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Extraction of Glucomannan from Porang (*Amorphophallus oncophyllus*) Flour Using Enzymatic Hydrolysis Pretreatment

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Abstract

Porang flour is made from porang tubers (Amorphophallus oncophyllus) which has a high glucomannan content. This research aimed to extract glucomannan from raw porang flour using α-amylase enzymatic hydrolysis pretreatment. The extraction process utilized three-neck flask, distilled water as a solvent, and 330 KNU/f of α-amylase enzyme (0.375, 0.625, 0.875 gr/gr). This process was 350 rpm stirred and 75°C heated for 60, 90, and 120 min. The hydrolyzed porang flour was analyzed for moisture and ash content, functional group (FT-IR), morphological structure (SEM), and crystallinity properties (XRD). The result showed that the water and ash content of hydrolyzed porang flour that contain highest level of glukomanan has reached the predetermined flour ash content standard. The lowest and highest glucomannan content were 51.937% and 95.191%, respectively. The presence of α-amylase and the longer extraction time can increase the glucomannan content of hydrolyzed porang flour. Spectra FTIR depicted all the functional groups of glucomannan compound. The SEM analysis showed a smoother surface of hydrolyzed porang flour. The XRD examination results showed a reduced degree of crystallinity, indicating that the water was originally sorbed in amorphous areas with fewer hydrogen bonds.

Keywords: enzymatic hydrolysis; extraction; glucomannan; porang flour

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1. Introduction

Porang (*Amorphophallus oncophyllus*) is a tuber plant growing in Indonesia's forests. These tubers contain high level of glucomannan which has many benefits in both food and functional food fields. Glucomannan is used as thickener for syrups, jelly, edible films, instant noodles, and sausage wrappers [1]. It is can be applied for basic ingredient of hydrogels as a DNA-controlled release matrix [2]. In addition, glucomannan also has positive consequences for reducing the risk of diabetes, cholesterol, and obesity [3]. The biggest challenge in improving glucomannan content in porang flour in Indonesia is that the flour after processing has low glucomannan content and high calcium oxalate concentration. It is well-known that calcium oxalate causes itching, skin irritation, renal crystallization, and other negative health consequences. It inhibits the application of glucomannan in many industrial fields in Indonesia. Therefore, efforts to increase glucomannan levels and reduce calcium oxalate in porang flour are very important to increase the selling value of porang flour [4].

Several methods have been developed to extract and purify glucomannan, either by mechanical (dry processing) or chemical (wet processing). The mechanical method involves grinding and refining through wind filtering, grinding and refining through sieving, and polishing. Chemical methods that have been carried out include centrifugation after flocculation using $Al_2(SO_4)_3$, the use of dimethyl sulfoxide, lead acetate, enzymatic hydrolysis, and ethanol to extract glucomannan [5, 6]. Glucomannan extracted with lead acetate is non-edible thus limiting its application in the non-food industry. In view of the above-mentioned shortcomings, the most common method of purification of porang flour is extraction using ethanol as solvent due to its simplicity and high efficiency [5]. A number of factors that affect the extraction yield and quality, including the type of extraction solvent, solvent concentration, particle size, temperature, pH, and extraction time. [7].

Enzymatic hydrolysis is an alternative method of starch modification that is widely used. The enzymatic hydrolysis process is more effective when compared to acid hydrolysis because the enzyme breaks the glycosidic bond

specifically, leaving no residue, and minimum color damage [8]. Therefore, in this study, enzymatic hydrolysis used α-amylase enzyme, an endoenzyme that breaks down α-1,4 glycosidic bonds on the inside of the starch polymer. However, this enzyme will not cleave the β-1,4-glycosidic bonds found in glucomannan with the constituent components of D-glucopyranose and D-manopiranose. It is due to the specific nature of the enzyme to the substrate with certain constituent bonds. This makes the α-amylase enzyme suitable for hydrolyzing starch and can produce high levels of glucomannan [9].

The objective of this study was to examine at the effect of α-amylase enzyme concentration and extraction time on the glucomannan content produced by the extraction process. The functional groups (FTIR), morphology (SEM), and crystallinity (XRD) properties of glucomannan granules are also discussed in this paper.

2. Materials and methods

Materials

The raw materials used in this study were porang flour obtained from PT Prima Agung Sejahtera-Surabaya (Indonesia) with an average particle size of 80 mesh. Distilled water, formic acid, hydrochloric acid, sulfuric acid, α-amylase enzyme, ethanol, phenol, anhydrous glucose, sodium bisulfite, sodium hydroxide, and sodium potassium tartrate were supplied by Sigma-Aldrich.

