectra obtained from extraction closely resembled commercial glucomannan spectrum, with only minor shifts observed. These shifts may be attributed to impurities present in extracted glucomannan in this study. Functional groups from proteins, starches, fats, and other substances could have interfered with glucomannan spectrum readings. Previoud study reported that in the FTIR spectrum of pure glucomannan, broad peak at 3217 cm^{-1} corresponds to stretching vibration of O-H group, indicative of moderately strong hydrogen bonding group. Peaks at 2808 cm^{-1}, 1604 cm^{-1}, and 1381 cm^{-1} attributed to –CH2 stretching vibration and two C-H bending modes, respectively.

3.3. Scanning Electron Miscroscope (SEM) Analysis

The size of the crude porang flour glucomannan granules was comparatively larger than the size of the porang flour glucomannan granules resulting from hydrolysis at the highest glucomannan content, according to the morphological analysis of the granules shown in Figure 7. Images A1, A2, A3, and A4. It is also evident from the image that the porang flour's surface is still relatively uneven. The surface of crude porang flour appears as needle- or fiber-like crystals with an uneven, non-uniform distribution. The significant amount of calcium oxalate covering the glucomannan granules as an impurity is responsible for this. Calcium oxalate is indicated by needle-shaped crystals [20]. In addition, the surface of crude porang flour is irregular, non-uniform, and less even, indicating the surface structure of oxalic acid. Meanwhile, the morphological examination of the porang flour glucomannan granules resulting from hydrolysis at the highest glucomannan content, as displayed in images B, shows that the surface of the porang flour glucomannan granules after hydrolysis is smoother.

The removal of calcium oxalate, an impurity covering the glucomannan granules, and the liquefaction of starch during hydrolysis are responsible for this smoother surface, indicating that impurity compounds covering the glucomannan granules have been effectively reduced [12]. The catalytic hydrolysis process occurs through 3 stages, namely gelatinization, liquefaction and saccharification. Gelatination is initiation stage before liquefaction occurs with the starch granules swelling due to heating which breaks the hydrogen bonds between molecules in the starch glycosidic bonds. This gelatinization process plays an important role because it determines the rate of the liquefaction process. Liquefaction is core process of catalytic hydrolysis controlled by HCl where starch is hydrolyzed into simpler molecules such as oligoscharides, maltose and dextrin. Saccharification is a further hydrolysis stage from liquefaction stage which involves HCl which plays a role in breaking down starch into final product of reducing sugar. Starch hydrolyzing catalyst used is hydrochloric acid (HCl). HCl plays an important role in determining speed of liquefaction process. Internal α-1, 4 glycosidic linkages of starch polymers can be hydrolyzed by HCl, an acidic hydrolysis catalyst.

When concentration and extraction time are optimal, HCl will not break β-1, 4-glycosidic bonds in glucomannan, which composed of D-glucopyranose and D-manopyranose. However, if extraction period is longer than optimal, HCl may also break these bonds. Research has shown that removing calcium oxalate and starch, which are impurity components covering the glucomannan granules, significantly increased the amount of glucomannan hydrolyzed from porang flour. This study also demonstrates that starch can be hydrolyzed with HCl to purify glucomannan from porang flour. Once starch is removed, glucomannan granules released, which also leads to removal of calcium oxalate, an impurity covering glucomannan granules [12]. Therefore, glucomannan concentration in porang flour resulting from hydrolysis also increased. For comparison, [12] images of hydrolyzed and crude porang flour obtained using a scanning electron microscope (SEM) were also displayed. Results show a significant similarity, with main difference being image magnification levels.

