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Effect of enzymatic hydrolysis and nitrogen on *Saccharomyces cerevisiae* β-glucan production from *Manihot utilissima* and *Maranta arunadinacea* waste

Efek hidrolisis enzimatis dan nitrogen pada produksi β-glukan Saccharomyces cerevisiae *dari onggok* Manihot utillissima *dan* Maranta arunadinacea

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Abstrak

*Penelitian ini memanfaatkan onggok ubi kayu (*Manihot utilissima*) dan onggok umbi garut (*Maranta arunadinacea*) sebagai media perbanyakan* Saccharomyces cerevisiae *untuk produksi β-glukan. Limbah tersebut dihidrolisis oleh enzim amiloglukosidase menjadi glukosa kemudian dilanjutkan fermentasi dalam medium nitrogen oleh* S. Cerevisiae. *Pelet β-glukan diekstraksi menggunakan larutan alkali NaOH 2% pada suhu 90°C selama 5 jam, dilanjutkan serangkaian proses sentrifugasi. Konsentrasi glukosa hasil hidrolisis tertinggi diperoleh dengan penambahan enzim amiloglukosidase* M. arundinacea *57,5 mg dengan konversi sebesar 95,93% dan penambahan enzim* M. utilissima *50 mg dengan konversi sebesar 64,70%. Produksi* S. cerevisiae *jumlah tertinggi diperoleh dengan menambahkan 4,75 g pepton ke semua sampel. Jumlah sel optimum diperoleh sebanyak 1,61 x 10^8 koloni pada t = 48 jam untuk onggok umbi garut dan 8,55 x 10^7 koloni pada t = 48 jam untuk onggok ubi kayu. Untuk produksi β-glukan, angka tertinggi diperoleh dengan menggunakan pepton 3,99 g dan 4,75 g masing-masing untuk onggok ubi kayu dan umbi garut dengan rendemen berturut-turut 1,20% dan 1,23%. Untuk pelet β-glukan, jumlah tertinggi adalah 1,77 g L^{-1}(0,18% b/v) dari media onggok ubi kayu dan 1,91 g L^{-1} (0,19% b/v) dari onggok umbi garut. Sel mutan dalam media Yeast Extract–Peptone–Glycerol (YPG) menghasilkan 6,56 g L^{-1} (0,66% b/v) pelet β-glukan sedangkan sel tipe liar dalam media YPG menghasilkan 1,84 g L^{-1} (0,18% b/v).*

[Kata kunci: amiloglukosidase, onggok ubi kayu, onggok umbi garut, pelet]

Abstract

This experiment utilised cassava (*Manihot utillissima)* and arrowroot (*Maranta arunadinacea)* wastes as the medium of propagation of *Saccharomyces cerevisiae* to produce β-glucan. The amyloglucosidase hydrolysed the waste, followed by fermentation in the nitrogenous medium by *S. cerevisiae*. The β-glucan pellet was extracted using 2% NaOH alkaline solution at 90°C for 5 hours, followed by a series of centrifugation processes. The highest glucose concentration from hydrolysis resulted from adding 57.5 mg amyloglucosidase enzyme for arrowroot waste with 95.93% conversion and 50 mg enzyme for cassava waste with 64.70% conversion. The highest amount was obtained for producing *S. cerevisiae* by adding 4.75 g peptone to all samples. The optimum number of cells was obtained at 1.61 x 10^8 colonies at t = 48 hours for arrowroot waste and 8.55 x 10^7 colonies at t = 48 hours for cassava waste. For β-glucan production, the highest number was obtained by using 3.99 g of peptone for cassava waste with a yield of 1.20% and by using 4.75 g of peptone for arrowroot waste with a yield of 1.23%. For β-glucan pellet, the highest number was 1.77 g L^{-1} (0.18 % b/v) from cassava waste medium and 1.91 g L^{-1} (0.19% b/v) from arrowroot waste. Mutant cells in the Yeast Extract–Peptone–Glycerol (YPG) medium produced 6.56 g L^{-1} (0.66% b/v) β-glucan pellet, while wild-type cells in the similar medium produced 1.84 g L^{-1} (0.18% b/v).

[Keywords: amyloglucosidase, arrowroot waste, cassava waste, pellet]

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Introduction

An unhealthy lifestyle, such as unbalanced meals, lack of exercise, high-stress levels, and depression, causes a high level of cancer and coronary heart disease in Indonesia (Ariyanti & Besral, 2019). If we do not act, cancer and coronary heart patients will surely come through stroke, coma, or even death (Shin et al., 2019). Experiments have been done to set food supplements that can decrease cholesterol levels in the blood, prevent free radicals, and reinforce the immune system in human bodies so that they can be exempt from some diseases that cause death. One of the experiments is about β-glucan (Earnshaw et al., 2017).

