Study of glucomannan extraction with hydrochloric acid catalyst and alcohol solvent based on porang tuber flour (Amorphophallus oncophyllus)

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Abstract. *Amorphophallus oncophyllus* or commonly known as porang is a group of Aracea tubers. Porang has a high economic value and prebiotic source of oligosaccharides because it contains glucomannan. Porang flour contains about 67.5% glucomannan. Glucomannan is used as an emulsifier and stabilizer in the food, beverage and cosmetics products industry and as an ingredient for supplements and food additives because of its high fibres content. In this study, glucomannan purification can be optimized by using chemicals with modified maceration techniques with a stirring and washing machine using ethanol and glucomannan analysis using phenol sulphuric acid test in order to determine the decrease in sugar in porang flour. The purpose of this study was to determine the highest glucomannan content from several parameters such as the concentration of hydrochloric acid catalyst, stirring time, temperature, and the ratio of samples to solvents. The optimum conditions for the extraction process were at 0.7 M hydrochloric acid catalyst concentration, stirring time for 1 hour, temperature at 70^{o}C and the ratio of samples to solvents was 1:5. Therefore, the optimum glucomannan content obtained from extraction using hydrochloric acid catalyst reached 95.85%.

1. Introduction

Indonesia has some local tuber like cassava, gembili, potatoes, porang for main food sources that generally grows in Indonesian forests [1-2]. Porang (*Amorphophallus oncophyllus*) with growing properties that are rarely owned by other plants, porang has the ability to live in the shade of other plant stands [3]. Porang is an Aracea plant which has abundant potential sources because of its high glucomannan content and its economic value [4]. Fresh porang tubers contain 50% - 65% glucomannan and porang tubers cannot be consumed directly because they contain calcium oxalate [5]. Porang flour as an oligosaccharide group is a processed product of porang tubers which contains about 67.5% glucomannan [6-7]. Glucomannan consists of D-glucose and D-manose monomer units linked by β-1,4-glycosidic bonds [8]. Glucomannan has a solubility of 86.43%, viscosity of 5.400 cP, degree of acetylation of 13.7%, polymerization rate of 9.4, water holding capacity of 34.50% (WHC), and purity of 92.69% [9]. Glucomannan is categorized as a prebiotic source of oligosaccharides [10]. Porang flour has the potential to be a major non-food, food and health food export commodity that is

produced according to needs due to its glucomannan content [11]. Glucomannan is also used as an emulsifier and stabilizer in the food, beverage and cosmetic products industry [12]. As well as ingredients for supplements and food additives because of their high fiber content [13]. In addition to the ability to form a good gel, glucomannan is also used for drug delivery systems in the pharmaceutical field [14].

Among various methods, including extraction methods using MAE (*Microwave Assisted Extraction*), extraction methods using *Reflux Condenser* and extraction methods using maceration modification with a stirring machine have more advantages in relatively lower operating costs, and the process saves more solvents than other extraction methods [15]. Several studies have shown that the extraction method using MAE (*Microwave Assisted Extraction*) requires a *curing* process and very expensive operating costs [16], whereas *Reflux Condenser* is only used for thermostable metabolites [17].

In this study, glucomannan purification can be optimized by using chemicals with modified maceration techniques with a stirring and washing machine using ethanol [18]. Modified extraction methods using acid catalysts produce a faster process than the usual extraction process [15]. The process includes hydrolysis using an acid catalyst, precipitating alcohol, washing with water and ethanol, and high energy centrifugation [19]. Because ethanol has high polarity, ethanol can dissolve other compounds such as impurities, calcium oxalate and so on except glucomannan [20]. the high polarity is suitable for resins, fats, oils, fatty acids, carbohydrates and other dissolved organic compounds [20]. Precipitation alcohol is used to produce high levels of purity glucomannan [21].

The purpose of this study was to determine the effect of acid catalyst concentration, stirring time, temperature, and ratio of samples and solvents to glucomannan levels, and to determine the purity of glucomannan and reduction of reducing sugars during glucomannan extraction from porang flour through acid hydrolysis.