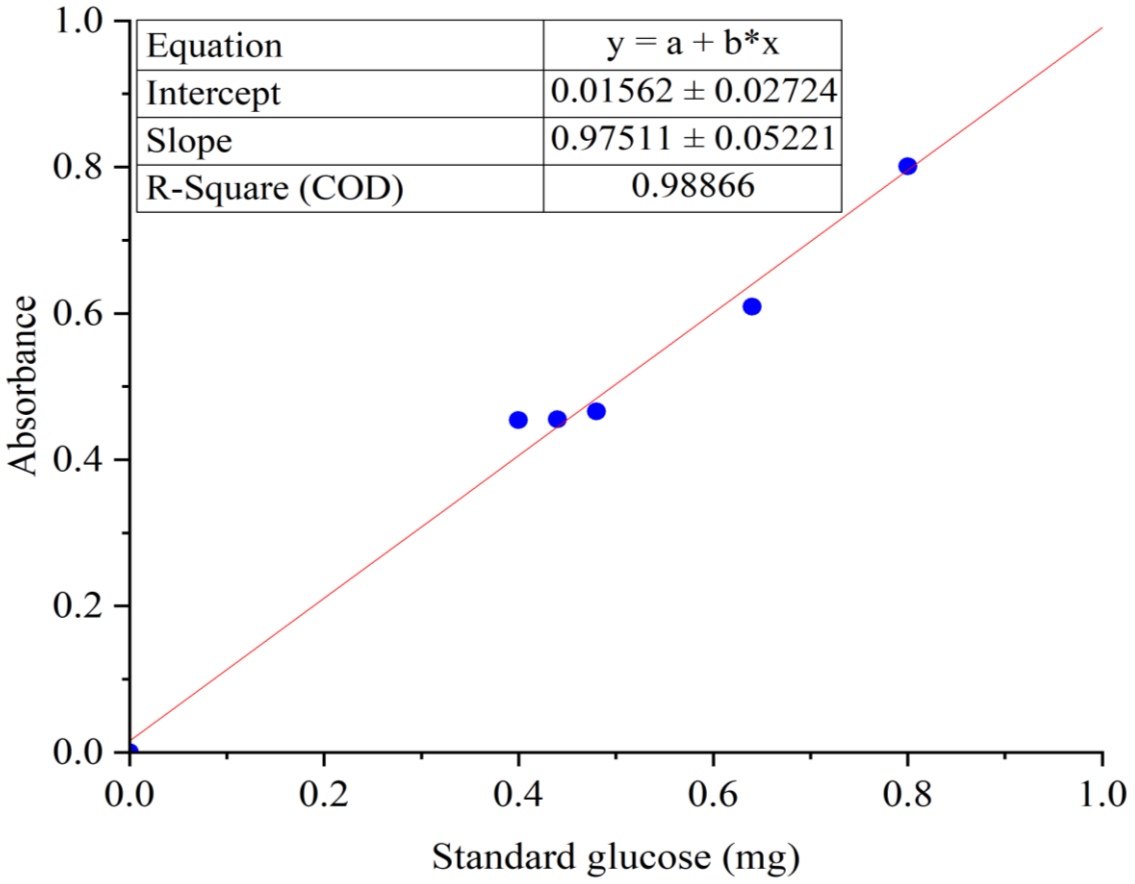

Figure 1. Glucose standard curve

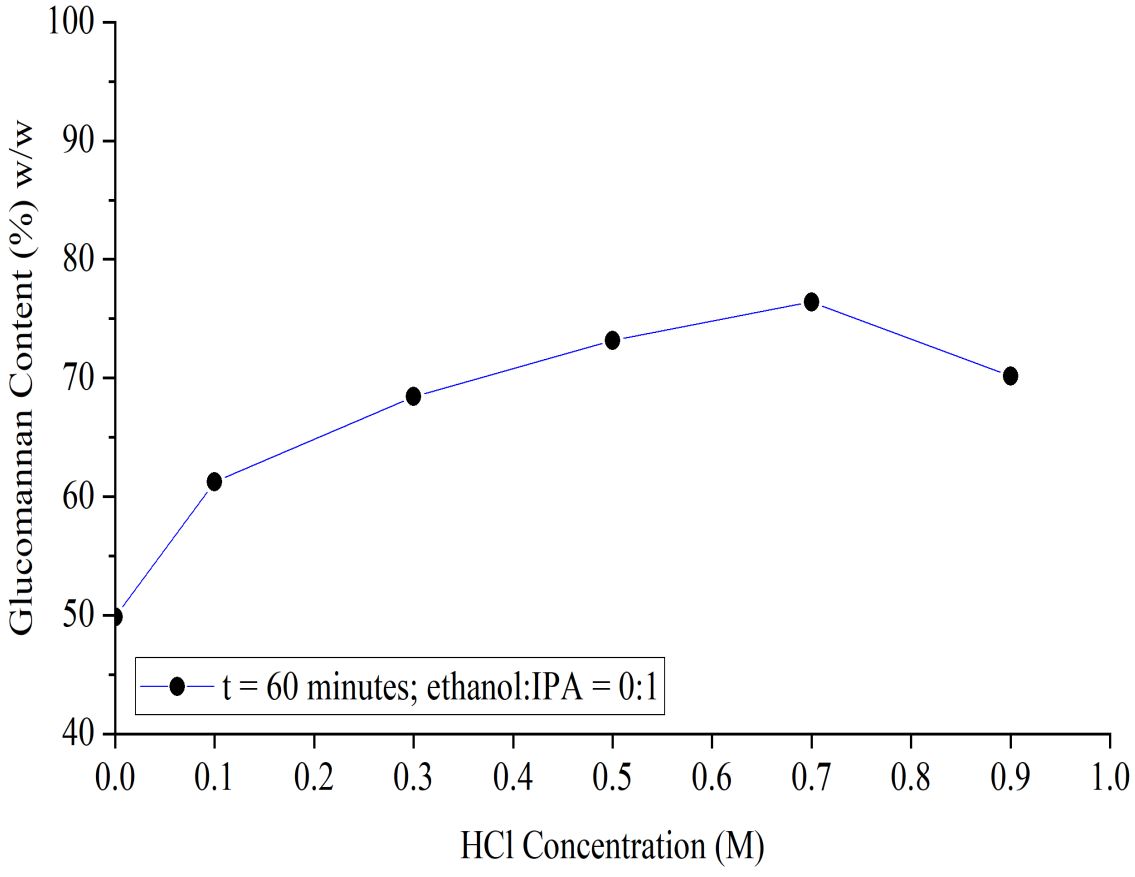

Figure 2. Graph of the relationship between the concentration of the HCl and the content of glucomannan produced