Extraction of Glucomannan using Enzymatic Hydrolysis

Pretreatment

Porang flour was dissolved using distilled water with a concentration of 2.5% (w/v) in a three-neck flask. Next, the mixture was reacted with the α-amylase enzyme (330 KNU/g) in varying concentrations (0.375 gr/gr, 0.625 gr/gr, 0.875 gr/gr) in a refrigerated three-necked flask. Then, the mixture was heated to 75 ºC for the desired time (60 min, 90 min, and 120 min) with 350 rpm mechanical stirring. After the extraction was complete and 3 thin layers were formed, the mixture was heated at 90 ºC for 15 minutes to inactivate the α-amylase enzyme, then ethanol with a concentration of 96% was added to the sample to precipitate glucomannan. After settling, the sample was centrifuged at 2500 rpm for 15 min 25 ºC. The precipitate formed was separated with cheesecloth and stopped after three repetitions.

Drying, Milling, and Sifting of Extracted Glucomannan

The extracted glucomannan was dried at 55 ºC for 24 hours. The cooled samples were ground with a mortar before being sieved using a 100-mesh screen.

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

The extract glucomannan was weighed of 0.2 grams and diluted by 50 ml of buffer solution (formic acid-sodium hydroxide) for 4 hours at 30 ºC then buffer solution was added to 100 ml of total volume. The mixture was then centrifuged at 2500 rpm for 15 min to obtain glucomannan extract. The hydrolyzate production was carried out by adding glucomannan extract (2 ml), H_2SO_4 (3M, 1 ml), and homogenized. The mixture was heated in a boiling water bath for 1.5 hours and then cooled. After that, NaOH (6M, 1 ml) and distilled water (10 ml) was added and homogenized. A 10 ml volumetric flask was filled with 0.8 ml of glucomannan extract, glucomannan hydrolyzate, 0.6 ml of 3.5-Dinitro Salicylic Acid (DNS), and distilled water. It was placed in a water bath for 5 min. The solution was cooled to room temperature, then distilled water was added up to 10 ml and ready for analysis. The absorbance value was measured at a wavelength of 540 nm. The glucose content (mg) that corresponds to its absorbance was estimated using the regression equation of the glucose standard curve. The glucomannan (GM) content was calculated using Equation (1).

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

Where T is mg of glucose in glucomannan hydrolysate; To is mg of glucose in extracted glucomannan; and m is weight of extracted porang flour.

Analysis of Water Content and Ash Content of Porang Flour

Analysis of water and ash content was determined according to the AOAC 1995 method. Water and ash content were calculated using Equation (2) and (3).

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

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

Fourier Transform Infra-Red Analysis (FT-IR)

Determination of specific functional groups from glucomannan granules in porang flour using Fourier Transform Infra-Red (FTIR, Thermo Scientific Diamond Nicolet IS 5, US).

Scanning Electron Microscope (SEM) Analysis

Determination of morphology from glucomannan granules in porang flour was determined using Scanning Electron Microscope (SEM, Philips XL series 30, Netherlands).

X-Ray Diffraction (XRD) Analysis

Determination of the phase structure and degree of crystallinity from glucomannan granules in porang flour using X-Ray Diffraction (XRD) analysis. The percent crystallinity was calculated using Equation (4).

$$\text{Percent crystallinity (\%)} = \frac{\text{crystalline area fraction}}{\text{crystalline area fraction+amorphous area fraction}} \quad (4)$$

3. Results and Discussions

The purpose of water and ash content determination is to observe the characteristics of porang flour. Based on experiments, the highest glucomannan content was obtained by 120 min of hydrolysis and 0.875% (w/w) of α-amylase enzyme. Water and ash content of crude and hydrolized porang flour was shown in Table 1. Based on the data in Table 1, it can be seen that the water content and ash content obtained from raw porang flour are higher than hydrolyzed porang flour. The standard value of water and ash content in porang flour that has been regulated by SNI 7939:2013 are less than 13% and less than 4%, respectively [10]. It also indicated that the water and ash content of hydrolyzed porang flour at the highest glucomannan content has reached the predetermined flour ash content standard.

