Acidic and enzymatic hydrolysis of glucomannan agroindustrial waste

M Nasrullah, Purwoko and T C Sunarti*

Department of Agroindustrial Technology, Faculty of Agricultural Engineering and Technology, IPB University (Bogor Agricultural University), Indonesia.

*E-mail: titi-cs@apps.ipb.ac.id

Abstract. Processing iles-iles tubers (Amorphophallus onchophyllus) into glucomannan flour leaves waste in the form of solid powder which is a by-product of the glucomannan separation process. The waste contains starch, fiber, and the remaining glucomannan which can still be used as simple sugar through hydrolysis. The purpose of this study is to examine the hydrolysis process of glucomannan agro-industrial waste using acidic and enzymatic methods as a comparison and to determine its effect on the products and by-products formed. Acid hydrolysis using conventional heating (autoclaves) and microwaves with dilute acids, HCl and H$_2$SO$_4$ at concentrations of 0.5 M and 1 M. Enzymatic hydrolysis using a consortium of $\alpha$-amylase (1.75 and 3.5 U/g), amyloglucosidase (0.3 and 0.6 U/g), and cellulase (1 and 2 U/g). The results showed the potential of waste with the main components consisting of starch (47.26 ± 0.29%), fiber (15.79 ± 0.62%), and residual glucomannan (12.64 ± 0.26%). Acid hydrolysis using HCl (0.40 - 0.56 g sugar/g dry matter) results slightly higher than H$_2$SO$_4$ (0.43 - 0.53 g sugar/g dry matter). Autoclaves hydrolysis produces sugars with a high DE (76.08 - 100.18), but with a lower yield (35.90 - 40.50 %), while the microwave hydrolysis produces sugars with a DP (1.62 - 2.22) and a higher yield. The higher the concentration of acid, the reducing sugar value, DE, and the higher HMF, but lower DP. In enzymatic hydrolysis, an increase in enzyme dose gives results that are not significantly different. The best treatment is obtained from enzymatic hydrolysis with normal doses because it has a reducing sugar (0.43 g reducing sugar/g dry matter) and total sugar (0.66 g sugar/g dry matter) high and a low HMF (0.19 mg HMF/g dry matter).

1. Introduction

Iles-iles (Amorphophallus onchophyllus) is a type of taro that is spread in almost all of Indonesia's forests. Plants produce tubers called iles-iles bulbs. The iles-iles bulbs are mostly used as raw materials for making glucomannan/konjac flour. Glucomannan flour processing consists of peeling the tuber skin, washing the tubers, slicing the tubers, soaking, and drying the tuber chips. Dry chips were ground to produce crude iles-iles flour. Then purified by mechanical means by blowing.

Crude iles-iles flour has a glucomannan content of 51.3 - 71.6 (%) [1]. Glucomannan flour processing leaves waste, composed of starch, fiber, and glucomannan flour which can still be utilized. If calculated based on the total amount of glucomannan content [1] available reserves of glucomannan agroindustrial waste amounted to 28.4 - 48.7 (%) in crude iles-iles flour. Glucomannan agroindustrial waste has not yet been utilized, so further processing needs to be done to make glucomannan agroindustrial waste more useful. An alternative use is to hydrolyze glucomannan waste into simple sugars.
There are several methods such as enzymatic hydrolysis, chemical, as well as combinations. Enzymatic hydrolysis can work specifically to produce a high yield but require a relatively long time. Chemical hydrolysis can use low concentrations of strong acids or dilute with conventional heating (autoclaves) or microwave heating. The duration of acid hydrolysis is relatively shorter. In the hydrolysis of glucomannan agro-industrial waste, a method that can hydrolyze the content of starch, fiber, and glucomannan residues simultaneously can produce higher yields in a relatively short time.