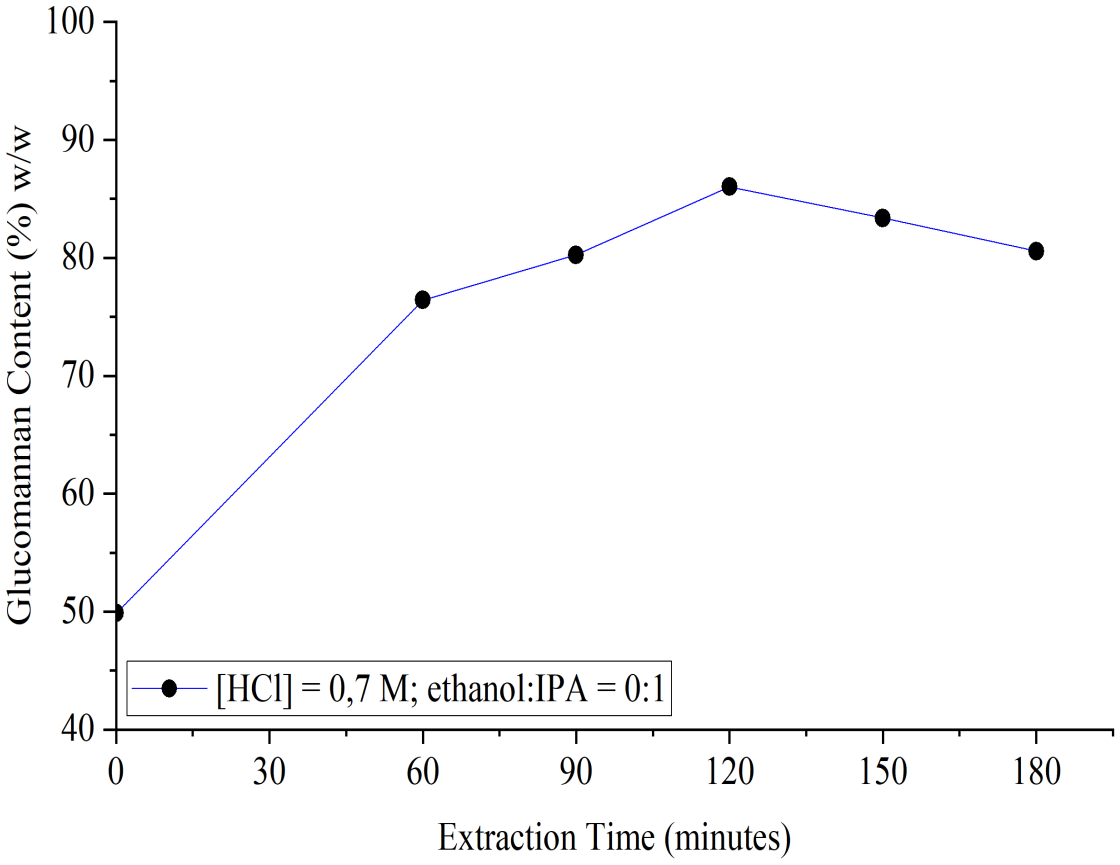

Figure 3. Graph of the relationship between extraction time and the content of glucomannan produced