The β-1,3-glucan is a polysaccharide composed of monomers with β-1,3 bonds. This polysaccharide is acquired from yeast, wheat, and bacteria. *Saccharomyces cerevisiae* is one of the yeasts that contains β-glucan in its cell (Kusmiati & Dhewantara, 2016). The cell walls of *S. cerevisiae* consist of two layers built from four primary molecules, mannoprotein, β-1,6-glucan, β-1,3-glucan, and chitin. In anaerobic conditions, *S. cerevisiae* takes fermentation in the metabolism process. On the other hand, *S. cerevisiae* respirators for cell multiplication.

Saccharomyces cerevisiae reaches its optimum growth when it stands in a medium with a high level of nitrogen and glucose level sources, where peptone acts as the best nitrogen provider from other sources (Utama et al., 2021). Sugar acts as an energy source, nitrogen saver, and metabolism regulator for *S. cerevisiae*'s propagation (Thontowi et al., 2007). Besides, nitrogen is a building block to synthesize parts of constituent cells.

One of the substances rich in glucose sources is industrial tapioca waste, cassava waste, and arrowroot waste (Rosyadi et al., 2014). The pulp contains 45-65% of starch, a potential polysaccharide, usually used for fodder (Lopez-Diago et al., 2018). So far, few experiments were developed using waste as a growth medium for yeast. In other experimenters, the medium used is already glucose or other substances with high sugar levels, such as molasses and papaya. The waste from cassava and arrowroot were used as *S. cerevisiae* propagation mediums for β-glucan production.

Starch in the pulp can be used as a glucose source to increase *S. cerevisiae* cell production (Hidayat et al., 2020). Amyloglucosidase is an enzyme that can split starch or dextrin into glucose (Warren et al., 2015). This enzyme was chosen as a hydrolysis enzyme because it does not need operation conditions and has a high-yield conversion. Subsequently, *S. cerevisiae* is allowed to propagate in a nitrogenous medium. The amount of nitrogen in the medium is variated to see its effect on cell growth. By doing starch hydrolysis, the *S. cerevisiae* production is expected to be high because the need for glucose for metabolism is already fulfilled. This experiment uses *S. cerevisiae* mutant cells so that the β-glucan obtained can be extracted easier in base than its wild type.

Materials & Methods

Materials

Materials used were β-glucan (Merck), ethanol standard (ethyl alcohol, 99.5% by Sigma-Aldrich), and biuret (Merck). The amyloglucosidase enzyme from *Aspergillus niger* A7420 was purchased from Sigma-Aldrich, *S. cerevisiae* InaCC Y93 was obtained from Indonesia Culture Collection (InaCC), Cassava waste and arrowroot waste samples were purchased from local market. Yeast Extract–Peptone–Glycerol (YPG) was prepared with yeast extract (Merck), peptone broth (Merck) and glucose, with the composition ratio of 1%: 2%:2%, respectively and agar 2%.

Preparation

Firstly, cassava waste and arrowroot waste samples were peeled before being cleanly washed. After that, they were granted until liquor waste was obtained. Grated results were then squeezed and filtered so that the starch was obtained. The retentate was kept for the experiment. Furthermore, cassava and arrowroot waste samples were dried in an oven at 40°C. Finally, these dry samples were shattered and sifted with a 60-mesh measure to obtain samples in homogenous conditions.

Ten grams of dry samples were gelatinized with an autoclave at 110°C for 60 minutes and diluted with 250 ml water. The results from these processes were hydrolyzed by adding amyloglucosidase enzyme at operation temperature 55°C and pH 4,5 with 50.0 mg, 52.5 mg, 55.0 mg, 57.5 mg, and 60.0 mg variations (for 40000, 42000, 46000, 48000, 500000, and 52000 unit) for 1 hour. The amount of glucose in the samples must be measured before and after hydrolysis to obtain the most optimum amyloglucosidase enzyme composition. The needs of the amyloglucosidase enzyme could be acquired by doing some theoretical calculations.

S. cerevisiae *preparation, production, and homogenization*

S. cerevisiae isolates were sub cultured in a Petri dish in solid YPG medium with the composition: 2% peptone, 1% yeast extract, 2% glucose, and 2% agar (Shokri et al., 2008). This culture was subsequently incubated at 37°C for 48 hours. One growing colony were moved to YPG agar media and incubated for 48

hours. One ose culture was grown to 4 ml liquid YPG media as a pre-culture and then incubated for 48 hours at 150 rpm at standard temperature. The incubated pre-culture was put into YP β-glucan media, which contains hydrolyzed starch with optimum enzyme composition, and subsequently incubated at a speed of about 150 rpm for 3 days at 36°C to obtain cell culture. Peptone added to the culture was variated: 3.985 g, 4.269 g, 4.544 g, 4.754 g, and 5.124 g for 150 ml medium, respectively, to determine the effect of nitrogen amount against cell growth. After 3 days, cells were cultivated by centrifuging at 5,000 rpm at 15°C temperature for 10 minutes.

During the incubation process, cell growth data, alcohol level, nitrogen level decrement, and glucose level in the medium were measured. The peptone variation amount was determined from the glucose value from starch hydrolysis. The value of peptone variation was obtained from a theoretical calculation.

