Development of Liquid Sugar HFS (High Fructose Syrup) from Lesser Yam Tubers Using Enzyme-Mix Inulinase and Amylase

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Abstract. Indonesia's sugar demand reaches 3.3 million tons/year, while domestic production is only 1.7 million tons or 51.5% of national demand, so imports are the choice. To reduce sugar imports, domestic sugar production needs to be continuously encouraged, in addition to looking for alternative sweeteners as sugar substitutes. Fructose syrup is an alternative to other sweeteners whose needs are increasing day by day. Apart from cassava, a local raw material that has been used as raw material for making liquid sugar is lesser yam tubers. The advantage of HFS liquid sugar produced from lesser yam tubers raw materials is that it only requires one process step, namely by using a mixture of enzymes inulinase and amylase (a mixture of gluco-amylase and alfa-amylase), where inulinase will hydrolyze inulin to fructose and amylase hydrolyzes starch become glucose. The research objectives were to find the optimum conditions for the production of liquid sugar HFS and to find the optimum concentration of liquid sugar from lesser yam tubers. The results of optimization of substrate and amylase enzyme showed that the optimum concentration of fresh lesser yam tubers substrate was 20%, alpha-amylase was 0.8% and gluco-amylase was 0.8%. The highest HFS liquid sugar was produced in the treatment of 20% inulinase enzyme concentration with a saccharification time of 48 hours, with a characteristic reducing sugar content of 22.958%; fructose content 3.96% and glucose content 23.04%.

Keywords. High fructose syrup; Lesser yam tubers; Inulinase; amylase

1 Introduction

Sweeteners are food additives that are added to food or beverages to create a sweet taste. The need for sweeteners always increases every year. Until now, Indonesia still has to adopt the material, sugar cane to meet domestic needs. Ministry of Agriculture Based on Agricultural Data and Information System, domestic sugar consumption in 2017 is projected to reach 5.07 million tons while production is only 2.47 million tons. This caused the sugar balance to experience a deficit of 2.6 million tons. Sugar consumption is projected to continue to increase to 5.26 million tons in 2021 while production will only reach 2.48 million tons, resulting in a deficit of 2.78 million tons (1). Therefore, it is necessary to develop other plants that can be used to meet the sweetener needs.

Some natural ingredients that have been used as a source of sweeteners include sugar cane, dates, corn, coconut sap and stevia. However, the use of lesser yam tubers as a sweetener has never existed, therefore an exploration of sweeteners is carried out as an alternative to natural sweeteners. The superiority of lesser yam plants compared to other plants is that the tubers contain a fairly high inulin content of 14.77% db (2). Lesser yam tubers also contain 82.82% starch consisting of 13.26% amylose and 69.56% amylopectin (3). This makes lesser yam very potential for raw material for producing HFS, namely liquid sugar that contains glucose and fructose.

Inulin is a water-soluble poly fructose (fructans), which is produced from the polymerization reaction of fructose in plants. Inulin can be utilized by some strains of Bifidobacteria as an energy source, because these bacteria produce the enzyme inulinase which can hydrolyze inulin into fructose. Inulinase is a -fructosidase that can hydrolyze the inulin molecule. Exo-inulinase (β-D-fructan-fructo-hydrolase, EC.3.2.1.80) cleaves the terminal fructose unit from the non-reductive end, this enzyme can also hydrolyze sucrose and raffinose molecules. In addition, endo-inulinase (2,1-β-D-fructan-fructo-hydrolase, EC.3.2.1.153) hydrolyzes the inulin molecule bonds from the inside to produce fructooligosaccharides such as inulotriose, -tetraose, and -pentaose as the main products (4).

Fructose syrup are two types of natural sweeteners that can be used to sweeten various types of food, beverages and pharmaceutical products, this is supported by the nature of fructose which has a sweetness level of 120-180% of sucrose (5). A mixture of glucose, fructose known commercially as high fructose syrup (HFS), usually contains 42% and 55%

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fructose. Fructose syrup or commonly called high fructose syrup (HFS) is a type of liquid sugar that is popular in the food industry. This sugar can be produced from all materials containing carbohydrates, such as corn, cassava, rice, potatoes, and others (6; 7).