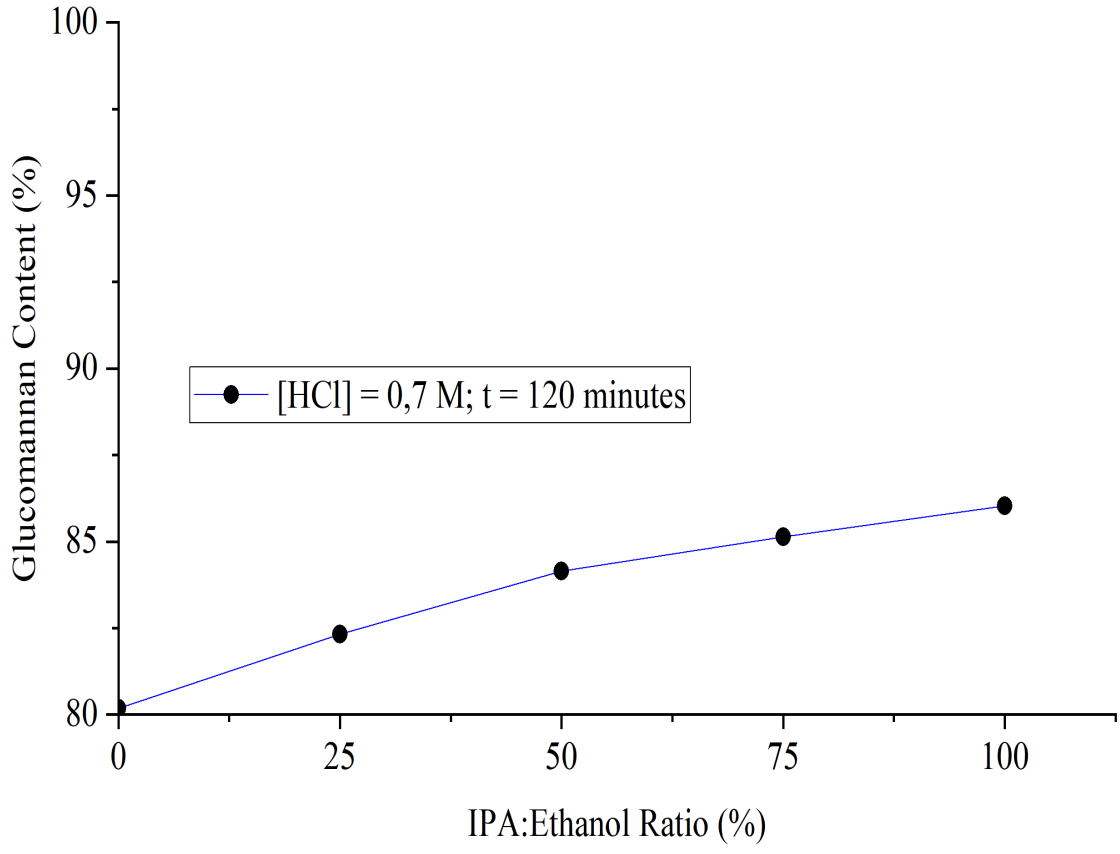

Figure 4. Graph of the relationship between nonsolvent ratio and the content of glucomannan produced

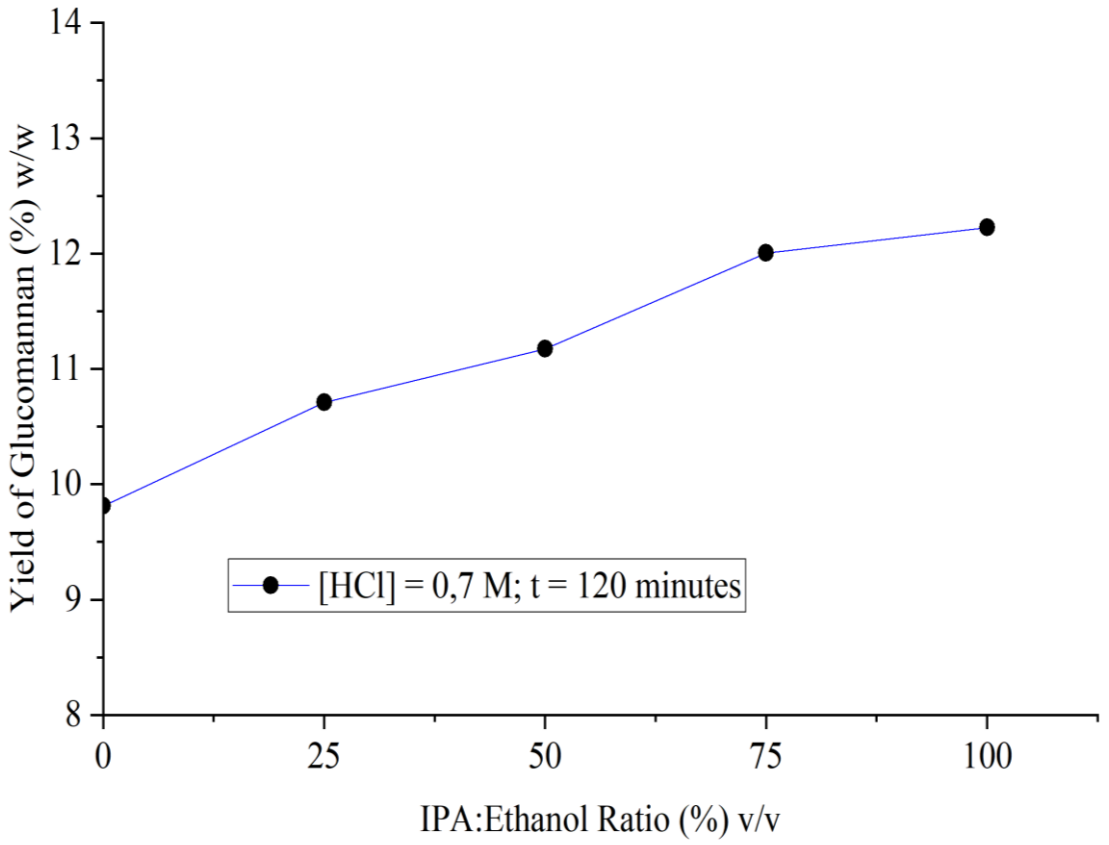

Figure 5. Graph of the relationship between nonsolvent ratio and the yield of glucomannan