Table 1. Characteristics of crude and hydrolized porang flour with highest glucommanan content

Sample	Water Content (%)	Ash Content (%)
Crude porang flour	12.55	1.93
Porang flour hydrolized with highest glucommanan content	3.86	0.78

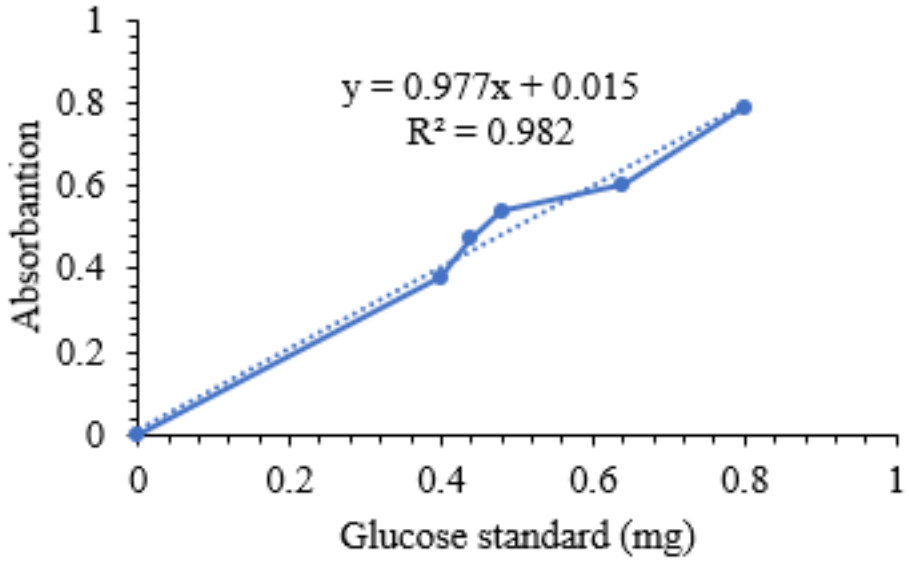

Figure 1. Glucose standard curve

Table 2. Glucomannan content in varying enzyme concentration and extraction time

Variable	Extraction Time (minutes)	Enzyme (gr/gr)	Glucomannan Content (%)
1	60	0.375	63.246
2	90	0.375	68.705
3	120	0.375	70.086
4	60	0.625	78.056
5	90	0.625	87.798
6	120	0.625	89.410
7	60	0.875	88.581
8	90	0.875	92.358
9	120	0.875	95.191
10*	0	0	51.937