Some previous researches have been conducted to convert starch, fiber, and glucomannan to sugar. Kato et al. [2] has reported the crude iles-iles flour can be hydrolyze with cellulase enzyme to produce oligosacharides. Kunlan et al. [3] has reported the starch can be hydrolyzed to glucose in acid with microwave irradiation in a sort time. Taherzadeh et al. [4] has reported lignocellulose can be hydrolyzed using acid. In this research, a study of the hydrolysis method between acid hydrolysis with conventional heating (autoclave), acid with microwave heating and enzymatic hydrolysis as a comparison. The use of different hydrolysis methods is thought to give different results.

2. Materials and methods

2.1. Materials
Glucomannan agroindustrial waste was provided by the glucomannan industry in Pasuruan, Indonesia. The enzymes were commercial food-grade enzymes for α-amylase, amyloglucosidase, and cellulase. Microwave oven sharp type R-348 C output 1000 W High power and 32 L capacity. HCl, H₂SO₄ and other chemicals used were the product with commercial grade.

2.2. Characterizations of raw material
Glucomannan agroindustrial waste was characterized by proximate analysis [5], starch content [6], glucomannan content [7], fiber content [8].

2.3. Hydrolysis
The hydrolysis method used is the acid hydrolysis method with conventional heating (autoclave), acid hydrolysis by microwave heating, and enzymatic hydrolysis. The material was suspended to 6(%) (w/w) using distilled water pH 5.2 for enzymatic hydrolysis and HCl solution and H₂SO₄ solution with concentrations of 0.5 and 1 M for acid hydrolysis.

Acid hydrolysis with conventional heating (autoclave) consists of two stages, namely gelatinization and hydrolysis. Gelatinization was carried out on a water bath with a temperature of 100°C for 10 minutes. Hydrolysis was performed on an autoclave at 121°C for 15 minutes. Acid hydrolysis by microwave heating using the method of Sunarti et al. [9] which consists of 2 stages: using a power level 30 (%) for 2 minutes and using a power lever of 70 (%) for 3 minutes.

Enzymatic hydrolysis consists of 2 stages, namely liquidation and saccharification. Liquidation using the α-amylase enzyme at a dose of 1.75 U/g starch [10] and 3.5 U/g starch for 3 hours. Saccharification uses amyloglucosidase enzyme at a dose of 0.3U/g starch [10] and 0.6 U/g starch and cellulase enzyme at a dose of 1 U/g fiber [10] and 2 U/g fiber for 48 hours.

2.4. Characterizations of hydrolysate
Product characterization is done by analyzing reducing sugar content [11], total sugar content [12], dextrose equivalent (DE), degree of polymerization (DP), filtrate volume, yield, hydroxymethylfurfural (HMF) content [13], and microscopic structure of residues.

3. Results and discussions

3.1. Characterization of raw material
Glucomannan agroindustrial waste has a moisture content (8.00 ± 0.04%), ash content (8.46 ± 0.09%), protein content (10.85 ± 0.14%), fat content (7.48 ± 0.32%), crude fiber content (15.79 ± 0.62%),
carbohydrate content (49.42%), starch content (34.62 ± 0.29%), glucomannan content (12.64 ± 0.26%), hemicellulose content (14.69 ± 0.26%), cellulose content (12.95 ± 0.75%), and lignin content (1.47 ± 0.05%). Similar research Dwiyono et al. [14] also reported the crude iles-iles flour has content of glucomannan (35.77 ±0.18%), starch (31.36 ± 4.3%), and fiber (9.06 ± 0.52%).

3.2. Characterization of hydrolysate

Treatment hydrolysis gives different results in the hydrolysate. The characteristics observed in this study were reducing sugars, total sugar, dextrose equivalent, degree of polymerization, filtrate volume, yield, hydroxymethylfurfural. The results of the characteristics provided in table 1.