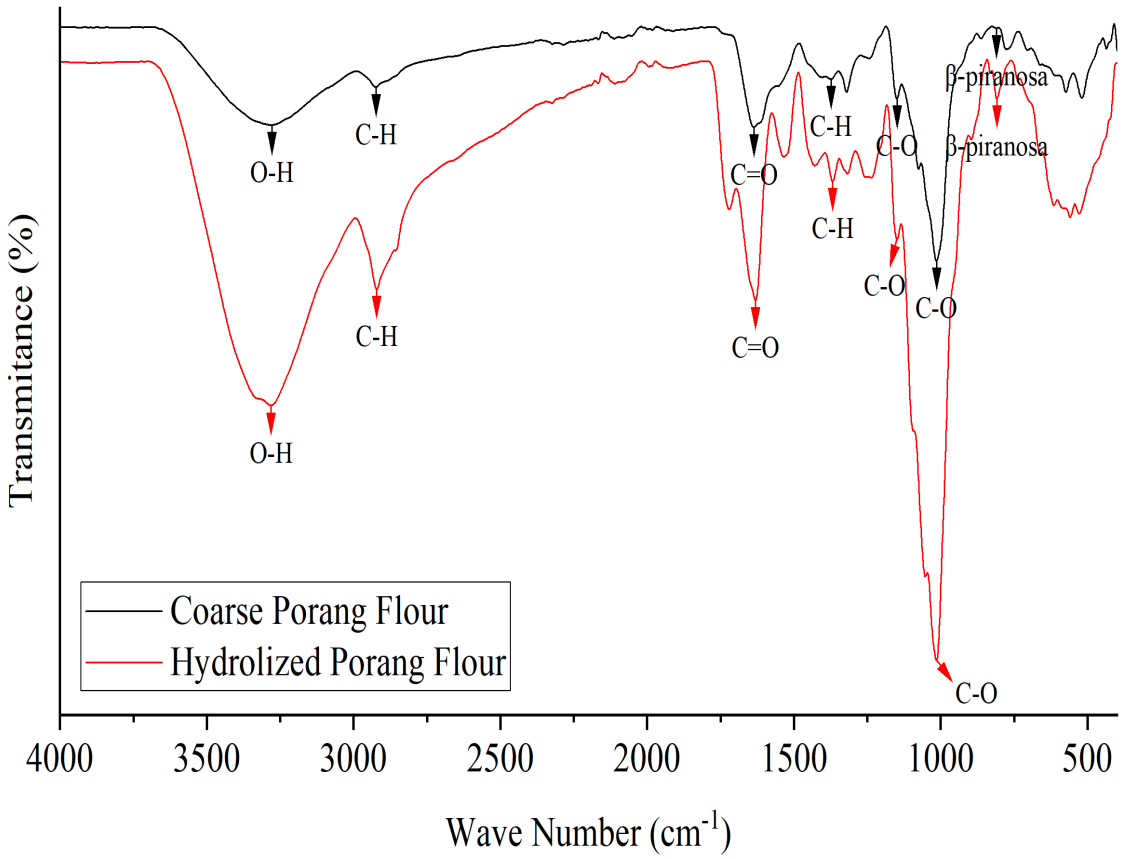

Figure 6. An assessment of the glucomannan granules from crude porang flour and porang flour, which has undergone hydrolysis at its highest glucomannan content, using Fourier Transform Infrared (FT-IR) analysis