The β-Glucan extraction and analysis

Shokri et al. (2008) centrifuged 30 ml culture β-glucan extract for 20 minutes with a speed about 7000 rpm at 15°C. The supernatant was discarded, and NaOH 2% were added to the biomass, subsequently heated for 5 hours at 90°C. The results from these processes were re-centrifuged for 10 minutes with a speed about 5000 rpm. The supernatant obtained was added with acetic acid 2M to set the pH to 6.8-7.0, and then precipitated by adding ethanol. After precipitation, centrifugation was done for 10 minutes with speed about 5000 rpm. The precipitate obtained was dried in a vacuum oven. The β-glucan was analyzed using HPLC after the sonicator distracted cell walls. The RID 10A detector was used in HPLC at 80°C column temperature, with 1 ml min^{-1} aquadest as its mobile phase.

Glucose in the samples was analyzed using HPLC. *Saccharomyces cerevisiae* growth was observed by taking a sample every 3 hours for 12 hours, 6 hours for the next 48 hours, and 12 hours for the next 72 hours. The RID 10A detector was used in HPLC at 80°C column temperature, with 1 ml min^{-1} aquadest as its mobile phase.

Cell growth measurement

Cell growth was measured by growing them on Malt Extract Agar and was incubated at 36°C for 48 hours. Dilution was done 8 times for every sample. The medium used for the dilution process is Buffered Peptone Water.

Protein analysis (Biuret method)

According to Cheng & Zheng (2014), the biuret analysis method identifies protein from the samples. In this way, 125 ml samples were put into a 100 ml volumetric flask and heated at 70°C until half of the agar diluted. Subsequently, the sample's pH was neutralized using H_2SO_4 0.05 M or NaOH 0.05 M with MM indicator. After that, 20 ml Tartrate Sodium Potassium and 20 ml $CuSO_4$ solution were added. The absorbance was then measured at 555nm using UV Spectroscopy. Tartrate Sodium Potassium solution dissolved 40 g NaOH and 50 g Tartrate Potassium in 1000 ml distillate water. On the other hand, the $CuSO_4$ solution was made by dissolving 15 g $CuSO_4.5H_2O$ in free-CO_2-distillate water.

The standard biuret was made from a crystallization process. A 10 g biuret was dissolved in 1000 ml absolute ethanol and then concentrated until 250 ml by the heating process. Cool the solution at 15°C, filtrate it, and wash it with alcohol twice. The results from these processes were dried in the oven at 105–110°C for 1 hour.

Ethanol analysis

Ethanol analysis was done using gas chromatography Agilent 6890 Flame Ionization Detector (FID). The injector temperature was 200°C, the column temperature was 80°C, and the flow rate was 5 ml min^{-1} with nitrogen as a carrier

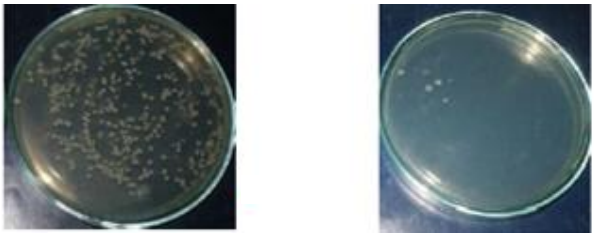
Results and Discussion

The purified *S. cerevisiae* was rinsed in sterile water twice, and subsequently, 200 µL colony suspension was taken and put into a Petri dish. Afterward, 50 J m^{-2} UV light was given, and the Petri dish was incubated for 24 hours to prevent it from light. All of these processes were done to make *S. cerevisiae* a mutant and shown in Fig. 1.

The results from the *S. cerevisiae* incubation process with UV light would differ from the *S. cerevisiae* incubation process without UV light. *S. cerevisiae* incubation with UV light will produce few colonies. On the other hand, *S. cerevisiae* incubation without UV light will produce more than 100 colonies. This happened because 99% of colonies would not survive with 50 J m^{-2} UV light. The one still alive are *S. cerevisiae* mutant cells (Ha et al., 2002). The β-glucan in *S. cerevisiae* mutant cell walls was easier to be extracted using the base solution (Perrine-Walker & Payne, 2021).

The amount of optimum enzyme for pulp hydrolysis

Starch and glucose levels were measured to determine each component's initial concentration before the process. Both arrowroot waste and cassava waste samples almost contained hardly glucose. Table 1 shows that the starch level in the samples was still high, approximately 80% for each sample. According to the literature, starch level in



(a) (b)

Figure 1. *S. cerevisiae* before (a) and after (b) UV light exposure
*Gambar 1.*S. cerevisiae *sebelum (a) dan sesudah (b) paparan sinar UV*

Table 1. Glucose and starch level of arrowroot waste and cassava waste
Tabel 1. Kadar glukosa dan pati pada onggok umbi garut dan onggok ubi kayu