*Crude Porang Flour

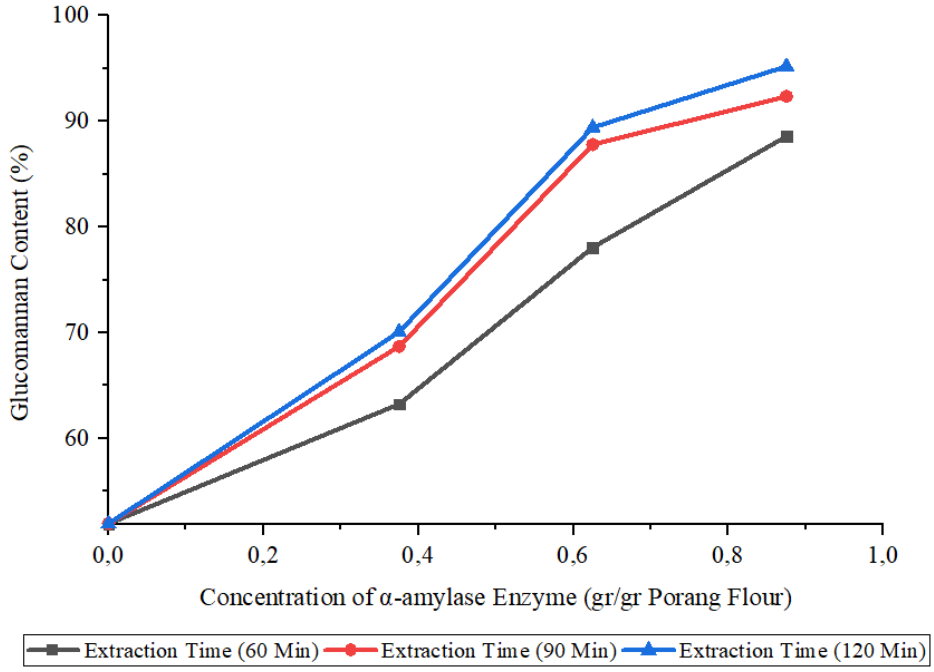

Figure 2. The glucomannan content in varying enzyme concentration

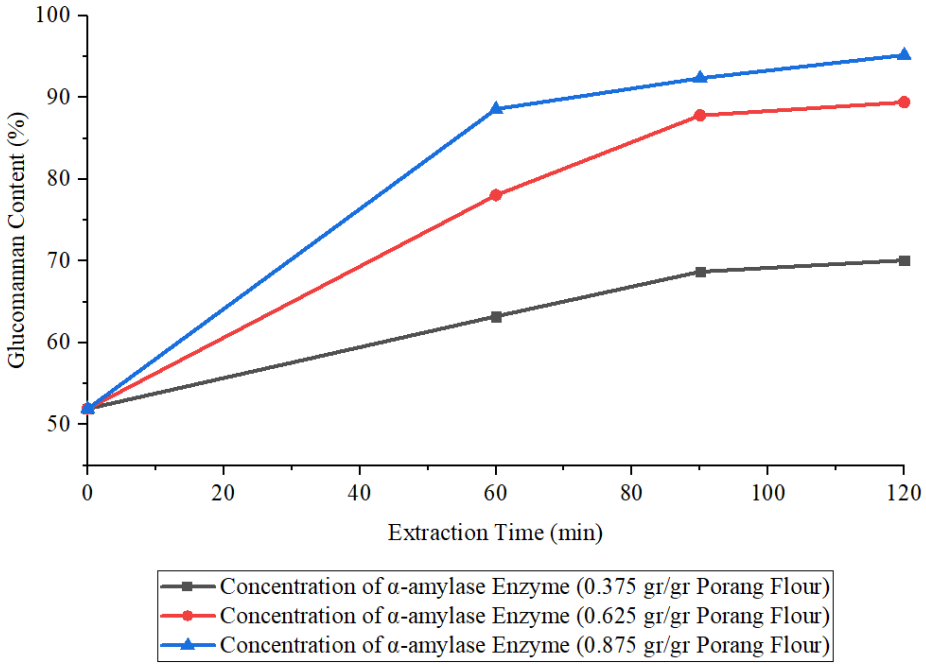

Figure 3. The glucomannan content in varying extraction time

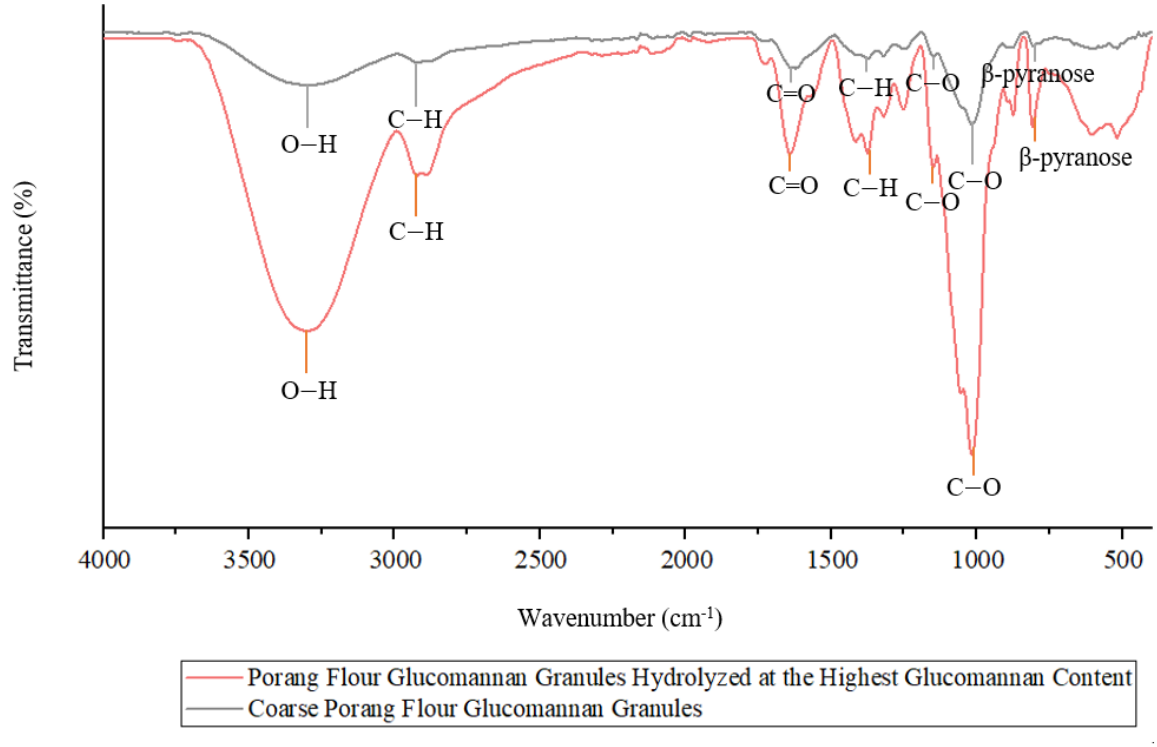

Figure 4. FTIR spectra of hydrolized porang flour (red line) and coarse porang flour (black line)