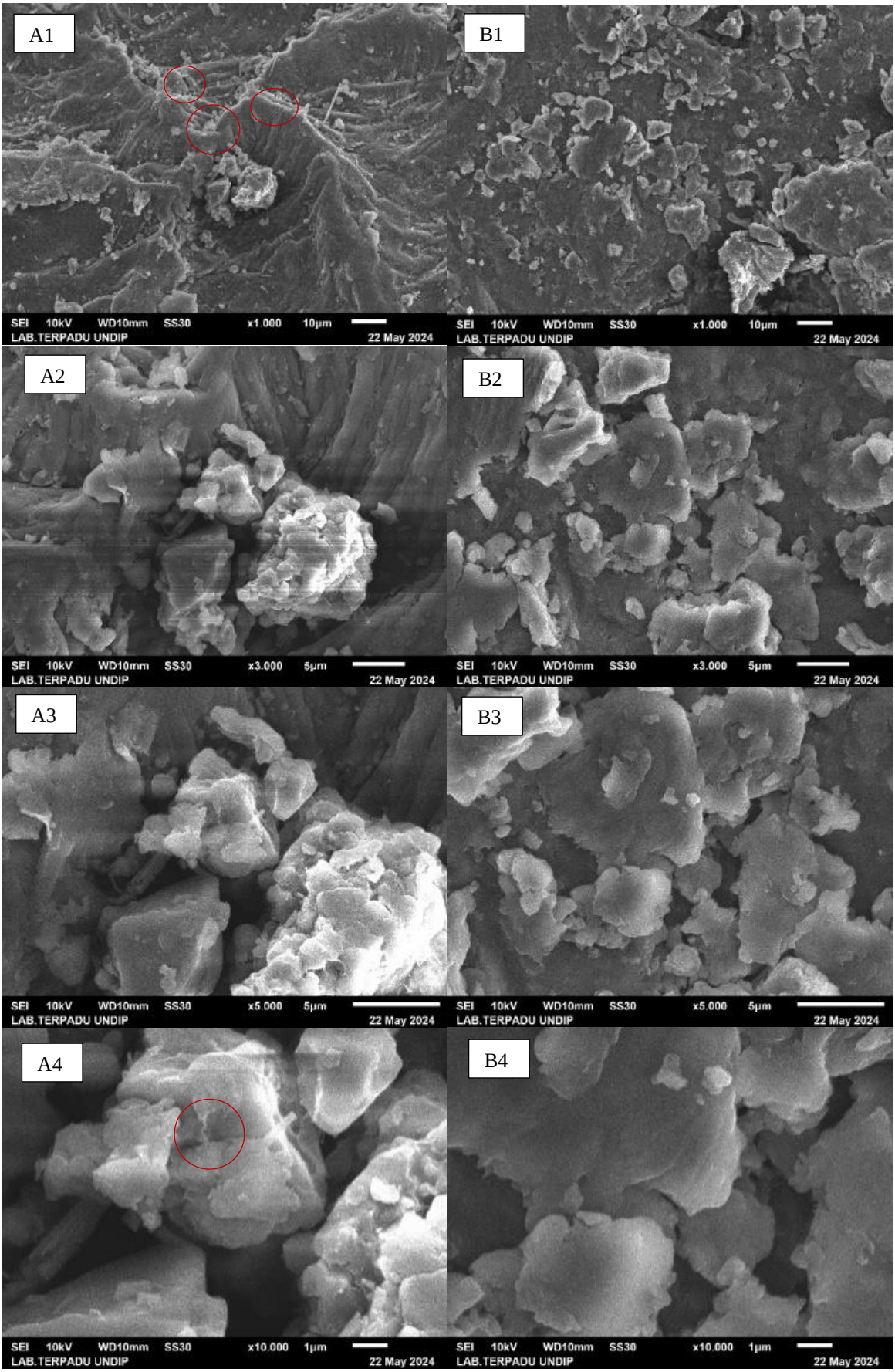

Figure 7. An evaluation using 1,000× (1), 3,000× (2), 5,000× (3), and 10,000× (4) magnifications of the results from scanning electron microscopy (SEM) analysis between crude porang flour glucomannan granules (A) and porang flour after hydrolysis at the highest glucomannan content (B)