Sample *Sampel*	Glucose Level (%) *Kadar glukosa (%)*	Starch Level (%) *Kadar pati (%)*
Arrowroot waste (*Onggok umbi garut*)	0.87	80.92
Cassava waste (*Onggok ubi kayu*)	0.76	79.67

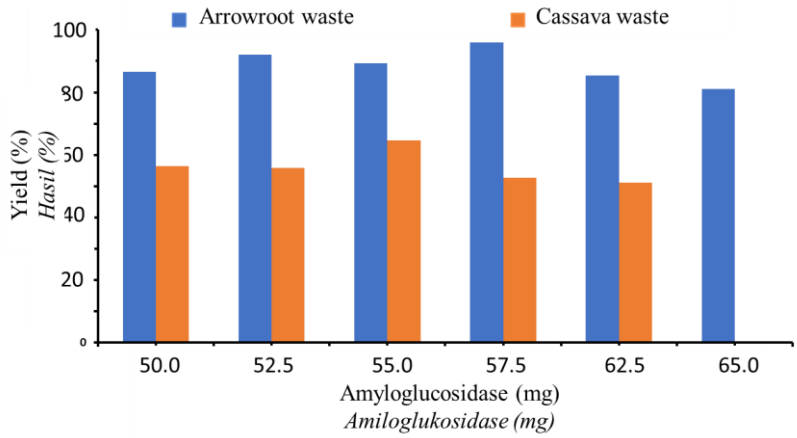


Figure 2. Hydrolysis of arrowroot and cassava waste with variations in the amount of amyloglucosidase enzyme
Gambar 2. Hidrolisis onggok umbi garut dan onggok ubi kayu dengan variasi jumlah enzim amiloglukosidase

both species should be about 45-65% (Ayala Valencia et al., 2015). These things happened because the samples used in the experiment were synthetic samples made in the laboratory. The incomplete separation process caused the starch level to be 80%. Based on the data, the experiment was done by varying the addition of the enzyme for both samples: 50.0 mg, 52.5 mg, 55.0 mg, 57.5 mg, 60.0 mg, 62.5 mg, and 70.0 mg, respectively, with 10 g basis of each sample. Adding the enzyme was to observe the optimum enzyme needed to hydrolyze starch in arrowroot waste and cassava waste samples. The hydrolysis process was done at the condition of the amyloglucosidase enzyme; 55°C temperature and pH 4.5. The result from the hydrolysis process is represented in Fig. 2 that shows a correlation between enzyme variations against glucose yield, which is a percentage number of samples converted into glucose so it can be used by *S. cerevisiae* as a carbon source in digestive metabolism.

Fig. 2 shows that the most significant conversion for the arrowroot waste sample was at 57.5 mg (≈ 50000 units) of enzyme; with the conversion was 95.93%. On the other hand, cassava waste sample shows the most significant conversion at 55 mg (≈ 48000 units) enzyme, with conversion was 64.7%. The cassava waste hydrolysis shows a more significant result than the arrowroot waste hydrolysis because the amount of

cellulose in arrowroot waste is higher than in cassava waste, so the different results of the hydrolysis process were obtained. The cellulose in cassava waste approximately 10–20% (Widiarto et al., 2019), while the cellulose in arrowroot waste is 20–40% (Tarique et al., 2022). The shape of the curve in Fig. 2 that does not conform to the general pattern of the hydrolysis process is most likely caused by irregularities in the samples and mixing process, so the conversion that happened was not optimum.

Determination of optimum nitrogen levels for the growth of S. cerevisiae

This experiment determined the optimum amount of peptone as a nitrogen source for the *S. cerevisiae* propagation process. The optimum value of the nitrogen added was measured by the growth of *S. cerevisiae*, the glucose consumption, and the ethanol formation. Nitrogen supplements are necessary to facilitate yeast growth and enhance fermentation. Culture medium supplementation with a nitrogen source led to an exponential growth of the yeast population. Glucose consumption increased in response to increasing nitrogen concentrations in the fermentation medium (Barahona et al., 2019). It is well established that the C/N ratio is a crucial parameter in fermentation processes, since it influences cell growth and metabolite production (Walker & Walker, 2018).

a. Effect of nitrogen variation on the number of S. cerevisiae *cells*

S. cerevisiae needs both carbon sources for its nutrition and protein and vitamins. In this experiment, protein sources come from various amounts of peptone. On the other hand, vitamin sources come from 2% (w/v) yeast extract in fermentation media. The purpose is to determine the amount of optimum protein needed for propagation so that the of *S. cerevisiae* cell production can be obtained as high as possible with a few peptones. Fig. 3 shows the cell growth of *S.cerevisiae* in cassava and arrowroot waste media with variations in the amount of nitrogen.

Fig. 3 shows that the cell growth is appropriate with the literature. The log phase occurred in the first 24 hours. In this phase, cell fission and population are doubled for every single-generation time. The amount of formed-cell depends on several factors; nutrient sources, temperature, oxygen level, light, and the presence of other microorganisms. After 24 hours, the cell growth is more stable and enters a stationary phase. The curve inclination from the 24th hour until the 72nd hour increases and starts to form a straight line. In this phase, cell fission's rate is directly proportional to the number of dead cells, so the amount of living cells remains constant.