The development of extraction methods was also carried out in the research of Wardhani, et al [22]. The study used porang tubers. The extraction method was carried out within 240 minutes to produce 72.8% glucomannan purity with a temperature of 75^{o}C, a ratio of 8: 1 (ml / g), and a concentration of 80%. Likewise, Kumoro A.'s research [23], the method using a hydrochloric acid catalyst and ethanol washing with a fixed variable temperature of 60^{o}C and an independent variable of 0.5 M acid concentration and a reaction time of 1 hour resulted in purity reaching 90.18%. Although there are several studies that report on the extraction of glucomannan from porang flour using hydrochloric acid catalyst and washing using ethanol, there is no method development with various parameters of temperature, acid catalyst concentration, stirring time, and the ratio of samples and solvents that play an important role in obtaining optimum results.

2. Method

2.1. Materials and Reagents

Porang tuber flour (*Amorphophallus oncophyllus*) is obtained from local farmers in the Central Java area (Bantul, Yogyakarta). This porang tuber flour is pure flour. Porang tuber flour has a high enough glucomannan content. Glucomannan has the main chain of monosaccharides in the form of D-glucose and D-mannose. There are several reagents used in this study such as Ethanol 96% (Hepilab-Indonesia), Phenol (Indrasari-Indonesia), hydrochloric acid (HCl) (Mallinckrodt, For Laboratory, Research of Manufacturing-United States of America), sulphuric acid (H_2SO_4) (Mallinckrodt, For Laboratory, Research of Manufacturing-United States of America).

2.2 Extraction Process

This study was designed using a Completely Randomized Design (CRD) consisting of 4 independent variables. The independent variable consists of the concentration of HCl as a catalyst that is 0.1 M, 0.3 M, 0.5 M, 0.7 M, 0.9 M, the temperature at the time of extraction is 50^{o}C, 60^{o}C, 70^{o}C, 80^{o}C, stirring time when extraction is 30 minutes, 60 minutes, 90 minutes, 120 minutes, 180 minutes and 240 minutes and the ratio of the sample to the solvent is 1:4, 1:5, 1:6, 1:7 and 1:8.

Porang Tuber Flour will be extracted by comparison of the sample of porang tuber flour with its solvent, the concentration of the hydrochloric acid catalyst (HCL), temperature and time in accordance with the variables that have been determined using a mixer with a speed of 700 rpm. After extracting, the sample solution will be centrifuged using CENTRIFUGE ROTOFIX 32 at a speed of 3000 × RCF for 10 minutes so that the solid can be deposited. Then the liquid sample will be washed using 96% alcohol twice as much as the sample solution. Glucomannan will form. Glucomannan will be filtered and dried in an oven at 55^{o}C for 5 hours.

2.3 Sugar Reduction Analysis

This sugar reduction test was performed using the *Phenol Sulfuric Acid* method [24]. This method is solved by take 1 ml sample solution into a reaction tube. Then add 1 ml of 5% *phenol* and 5 ml of concentrated H_2SO_4 into a reaction tube containing 1 ml of sample solution. Reaction tubes that are ready will be incubated with water for 5 minutes at 90^{o}C. Then cooled and placed into a cuvette to measure its absorbance using the GENESYS 10 UV-Vis spectrophotometer at 490 nm.