| Treatment | Reducing sugar (g/g) | Total sugar (g/g) | DE | DP | Volume filtrate (ml) | Yield (%) | HMF (mg/g) |
|-----------|----------------------|------------------|----|----|---------------------|----------|-----------|
| HOC1      | 0.35c                | 0.40a            | 88.68d  | 1.13ab | 121.10c            | 35.90c   | 0.69c     |
| HOC2      | 0.45d                | 0.45ab           | 99.67e  | 1.00a  | 115.65de           | 40.50ab  | 1.57f     |
| HOS1      | 0.33c                | 0.44ab           | 76.08c  | 1.32abc | 112.80ad          | 39.57ab  | 0.55b     |
| HOS2      | 0.43d                | 0.43abc          | 100.18e | 1.00a  | 108.73c            | 38.82ab  | 1.16e     |
| HMC1      | 0.23a                | 0.47b            | 49.85a  | 2.04cf | 76.68ab            | 42.02b   | 0.31a     |
| HMC2      | 0.35c                | 0.56d            | 61.88b  | 1.62cd | 78.50ab            | 50.28d   | 0.81d     |
| HMS1      | 0.22a                | 0.48bc           | 45.65a  | 2.22f  | 70.65a             | 43.29bc  | 0.29a     |
| HMS2      | 0.29b                | 0.53cd           | 55.35ab | 1.81de | 79.05b             | 47.98cd  | 0.51b     |
| HEN1      | 0.43d                | 0.66c            | 64.81b  | 1.54cd | 130.55f            | 59.43c   | 0.19a     |
| HEN2      | 0.46d                | 0.69e            | 66.66bc | 1.50bcd | 132.30f           | 62.12c   | 0.20a     |

*a dry base
HO : Hydrolysis with autoclaves
HM: Hydrolysis with microwaves
C : HCl (C1: concentration of 0.5 M, C2: concentration of 1 M)
S : H2SO4 (S1: concentration of 0.5 M, S2: concentration of 1 M)
HEN : Enzymatic hydrolysis (HEN1: normal enzyme dose (α-amylace 1.75 U/g, amyloglucosidase 0.3 U/g, cellulase 1 U/g), HEN2: increased enzyme dosage (α-amylace 3.5 U/g, amyloglucosidase 0.6 U/g, cellulase 2 U/g))

3.2.1. Acidic hydrolysis with autoclave. Acid hydrolysis is a chain scission process starch by acids at random and is not affected by the bond of α-1, 6-glycosidic [15]. The hydrolysis process that is commonly used is the conventional heating using an autoclave. The hydrolysis process is divided into two stages; gelatinization and hydrolysis. Gelatinization aims to break down the starch granules so that the acid easily reaches the starch molecule. Acid hydrolysis by autoclaving heating can produce high reducing sugars but is less able to hydrolyze the fiber, so that the fiber is left as a residue. Autoclaving hydrolysis also produces a byproduct that is high hydroxymethylfurfural (HMF).

3.2.2. Acidic hydrolysis with microwaves. Acid hydrolysis can also use microwaves. The use of microwaves is done in a shorter time, the time used is less than 15 minutes. The longest hydrolysis time is less than 10 minutes, but during the process a secondary product is formed resulting from the decomposition of the hydrolysis product, causing a dark color [16]. The mechanism of heat transfer in acid hydrolysis with microwaves takes place by radiation. According to Taylor [17], the mechanism of microwave heating is the movement of a magnetic or electric field that causes agitation of moving polar molecules. Magnetic and electric fields cause particles to be limited by the limiting force so that they form heat by random motion. Microwave radiation can provide even heating on a mixture of materials.

3.2.3. Enzymatic hydrolysis. The enzymatic hydrolysis process consists of three stages, namely gelatinization, liquidation, and saccharification. Gelatinization is the formation of a thick suspension of
starch granules. Liquidation is the process of melting starch gel into dextrin (oligosaccharides) by the α-amylase enzyme. Saccharification is the process of converting oligosaccharides into glucose with the help of the enzyme amyloglucosidase. The α-amylase enzyme will hydrolyze starch to glucose, maltose, and malto-oligosaccharides. The enzyme added is an enzyme consortium consisting of the enzyme amyloglucosidase to break the amylose chain and the cellulase enzyme to hydrolyze the cellulose fiber portion.