The growth in cassava waste medium is similar to the growth phase of yeast. The effect of variated nitrogen did not give a significant difference, but the optimum curve still can be observed. The highest amount of *S. cerevisiae* was obtained at the fourth variation when peptone added was 4.754 g with 8.5x10^7 CFU colonies. The highest growth of microbe happened at the 48th hour and formed a straight curve. On arrowroot waste medium, the highest amount of *S. cerevisiae* was obtained at the fourth variation, when peptone was added as much as 4.754 g with 1.105x10^8 CFU colonies. For both kinds of carbon sources used, the value of microbial growth is similar. It is shown that the amount of glucose in both media is enough to propagate *S. cerevisiae*. The high viscosity of cassava waste caused an incomplete agitation, so the contact between yeast and medium was not as good as the arrowroot waste medium.

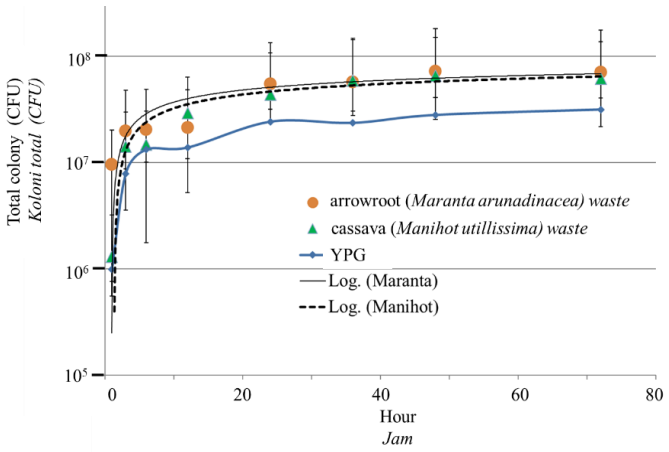


Figure 3. Growth curve of *S. cerevisiae* in cassava and arrowroot waste media with variations in the amount of nitrogen

Gambar 3. Kurva pertumbuhan S. cerevisiae *pada media onggok ubi kayu dan onggok umbi garut dengan variasi nitrogen*

According to the graph of the relationship between the numbers of cells versus time we concluded that the time needed to produce *S. cerevisiae* for both samples is less than 48 hours because the optimum cell growth occurred at the end of the log phase, which happened between the 24th until 36th hours.

b. Effect of nitrogen variation on the glucose consumption

Fig. 4 represents the glucose level as a fermentation result for 72 hours when the nitrogen was variated. Consumption at the beginning of the fermentation was rapid, so the curve inclination decreased until the 24th hour and formed a sloping line until the end of the fermentation process. On the cassava waste sample, the highest amount of cells was at the fourth variation. The highest glucose consumption was also obtained in the fourth variation. The highest consumption rate stopped in the 24th hour when the initial glucose level value was 2.4% until 1.9%.

The curve of glucose content in arrowroot waste and cassava waste medium are different. In the cassava waste medium, a decrement in glucose level occurred at the third and fourth variations, and it took place until the 36th hour. Conversely, glucose consumption reached its maximum limit at the 24th hour. *S. cerevisiae* cell growth in arrowroot waste medium was likely to arise and still not reach its stationary phase. Because the consumption rate still occurred until the 36th hour, glucose formed a stable curve. The highest amount of cell and glucose consumption was obtained by arrowroot waste in the fourth variation. In addition, the highest glucose consumption occurred at the 36th hour, when the glucose level was 2.4% until 1.56%.

c. Effect of nitrogen variation on ethanol formation

As explained before, the more nitrogen added, the more *S. cerevisiae* obtained. On the other hand, this thing does not apply to the ethanol level obtained from the process. The amount of ethanol formed according to its yield conversion against raw material can be seen in Fig. 5.

Changes in the amount of ethanol curve should be directly proportional to the cell growth curve. All ethanol obtained from the combinations shows the same inclination. The highest ethanol was obtained at the 12th hour, and the others were obtained at the 24th hour. After reachin its maximum value, the curve inclination decreases. If starch did not change sugar into ethanol anymore, the amount of ethanol in the substrate should be constant. The decrease in ethanol concentration shows a reaction that changed ethanol into acetic acid. Changes in starch metabolism from anaerobic to aerobic are the possible explanation for this phenomenon.