3. Results and Discussion

3.1 Effects of Stirring Time on Glucomannan Extraction from Porang Flour (Amorphophallus oncophyllus)

The variables studied were stirring time when extraction lasted 30 minutes, 60 minutes, 90 minutes and 120 minutes. Research with time variables like that has also been done by Anindita F. [25]. This extraction was carried out with an optimal concentration of 0.5 M hydrochloric acid (HCl) catalyst and an optimal temperature of 70^{o}C in a study conducted by Kumoro A. [19]. While the optimal variable ratio of the sample to a solvent of 1: 8 in a study conducted by Wardhani D H. [22]. Research data on the effect of stirring time with a concentration of 0.5 M hydrochloric acid (HCl) catalyst, 70^{o}C temperature and 1:8 sample / solvent ratio on glucomannan levels are presented in Figure 1. From Figure 1, it was found that glucomannan levels from each mixing time varied. glucomannan levels to the stirring time produced with 0.5 M hydrochloric acid (HCl) catalyst concentration, temperature 70^{o}C and a sample / solvent ratio of 1:8 in 30 minutes resulted in glucomannan levels reaching 40.87%; at 60 minutes the resulting glucomannan levels reached 68.20%; at 90 minutes the resulting glucomannan levels reached 67.87%; at 120 minutes the resulting glucomannan levels reached 57.76%. During the first 60 minutes it gives a tendency to increase in glucomannan levels.

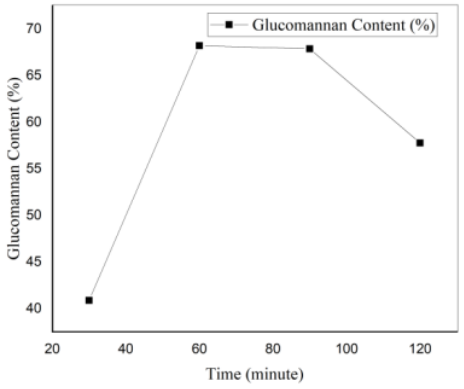

Figure 1. Effect of Stirring Time on Glucomannan Extraction

In the stirring time of more than 60 minutes there was a decrease in glucomannan levels due to the degradation of glucomannan to monosaccharides so that the long reaction time only slightly increased the concentration of sugar [19]. Extending the reaction time can reduce the concentration of reducing sugars which indicates the decomposition of glucose into degradation products. In the hydrolysis of polysaccharide acids, catalyzing acids not only convert polysaccharides to monosaccharides, but also there is a further degradation reaction of monosaccharides to furan acids and carboxylic acids [26]. The cause of decreased monosaccharides from polysaccharide acid hydrolysis due to prolonged degradation and increased acid concentration has also been previously reported [27]. The optimal time for this extraction process is 60 minutes. The same study was also conducted by Kumoro A. [19] which stated that the optimal stirring time during extraction was 60 minutes.

3.2 The Effect of Chloride Acid (HCl) Catalyst Concentration on Glucomannan Extraction from Porang Flour (Amorphophallus oncophyllus)

The next variable is the concentration of hydrochloric acid (HCl) catalyst, namely 0.1 M, 0.3 M, 0.5 M, 0.7 M and 0.9 M. 60 minutes and an optimal temperature of 70^{o}C in a study conducted by Kumoro A. [19]. While the optimal sample / solvent ratio variable is 1: 8 in a study conducted by Wardhani D H. [22]. Research data on the effect of hydrochloric acid (HCl) catalyst concentration with stirring time at extraction of 60 minutes, temperature of 70^{o}C and ratio of sample / solvent 1: 8 to glucomannan levels are presented in Figure 2. It can be seen in Figure 2, that the glucomannan content of each hydrochloric acid (HCl) catalyst concentration varies. Glucomannan concentration to the concentration of hydrochloric acid (HCl) produced with a stirring time of 60 minutes, a temperature of 70^{o}C and a sample / solvent ratio of 1: 8 at 0.1 M hydrochloric acid (HCl) concentration resulted in glucomannan levels reaching 88.61%, at concentrations of hydrochloric acid (HCl) 0.3 M produces glucomannan levels reaching 90.11%, at 0.5 M hydrochloric acid concentration (HCl) produces glucomannan levels reaching 92.24%, at concentrations of hydrochloric acid (HCl) 0.7 M produces glucomannan levels reached 94.91% and at a concentration of 0.9 M hydrochloric acid (HCl) produced glucomannan reached 91.47%.