3.2.4. Reducing sugar. The value of reducing sugars ranges from 0.22 - 0.46 g/g dry matter. This shows that around 22 - 46 (%) of the material is converted to reducing sugars. Analysis of variance showed results that significantly affected the value of reducing sugars in each treatment. The value of reducing sugars in autoclave hydrolysis is higher than microwave hydrolysis. Autoclave hydrolysis can convert more material into reducing sugars than microwave hydrolysis. The use of high temperatures and a long time in acid hydrolysis is thought to cause more material to be converted to reducing sugars.

The temperature in the microwave hydrolysis is thought to be higher than in the autoclave hydrolysis it is thought to be due to the high evaporation of the microwave hydrolysis. However, the use of high temperatures in microwave hydrolysis has not been able to increase reducing sugar levels because of the use of shorter microwave hydrolysis time.

![Reducing sugar content hydrolysate](image)

**Figure 1.** Reducing sugar content hydrolysate.

The value of reducing sugars value in enzymatic hydrolysis has increased but not significantly different. That is because the enzymes are already in their optimum state to catalyze substrate into products. Enzyme hydrolysis with normal enzyme dosage (HEN1) is hydrolysis using optimum enzyme dosage. In this study, cellulase enzymes are targeted to be able to hydrolyze fibers, but cellulase enzymes can also hydrolyze glucomannan which is not hydrolyzed by the α-amylase enzyme. According to Mutia [18], the α-amylase enzyme is thought to be unable to hydrolyze glucomannan because there is no change in the form of glucomannan on microscope observation. Cellulase can cut glucomannan because glucomannan has β-1,4 glycosidic bonds. Kato et al. [2] has reported the glucomannan can be hydrolyzed by cellulase enzyme to produce mannose, glucose, mannobiase, mannosil glucose, mannotriosa, glucose mannobiosil, mannotetraosa, glucose mannotriosil and mannotetraosilglucose.

3.2.5. Total sugar. Total sugar values range from 0.40 to 0.69 g/g dry matter. This shows that around 40 - 69 (%) of the ingredients were converted to sugar. Analysis of variance showed results that significantly affected the value of total sugar in each treatment. The total sugar value in microwave hydrolysis is higher than autoclave hydrolysis. This shows that microwave hydrolysis is more able to break down substrates into simple sugars than autoclaves hydrolysis.

The highest total sugar value is enzymatic hydrolysis. That's because the enzymes work specifically. The total sugar value tends to increase with each treatment when the acid and enzyme doses increase, but there is a decrease in the autoclave hydrolysis treatment with H₂SO₄ acid. The decrease can be caused
by the degradation of sugar into furfuraldehyde derivatives (such as furfural and hydroxymethylfurfural), so that the total sugar value measured is lower.

Figure 2. Total sugar content hydrolysate.

3.2.6. **Dextrose equivalent and degree of polymerization.** Dextrose Equivalent values range from 49.85 - 100. Analysis of variance showed results that significantly affected the value of DE in each treatment. DE autoclave hydrolysis is higher than microwave hydrolysis and enzymatic hydrolysis. That is due to the high value of reducing autoclave hydrolysis sugar. A low DE value shows that most of the hydrolyzate is composed of a component of sugars with relatively high molecular weights. There was an increase in DE in all treatments when the acid concentration and enzyme dose were increased. This shows that an increase in acid concentration and enzyme dosage can increase the conversion of ingredients into simpler sugar components. The use of different acids does not provide the difference in the value of DE.

Degree of polymerization (DP) values range from 1 - 2.2. Analysis of variance showed results that significantly affected the value of DP in each treatment. Microwave hydrolysis has a higher DP value than autoclave and enzymatic hydrolysis. This shows that the sugar contained is still in the form of simple sugars but has not been fully converted to reducing sugars.

3.2.7. **Yield.** The yield value ranges from 35.90 - 62.12 (%). This shows that there are around 35.90 - 62.12 (%) material that has been converted to a product. Analysis of variance showed results that significantly affected the value of yield in each treatment. Yield value correlates with the total value of sugar because the yield value is calculated based on the total sugar produced per unit of material. Enzymatic hydrolysis has the highest yield value because the enzymes in the hydrolysis process work specifically. While on acid hydrolysis, acid works randomly. Acid hydrolysis with microwaves has a higher yield than acid hydrolysis with autoclaves because heating in the autoclave takes place from outside into the material while heating in the microwave takes place in the material due to the influence of microwave radiation so that the material is more easily degraded.