Table 3. The degree of crystallinity of porang flour

Sample	Percent Crystallinity (%)
Porang flour glucomannan granules	21.888
Porang flour glucomannan granules hydrolyzed at the highest glucomannan content	20.354

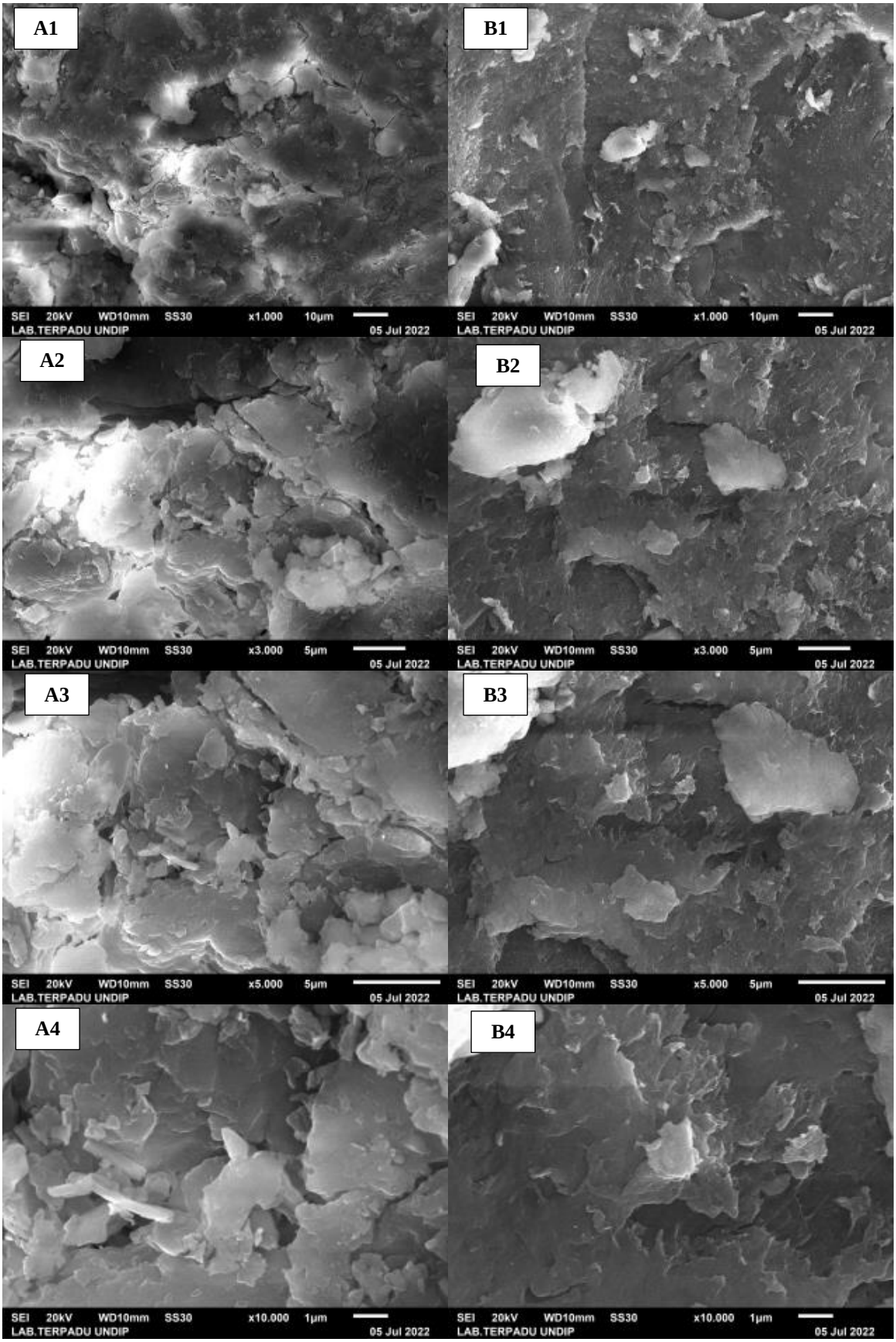

Figure 5. The morphological properties of coarse porang flour (A) and hydrolyzed porang flour at the highest