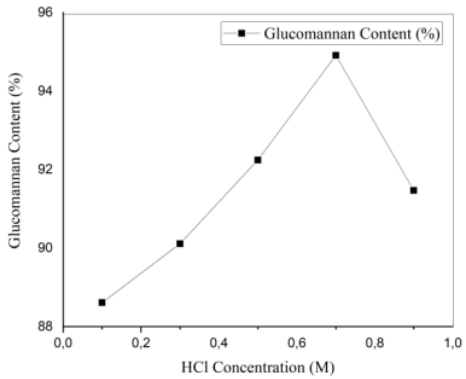

Figure 2. Effect of Stirring Time on Glucomannan Extraction

Homogeneous acid hydrolysis of starch is regulated by the acidity (pH) of the solution which is directly proportional to the concentration of hydronium ions (H_3O+) present in the solution [28]. In general, the rate of hydrolysis reaction and sugar formation is directly proportional to the concentration of acid, the more acidic concentration increases, the rate of hydrolysis reaction and

sugar formation will also increase due to increased activity of hydrogen ions that take part in the reaction as catalysts [29]. Concentration of hydrochloric acid (HCl) catalyst increased at 0.1 M, 0.3 M, 0.5 M, 0.7 M and decreased at 0.9 M hydrochloric acid (HCl) concentration. This could occur due to treatment acid to high temperatures which can cause degradation of polysaccharides separately to form side products in the form of furfural and hydroxymethylfurfural so as to reduce glucomannan levels [30]. Decomposition of excess levels of glucomannan is transformed into another product that causes this phenomenon [31].

3.3 Effect of Temperature on Glucomannan Extraction from Porang Bulbs Flour (Amorphophallus oncophyllus)

The effect of temperature on glucomannan was observed using extraction methods. Research with the optimal stirring time variable that has been produced from this research is 60 minutes and the optimal temperature of 70^{o}C in research conducted by Kumoro A. [19]. Optimal ratio of solvent to 1: 8 (mg / ml) ratio in a study conducted by Wardhani D. H. [22]. Research data for ratio of sample to solvent 1: 8 and the optimal concentration of 0.7 M hydrochloric acid catalyst at 50^{o}C to 80^{o}C for 60 minutes against glucomannan levels are presented in Figure 3. It can be seen in Figure 3, that glucomannan levels at various temperature variations have different glucomannan results. At a temperature of 50^{o}C the resulting glucomannan levels reaching 92.25%, at 60^{o}C produces a glucomannan levels reaching 94.69%, while at 70^{o}C produces a glucomannan levels reaching 95.11%. But at a temperature of 80^{o}C, the resulting glucomannan content decreased significantly, reaching 90.76%.

Higher extraction temperatures help remove more impurities and obtain high purity [32]. However, Figure 3.3 shows that glucomannan levels are relatively insignificant. At a temperature of 80^{o}C there is a decrease in glucomannan levels, this is caused by the glucomannan undergoing a gelatinization process so that glucomannan becomes damaged. At high temperatures, glucomannan will break down into smaller molecules or undergo hydrolysis [33].

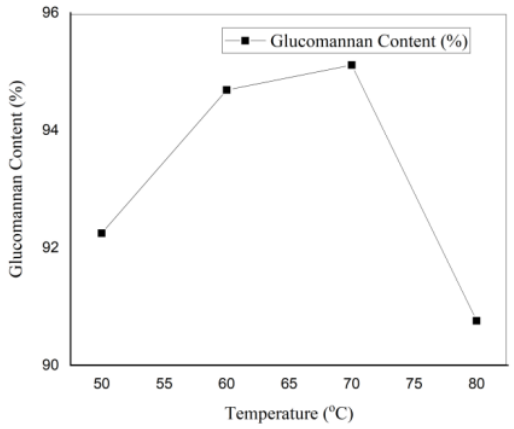

Figure 3. Effect of Temperature on Glucomannan Extraction

According to ISHS Acta [34], the glucomannan decomposition temperature is 280^{o}C, but it does not rule out that glucomannan will be damaged at 80^{o}C. Extremely high temperatures in the extraction process can cause a reduction in the amount extracted and also due to the nature of glucomannan which is very easily damaged when at temperatures over 80^{o}C for a long time [35]. The results of a similar study conducted by Kumoro A. [19], the possibility of degradation of glucomannan through acid hydrolysis is used to form byproducts because the extraction conditions at high temperatures