Figure 3. Yield hydrolysate.
3.2.8. Filtrate volume. Microwave hydrolysis has decreased in volume due to evaporation during hydrolysis. In enzymatic hydrolysis and autoclave hydrolysis also occurs evaporation, but the evaporation is not as big as in hydrolysis of microwaves. The temperature in the microwave hydrolysis is thought to be higher than in the autoclave, it is allegedly due to the high evaporation of the microwave hydrolysis.

3.2.9. Hydroxymethylfurfural. Hydroxymethylfurfural (HMF) values range from 0.19 - 1.57 mg/g dry matter. Analysis of variance showed results that significantly affected the value of hydroxymethylfurfural in each treatment. Autoclaving hydrolysis produces higher HMF than microwave hydrolysis and enzymatic hydrolysis. According to Kunlan et al. [19], heating using microwaves can produce lower by-products because heating is done in a short time. Enzymatic hydrolysis treatment produced the least HMF value because it took place at pH 5.2 (not too acidic) and temperature of 95°C and 60°C (not too high). The increase of acid concentration in the autoclave and microwave hydrolysis treatment gives a significantly different and quite significant increase. That is due to the degradation of glucose by acids. According to Tsao et al. [20], strong acids can cause glucose degradation from hydrolysis. The use of different acids gives different results on the value of HMF. Hydrolysis using HCl produces more HMF compared to H2SO4. That is because the higher HCl acid reactivity (compared to H2SO4) causes more glucose to be degraded.

![Figure 4. Hydroxymethylfurfural content hydrolysate.](image)

3.2.10. Appearance of residues. In glucomannan agroindustry waste without treatment (figure 5 NA) visible components of glucomannan, starch, fiber, and calcium oxalate. Fiber that has not been treated looks tight. After undergoing treatment the fibers begin to drift apart or break. The tenuous structure of the fibers indicates that the fibers have turned into amorphous. In autoclave hydrolysis (figure 5 HOC HOS), the fibers look amorphous in shape with some parts still tight. In microwave hydrolysis (figure 5 HMC HMS), the fibers appear to be amorphous in shape with very little denseness. In enzymatic hydrolysis (figure 5 HEN), the fibers appear to be smooth or destroyed. Microwave hydrolysis is more effective in hydrolyzing fibers compared to autoclave hydrolysis, this is indicated by the more amorphous fiber structure in microwave hydrolysis. According to Taylor [17], heating microwaves can cause random movements in polar molecules so that it produces heat. According to Sunarti et al. [10], autoclave treatment can gelatinize starch but cannot degrade fiber. In microwave heating, the fiber can be degraded into an amorphous form. Enzymatic hydrolysis produces a smooth residue, it shows enzymatic hydrolysis can hydrolyze the material to the crystalline structure of the fiber.
4. Conclusion

The main components of glucomannan agro-industry consist of starch (47.26 ± 0.29%), fiber (15.79 ± 0.62%), residual glucomannan (12.64 ± 0.26%). Acid hydrolysis using HCl (0.40 - 0.56 g sugar/g dry matter) results slightly higher than H₂SO₄ (0.43 - 0.53 g sugar/g dry matter). Autoclaves hydrolysis produces sugars with a high DE (76.08 - 100.18), but with a lower yield (35.90 - 40.50 %), while the microwave hydrolysis produces sugars with a DP (1.62 - 2.22) and a higher yield. The higher the concentration of acid, the more reduced the sugar value, DE, and the higher the HMF, but the lower the DP. In enzymatic hydrolysis, an increase in enzyme dose gives results that are not significantly different. The best treatment is obtained from enzymatic hydrolysis with normal dose because it has a high reducing sugar (0.43 g reducing sugar/g dry matter) and total sugar (0.66 g sugar/g dry matter) and a low HMF (0.19 mg HMF/g dry matter).

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