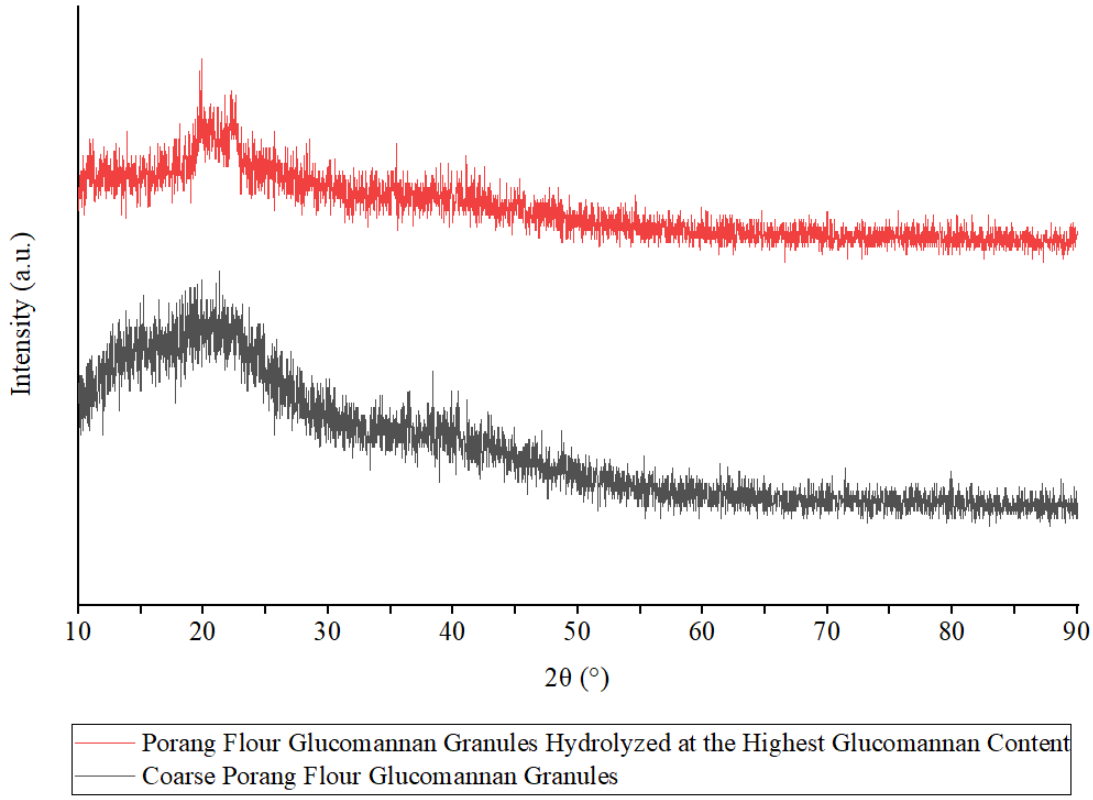

Figure 6. X-Ray Diffraction (XRD) analysis of hydrolyzed porang flour (red line) and coarse porang flour (black line) glucomannan content (B) with magnifications of 1,000× (1); 3,000× (2); 5,000× (3); and 10,000× (4)

Glucomannan Content

Determination of the glucomannan content was carried out by measuring the glucomannan content in the extract and hydrolysate. The analysis process began by making a calibration curve of standard glucose using the spectrophotometric method. Glucose was chosen because it is a monomer of glucomannan which provides more accurate and precise measurement results compared to mannose. This is based on the results of previous work that the glucose standard provides a higher sensitivity than mannose with a more linear correlation coefficient value [5, 11]. Absorbance measurements were carried out at a wavelength of 540 nm. The standard curve of glucose was shown in Figure 1. Figure 1 depicts the linear regression equation y = 0.977x + 0.015 with a value of R^2 = 0.982 is obtained. The glucomannan content in the extract and glucomannan hydrolysate was then calculated using the linear regression equation. Table 2 represents the effects of different enzyme concentration and extraction time on glucomannan levels. The lowest glucomannan content found in crude porang flour was 51.937%; while the largest concentration was found in hydrolyzed porang flour was 95.191% with an enzyme concentration of 0.875 gr/gr and a time of 120 minutes. The results shows that enzymatic hydrolysis treatment, using water and the α-amylase enzyme, had a great influence to increase the glucomannan content of porang flour. α-amylase is an endoenzyme which capable for hydrolyzing α-1,4 glycosidic bonds on the inside of starch polymers. This enzyme will not cut the β-1,4-glycosidic bond found in glucomannan due to the specific nature of the enzyme on the substrate with certain constituent bonds. It causes the glucomannan content obtained after enzymatic hydrolysis is greater [12]. The higher α-amylase enzyme concentration can also increase glucomannan content, as shown in Figure 2. The presence of α-amylase will degrade starch to maltodextrin and other derivative compounds that have α-1,4 bonds. The more enzymes used means the more degradation products from starch which make the yield of heterosaccharide products such as glucomannan increase [13]. The extraction time can affect the glucomannan content of hydrolyzed porang flour, as depicted in Figure 3. For each variation in the concentration of α-amylase enzyme, the same results were obtained where the longer the extraction time, the higher the glucomannan content obtained. It is because the longer the hydrolysis time, the higher the opportunity for the α-amylase enzyme to interact with the substrate, so that the starch covering the glucomannan will be more easily hydrolyzed and more glucomannan will be extracted [12].