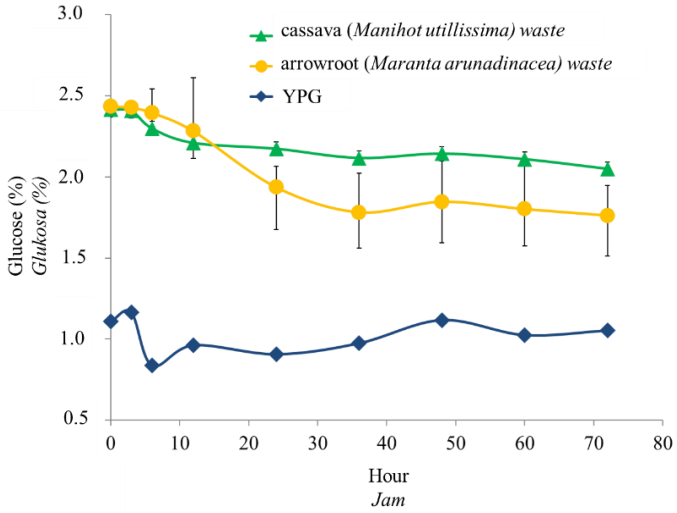


Figure 4. Effect of incubation time on glucose content in cassava waste and arrowroot waste media

Gambar 4. Pengaruh waktu inkubasi terhadap kadar glukosa pada media onggok ubi kayu dan onggok umbi garut

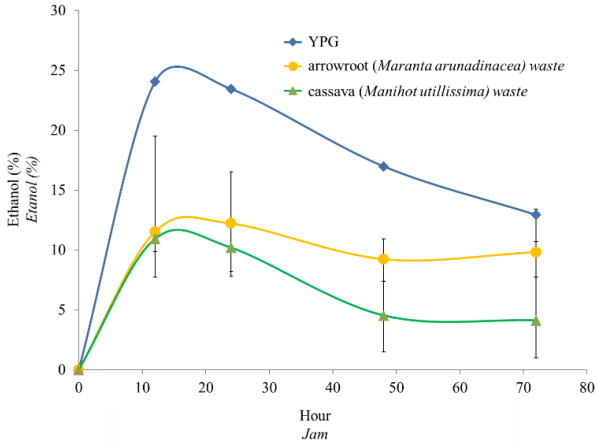


Figure 5. Effect of fermentation time on the formation of ethanol in arrowroot waste and cassava waste media

Gambar 5. Pengaruh lama fermentasi terhadap pembentukan etanol pada media onggok umbi garut dan onggok ubi kayu

Fig. 5 shows that the curve of alcohol variation in cassava waste gives the same inclination at the 12th or 24th hour with the yield value of 9-13%, especially at the first, second, fourth, and fifth variations. In the YPG medium, alcohol formation is much higher than in other mediums. This happens because alcohol basis formation is the amount of glucose in the medium, so starch conversion into alcohol quickly occurs. According to these things, YPG medium used pure glucose as its carbon source. The starch contained in the medium could be used for metabolism and obtain alcohol without adaptation. After reaching its maximum value, ethanol yield decreased because of cell respiration.

The unique pattern was shown in some variations where the curves formed differed from the others. An example is cassava waste medium at its third variation. After alcohol reaches its maximum point, yield drops slightly but rises over time. This happened because of the limit of medium contact with air, so the fermentation metabolism continued without changing the metabolism system into respiration. For the sixth variation, where nitrogen did not add, there was no change in alcohol level significantly. Nitrogen greatly impacts cell propagation, so that cell was hardly improved. The previous experiment also stated that peptone is the best nitrogen source for yeast.

Effect of fermentation on the amount of nitrogen consumption

Nitrogen plays an important role as a nutrient during the fermentation process because it is involved in the biosynthesis of proteins, amino acids, nucleotides, and other metabolites (Gobert et al., 2019). A study by Gozan et al. (2013) also shows that the type of nutrient source (C/N ratio) affects changes in nitrogen consumption. Therefore, it is necessary to observe the concentration of nitrogen in the fermentation process in this study. Fig. 6 shows the average values of nitrogen levels from six variations of arrowroot and casava wastes, which form almost straight lines. Nitrogen sources are essential thing for cell metabolism. The peptone addition in arrowroot waste had the highest initial value of 19777 mg L^{-1}, and the final value was 12330 mg L^{-1}. The second variation gave a fluctuating curve where the curve arose again at the 24th hour. The nitrogen consumption in cassava had the highest initial value of 19879 mg L^{-1}, and the final value of 13913 mg L^{-1}. All variations show that the longer the fermentation process occurs, the more nitrogen in the sample decreases. Nevertheless, this decrement forms a parallel path for all variations.

Effect of nitrogen variations on the β-glucan level

After 72nd hour, *S. cerevisiae* obtained from fermentation was cultivated and centrifuged to separate the cells from the medium. Subsequently, a 100 ml sample was sonicated to destroy *S. cerevisiae* cell walls so that the amount of β-glucan could be measured. Sonication is a method where we can obtain dissolved β-glucan in the medium. This method is more effective than dissolution using alkaline. The analysis shows that the dissolved β- glucan Level was relatively high by 10-minutes-sonification.

On the other hand, 5 hours were needed to extract β-glucan using the base solution. The difference in β-glucan obtained from both mediums was quite significant. The amount of β-glucan obtained from the cassava waste medium was only half of the β-glucan obtained from arrowroot waste medium. The result of the β-glucan level analysis can be seen in Fig. 7.