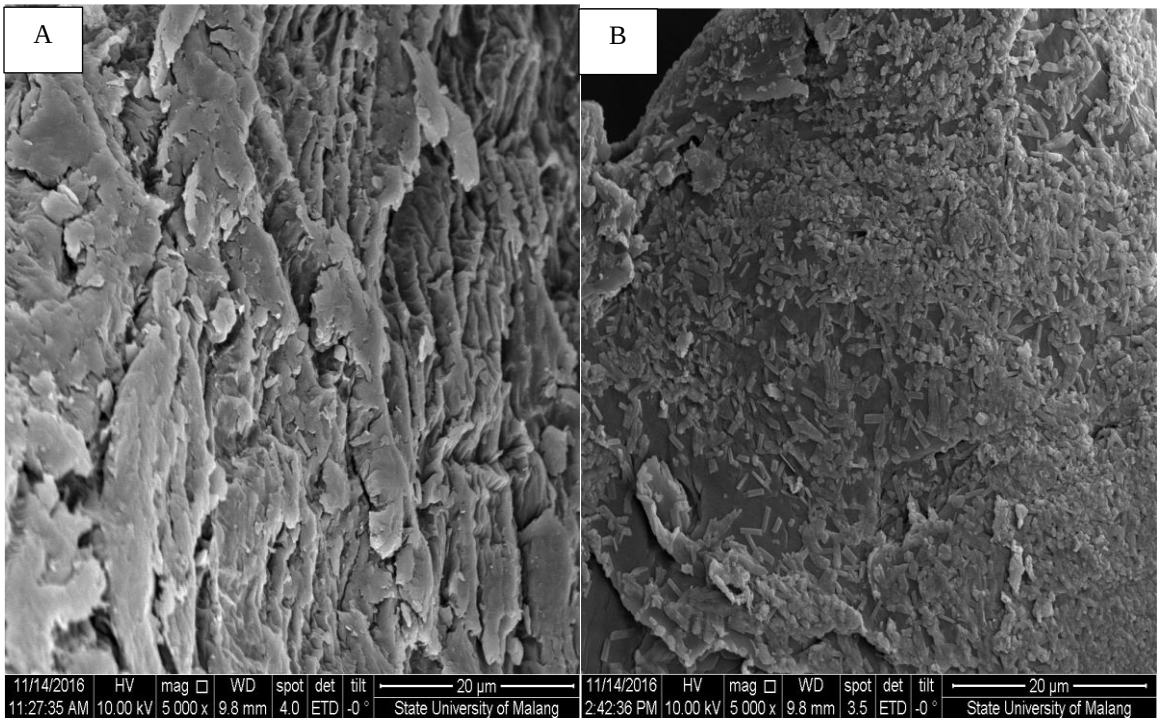

Figure 8. Result comparison of a scanning electron microscope (SEM) analysis between porang flour obtained after hydrolysis at the highest glucomannan content (B) and crude porang flour glucomannan granules (A).

Table 1. Characteristics of porang flour with the highest glucomannan content, both hydrolyzed and crude

Sample	Water Content (%)	Ash Content (%)
Crude porang flour	11.87	2.78
Porang flour hydrolzed with highest glucomannan content	5.97	1.83

4. Conclusions

The glucomannan content of porang flour increased from 49.88% to 86.03% during the hydrolysis process. This increase is attributed to higher concentrations of hydrochloric acid and longer hydrolysis times, which allow more catalysts to degrade starch and produce more glucomannan. Additionally, prolonged hydrolysis provides more interaction time between hydrochloric acid and the substrate. However, excessive acid concentration and overly extended hydrolysis times can degrade glucomannan. The presence of all distinct glucomannan peaks was confirmed by the FT-IR spectra, and the hydrolyzed porang flour's smoother surface was verified by SEM examination.

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