Fourier Transform Infra-Red (FT-IR) Analysis

Fourier Transform Infra Red (FT-IR) analysis was used to determine the specific functional groups of glucomannan granules of crude porang flour and hydrolyzed porang flour at the highest glucomannan content. A comparison of functional groups from Fourier Transform Infra-Red (FT-IR) analysis between crude porang flour and

hydrolyzed porang flour at the highest glucomannan content is shown in Figure 4.

Figure 4 shows the FTIR spectra of hydrolized porang flour and coarse porang flour in the wavelength range of 4000−400 cm^{-1}. According to previous research, the broad band detected at 3000-3700 cm^{-1} might be described to glucomannan O-H stretching [14]. Meanwhile, methyl groups at ~2900 cm^{-1} were assigned to the -CH stretch vibration, while carbonyl groups at 1720 cm^{-1} were assigned to glucomannan acetyl groups [15]. The carbonyl (C=O) stretch vibration at 1650 cm^{-1} revealed the presence of β-1,4 linked glucose and mannose of glucomannan [16]. The bands at 1413 cm^{-1} and at 1377 cm^{-1} correspond to the angular deformation of C–H. The C–O ether bond stretched around 1150 cm^{-1} whereas the C–O alcohol bond stretched around 1079 cm^{-1} and 1022 cm^{-1}. The β-pyranose between mannose and glucose unit had been attributed to the distinctive peaks found at 808–900 cm^{-1} [17]. FTIR spectra of course porang flour shows an absorption band at the strain 3278.04 cm^{-1} (O-H), 2919.32 cm^{-1} (C-H), 1621.79 cm^{-1} (C=O), 1373.79 cm^{-1} (C-H), 1016.45 cm^{-1} (C−O primary alcohol), and 805.49 cm^{-1} aimed at β-pyranose between mannose and glucose units. The spectrum of the Fourier Transform Infra-Red (FT-IR) hydrolyzed porang flour at the highest glucomannan content showed an absorption band at the strain 3302.91 cm^{-1} (O-H); 2922.49 cm^{-1} (C-H); 1642.64 cm^{-1} (C=O); 1415.67 cm^{-1} (C-H); 1374.86 cm^{-1} (C-H); 1319.45 cm^{-1} (C-H); 1149,22 cm^{-1} (C-O ether); 1016,66 cm^{-1} (C−O primary alcohol); 874.03 cm^{-1} and 808.01 cm^{-1} were assigned to β-pyranose between mannose and glucose units. FTIR spectra indicated that all the characteristic peaks of glucomannan in this article appeared. The FTIR spectra of all samples displayed almost identical absorption band patterns, although with varying intensities, where there is an increase in the intensity of the entire FTIR spectrum of hydrolyzed porang flour at the highest glucomannan content as a result from the enzymatic hydrolysis process. This indicates that most of the common impurities are broadly Infra-Red (IR) inactive, which means that impurities such as starch and calcium oxalate are present at low levels [5].

Scanning Electron Microscope (SEM) Analysis

Scanning Electron Microscope (SEM) analysis was used to observe the morphology or characteristics of glucomannan granules of coarse porang flour and hydrolyzed porang flour at the highest glucomannan content. Comparison of the results of the Scanning Electron Microscope (SEM) analysis between glucomannan granules of crude porang flour (A) and hydrolyzed porang flour at the highest glucomannan content (B) is shown in Figure 5. Based on the morphological analysis, it was found that the size of the glucomannan granules of crude porang flour was relatively larger than the size of the glucomannan granules of hydrolyzed porang flour. Figure 5 also shows that the surface of the porang flour is still very rough. The surface of coarse porang flour is in the form of needle crystals or fibers with an irregular, non-uniform, and uneven distribution. This is due to a large amount of calcium oxalate as an impurity compound that covers the glucomannan granules. Needle-shaped crystals indicate the presence of calcium oxalate [6]. In addition, the irregular, non-uniform, and uneven surface of coarse porang flour shows the surface structure of oxalic acid [18]. While the glucomannan granules of porang flour as a result of hydrolysis at the highest glucomannan content tend to be oval or round in shape which is not the same size with fairly even distribution and obtained granules glucomannan with a smoother surface. This smoother surface is caused by the liquefaction effect of starch during hydrolysis and the removal of calcium oxalate as an impurity compound that surrounds the glucomannan granules which indicates that starch and calcium oxalate as impurities that surround the glucomannan granules have been successfully reduced [19].