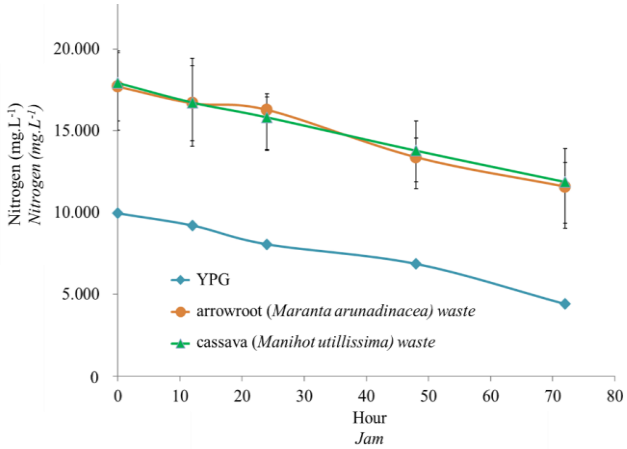


Figure 6. Nitrogen consumption in cassava waste medium and arrowroot waste medium
Gambar 6. Konsumsi nitrogen dalam onggok ubi kayu dan onggok umbi garut

Fig. 7 shows that β-glucan in the cassava waste medium formed an optimum curve with the highest point at the third variation with the value of 1.23%. On the other hand, the highest β-glucan Level in arrowroot waste medium was obtained at the third variation with a value of 0.51%. It forms a stable curve at the following variations. The control variations for both cassava waste and arrowroot waste mediums with nitrogen added were higher than both mediums without nitrogen added into them. The amount of β-glucan contained in the sixth variation from both mediums was 0.44% and 0.30%, respectively. In addition, YPG medium only produced 0.88% of β-glucan, and non-mutant *S. cerevisiae* obtained 0.23% of β-glucan.

An ultrasonic processor is an equipment needed for the sonication process. Sonication is a process that changes the electrical signal into mechanical vibration that can be directed to destroy cells (Ranjha et al., 2021). The main part of the sonicator is an ultrasonic electrical generator. This equipment produces a signal (≈20 kHz) which can activate a transductor. While low power and high frequency ultrasonic systems were employed for non-destructive evaluation of the physicochemical qualities of the samples, high-power and low frequency ultrasonic systems aim to improve product quality (Ranjha et al., 2021). Subsequently, the transductor converts the electrical signal using a piezoelectric crystal, which responds electrical signal by producing electrical vibrations. The sonicator keeps these vibrations until they pass the probe. Probe sonicator movement can obtain a cavitation effect when microscopic bubbles appear (Mussatto, 2015). The formation and destruction of the bubbles obtain vibration waves that can destroy cells (Weggler et al., 2020).

Sonicators can destroy cell walls to achieve homogeneity, which affects the results (Herrero et al., 2012). Cassava waste medium is more homogenous than arrowroot waste medium, and its texture is softer. When sonication is done using an ultrasonic processor, *S. cerevisiae* and medium will split its molecule because of the high vibration. This vibration caused destruction in cell walls, and the β-glucan molecule could be extracted and dissolved into the medium. A different situation happened with arrowroot waste medium. This medium is likely formed in 2 phases where the solid phase still can be seen clearly. The results seemed less homogenous when sonication was done to the medium. These things caused β-glucan cannot come out from arrowroot waste medium, and the amount of β-glucan produced was less than the cassava waste medium.

The β-glucan extraction from S. cerevisiae

The β-glucan is a polysaccharide composed of monomers that bond to one another. Glucose enters cell walls through active transport. Subsequently, cells started to synthesize polysaccharides and obtained β-glucan. The results of the extraction can be seen in Fig. 8. The extraction process showed that the β-glucan slurry obtained was not appropriate with the β-glucan Level measured. The yeast mutant obtained the highest β-glucan extraction result, with a value of 6.565 g L^{-1} (0.66% w/v). The amount of β-glucan Level obtained from wild-type yeast was 1.843 g L^{-1} (0.18% w/v). The amount of β-glucan slurry from both sources was similar to the results measured by HPLC, where the β-glucan Level measured were 0.88% and 0.22%, respectively.

The results of β-glucan extraction from *S. cerevisiae* in the medium differed from the results of HPLC. Only 10% of the β-glucan measured