cause a decrease in glucomannan levels. The resulting by-products such as reducing sugars and other low molecular weight fractions are soluble in ethanol and do not reappear during the ethanol deposition stage leading to decreased glucomannan content [36].

3.4 Effect of Comparison of Sample and Solvent Ratios on Glucomannan Extraction from Porang Bulbs Flour (Amorphophallus oncophyllus)

The effect of the ratio of sample and solvent ratio on glucomannan was observed using the extraction method. Research with variable ratio of sample and solvent ratio has also been conducted by Wardhani D. H. [21] with a solvent comparison sample of 1: 8 (mg/ml). Research data on the effect of ratio of samples and solvents 1:4 (mg/ml) to 1: 8 (mg/ml) with stirring time at extraction 60 minutes, temperature 70^{o}C and concentration of hydrochloric acid (HCl) catalyst 0.7 M to Glucomannan levels are presented in Figure 4. Figure 4, shows that glucomannan levels at various ratio ratios of samples and solvents have different glucomannan results. For a ratio of 1: 4 (mg/ml) glucomannan levels reached 95.38%, while at a ratio of 1: 5 (mg / ml) the highest levels of glucomannan reaching 95.85%. At a ratio of 1: 6 (mg/ml) decreased with glucomannan levels reaching 92.73%, at a ratio of 1:7 (mg/ml) the resulting glucomannan levels decreased to 91.98% and for a ratio of 1: 8 (mg/ml) experienced a significant decrease in glucomannan levels, reaching 89.75%.

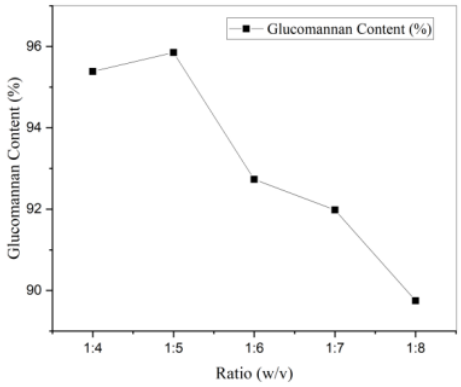

Figure 4. Effect of Comparison of Sample and Solvent Ratio on Glucomannan Extraction

The ratio of sample to solvent ratio tends to show that the more solvents are used, the greater the level of glucomannan produced [37]. However, Figure 4, shows that glucomannan levels are relatively insignificant. Variable ratio of 1: 6 (mg/ml) to 1:8 (mg/ml) has decreased levels of glucomannan. This is due to the ratio of the solvent that is too much water in the extraction process will result in a lower concentration of extracted glucomannan and an increase in the amount of water that coats the glucomannan particles. In this case when the extraction results are precipitated with anti-solvent, the water molecule lining the glucomannan molecule is not completely precipitated, causing a decrease in glucomannan levels [35]. In this study, a ratio of samples: 1: 5 (w/v) gave 91.32% glucomannan. This result is higher than Wardhani D.H. [22] who obtained 72.8% glucomannan from porang purification using a solvent-solid ratio of 8:1 (v/w) with 80% ethanol solution for 4 hours.

4. Conclusion

The optimum conditions for the extraction process from porang flour (*Amorphophallus oncophyllus*) using hydrochloric acid catalyst (HCl) were at 0.7 M hydrochloric acid catalyst concentration, stirring time for 1 hour, temperature at 70^{o}C and the ratio of samples to solvents was 1:5. Therefore, the

optimum glucomannan content obtained from extraction using hydrochloric acid catalyst reached 95.85%.

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