In the research that has been carried out, the removal of starch and calcium oxalate as impurities that enveloped the glucomannan granules significantly increased the glucomannan content of hydrolyzed porang flour. This article also shows that purification of glucomannan from porang flour can be carried out by hydrolyzing starch using α-amylase enzyme. The removal of starch will release the glucomannan granules so that calcium oxalate as an impurity compound that covers the glucomannan granules is also released [13]. Therefore, the glucomannan content in the hydrolyzed porang flour also increased.

X-Ray Diffraction (XRD) Analysis

X-Ray Diffraction (XRD) analysis was used to determine the phase structure and degree of crystallinity of glucomannan granules of coarse porang flour and porang flour hydrolyzed at the highest glucomannan content using X-ray electromagnetic radiation. Comparison of the analysis results of the diffractogram pattern X-Ray Diffraction (XRD) between glucomannan granules of coarse porang flour and hydrolyzed porang flour at the highest glucomannan content is shown in Figure 6. The overall diffractogram pattern resulting from the X-Ray Diffraction (XRD) analysis of this article for glucomannan granules of coarse porang flour and hydrolyzed porang flour at the highest glucomannan content was found at $2\theta = 10^{o} – 90^{o}$ as shown in Figure 6. The glucomannan granules of coarse porang flour had the highest intensity of diffraction peaks at $2\theta = 21.3^{o}$ at 134 a.u. and diffraction peaks with the lowest intensity at $2\theta = 83^{o}$ and 87.78^{o} at 6 a.u. While the glucomannan granules of porang flour hydrolyzed at the highest glucomannan content showed the diffraction peak with the highest intensity at $2\theta = 19.9^{o}$ with an intensity of 104 a.u. and the diffraction peaks with the lowest intensity at $2\theta = 66.58^{o}$ and 87.3^{o} with an intensity of 2 a.u. The same patterns were also found in native konjac glucomannan [20] and *Amorphophallus corrugates* glucomannan [17].

Based on the previous work, native glucomannan had low degree of crystallinity around or less than 5.43% which indicates that all of them almost fully amorphous [21]. The degree of crystallinity can be determined by comparing the crystalline area fraction with the total of the crystalline area fraction and amorphous area fraction. The data taken for the calculation of the percent crystallinity in this article were not all due to sharp or narrow diffraction peaks indicating the formation of crystals in the sample only occurred at certain diffraction angles. So that the crystalline area fraction is obtained by removing the amorphous area fraction first. The area of the crystalline fraction and the area of the amorphous fraction are entered into Eq. (4) to obtain the percent crystallinity (%) in Table 3. The removal of amorphous regions, determination of crystalline area fraction, amorphous area fraction, and degree of crystallinity were carried out using Origin Lab 2019b software. It can also be seen that

there is a decrease in the degree of crystallinity where the degree of crystallinity of the glucomannan granules of crude porang flour was 21.888%, whereas after the enzymatic hydrolysis pretreatment using the α-amylase enzyme, the degree of crystallinity of the hydrolyzed glucomannan granules of porang flour at the highest glucomannan content was 20.354%. Previous research indicated that water was originally sorbed in amorphous areas with less hydrogen bonds [22]. Based on these findings, it is possible to assume that the glucomannan chain may extend and that the water molecule could break down the glucomannan's inter and intramolecular hydrogen bonds. Because hydrogen bonds are weak, water can create hydrogen bonds with molecules, increasing the amorphous space area.

4. Conclusions

The hydrolysis process of porang flour was able to increase the glucomannan content from 51.937% to 95.191%. Increasing the concentration of α-amylase enzyme and the length of time can increase glucomannan levels because more enzymes work to degrade starch which results in more glucomannan products and the longer hydrolysis time gives the opportunity for α-amylase enzymes to interact with the substrate higher. The FT-IR spectra indicated that all the characteristic peaks of glucomannan appeared. The SEM analysis showed a smoother surface of hydrolyzed porang flour. The XRD analysis showed a decreased degree of crystallinity which indicated that the water was initially sorbed in the amorphous regions with less hydrogen bonds.

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