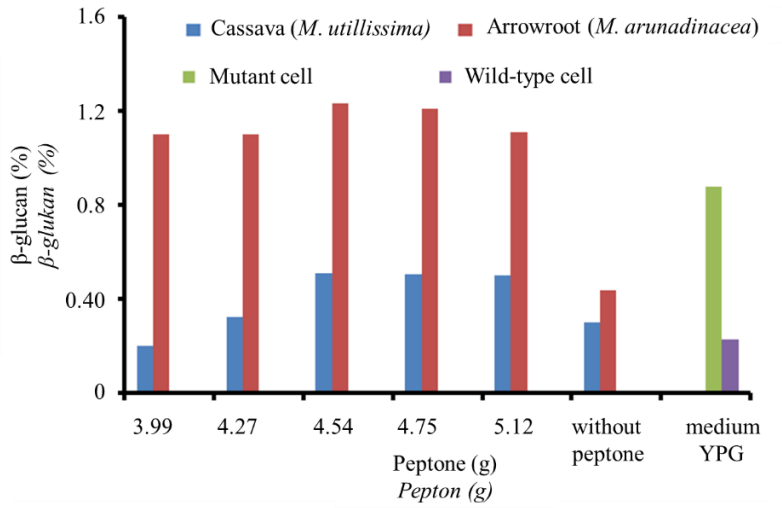


Figure 7. Effect of peptone addition on concentration of β-glucan

Gambar 7. Pengaruh penambahan pepton terhadap konsentrasi β-glukan

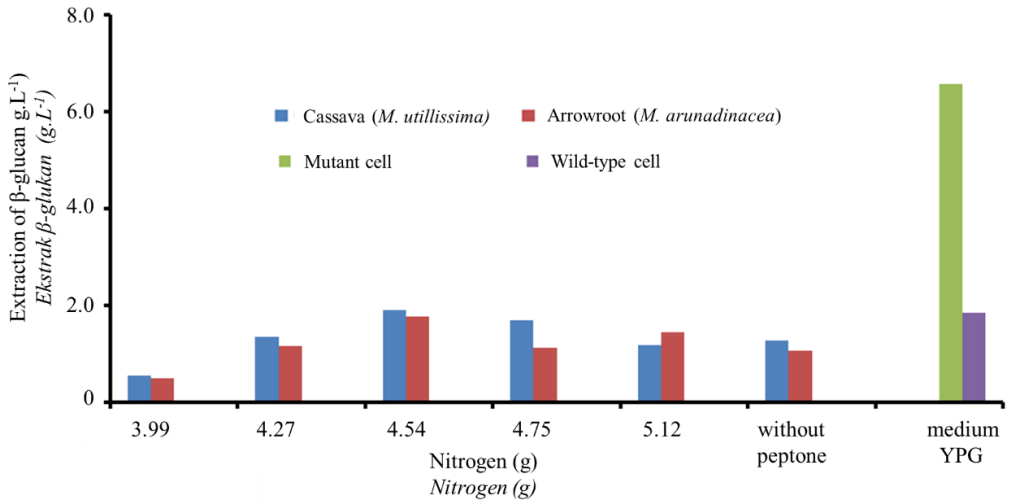


Figure 8. The *β*-glucan extraction yield in various N addition on cassava waste, arrowroot waste, and YPG medium

Gambar 8. Hasil ekstraksi β-glucan dengan berbagai variasi kadar N pada onggok ubi kayu, onggok umbi garut, dan medium YPG

could be extracted. From the arrowroot waste, the highest amount of β-glucan was obtained from the third variation with a 1.91 g L^{-1} (0.19% w/v) value. From the cassava waste, the highest value was obtained at the third variation with a 1.767 g L^{-1} (0.18% w/v) value. These things happened because the extraction process was done with a conventional method, gravimetric. The difference in slurry obtained was also caused by particle precipitation discarded during the process.

The extraction method used in this experiment was β-glucan dissolution in base solution and continued with the centrifugation process at 15°C. In a solid medium, other methods were needed. The slurry medium caused the sampling process to become more complex. Sampling was done by putting the slurry into a centrifuge tube while the *S. cerevisiae* cell precipitated in the bottom of the Erlenmeyer. Solid samples prevented *S. cerevisiae* precipitated from being fetched. Hence, it requires a better homogenization method so that the particle would not disturb the sampling process until the optimum amount of β-glucan slurry was obtained. On the other hand, it needs the optimum temperature to separate β-glucan through a centrifugation process from its medium. Precipitation could have happened incompletely if the temperature was not suitable. Moreover, it also needs the optimum time and frequency of sonication to destroy cell walls.

Other study using banana waste, papaya waste, and napa cabbage waste as fermentation medium have also succeeded in producing β-glucan (Utama et al., 2020). As expected, the highest level of glucose among the wastes led to papaya as the highest β-glucan producer of the three mediums. Another study showed that the type of reactor would greatly affect the yield of β-glucan (Chotigavin et al., 2021). The study showed that β-glucan production in the stirred tank reactor (STR) was 1.4 times higher than that of the shaken flask (SF) culture. This research generally provides benefits for the production of prebiotics and functional foods for human health and animal feeds.

Conclusion

Production of β-glucan achieved by S. cerevisiae using cassava (*M. utillissima*) and arrowroot (*M. arunadinacea*) wastes as a medium. A slightly higher amyloglucosidase enzyme was needed for hydrolyzing arrowroot waste than cassava waste. The hydrolysis yield of cassava and arrowroot is 95.93% and 64.7%, respectively. However, the maximum production of β-glucan from cassava is higher than arrowroot. The β-glucan level from cassava and arrowroot is 1.23% and 1.20%, respectively. Adding peptone to all samples increased the production of β-glucan in all samples.

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