Liquid sugar made from raw materials of cassava and corn starch requires 2 stages of processing, namely: stage I; hydrolysis of starch using amylase enzymes, namely glucoamylase and/or alpha-amylase; stage II is the conversion of glucose from stage I using the enzyme glucose isomerase, where this enzyme acts in the process of converting glucose into fructose.

The advantage of liquid sugar produced from lesser yam tuber raw materials requires only one process step, namely by using a mixed enzyme inulinase and amylase (a mixture of gluco-amylase and alpha-amylase), where inulinase will hydrolyze inulin to fructose and amylase hydrolyze starch into glucose.

2 Research Methods

2.1 Research Materials and Tools

The materials used in this study were lesser yam tubers from Nganjuk Resident, aquadest, inulinase enzyme (Exo-inulinase produced by Megazyme), -amylase (Liquuozyme Supra produced by Novozyme), glucoamylase enzyme (Dextrozyme produced by Novozyme), standard glucose, standard fructose , H_2SO_4, Carbazole, Cysteine, NaOH, HCl, and Acetate buffer pH 6.5 and 4.5.

The tools used in this research are digital scales, analytical balance, knife, Erlenmeyer flask, funnel, filter paper, stirrer, measuring cup, pipette, pH meter, blender, beaker, thermometer, water bath shaker, cabinet dryer, desiccator, hot plate, porcelain crucible, test tube, refractometer, and HPLC with Aminex Column HPX-18C (250mmx4mm), refraction index water model 410 and LCHE Waters pump model M-45.

2.2 Research Design

This research was carried out in 2 stages, namely optimization of the liquid sugar production process from lesser yam tubers which included optimization of substrates, alpha-amylase and gluco-amylase enzymes, the second stage was to determine the effect of inulinase enzyme concentration and saccharification time on reducing sugar, glucose and fructose levels.

2.3 Data Analysis

The research data were analyzed using ANOVA (Analysis of Variance) if there were significant differences, further tests were carried out using the DMRT (Duncan't Multiple Range Test) 5% method.

2.4 Colour illustrations

a) Selected lesser yam tubers that were still fresh and healthy.
b) Lesser yam tubers are peeled, after being peeled, they were immediately immersed in clean water to prevent browning. Then washed with clean and running water.
c) Lesser yam tubers were cut into several pieces and then blended using hot water at 80-90 °C ratio 1:2 (lesser yams:water), lesser yam slurry was produced.
d) The lesser yam tuber slurry was heated at 100°C for 15 minutes to allow starch gelatinization to occur.
e) After the slurry was cold, it was then treated according to the treatment, namely the first optimization of the ratio of inulinase: amylase (10:90; 20:80; 30:70; 40:60; 50:50 and 60:40).
f) The second optimization is the enzyme concentration from the best treatment, which was 0.01%; 0.015%; 0.02%; 0.025%; 0.03%; 0.035%; 0.04%; 0.045% and 0.05% compared with control without enzyme (0%)
g) The third optimization was substrate concentration from 10%, 15%; 20%; 25%; 30%, 35%, 40%; 45% and 50% (ratio of water: lesser yam =50:50).

3 Result and Discussion

3.1 Optimization of Substrate Concentration

Substrate concentration is one of the factors that affect the work of enzymes, so that it affects the reaction results. Therefore, the initial stage of this research was to optimize the substrate concentration of lesser yam tubers for the formation of reducing sugars produced. The results of substrate optimization are presented in Fig 1.

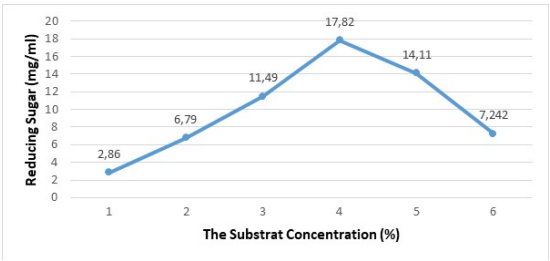

Fig. 1. Effect of substrate concentration of lesser yam tubers on the reducing sugar level of HFS produced.

In Fig. 1, it can be seen that the higher the substrate concentration of lesser yam tubers, the faster the amylase enzyme reaction, which is indicated by the increasing levels of reducing sugar produced. However, if the substrate concentration was increased above 20%, the resulting reducing sugar content decreased. This is due to the fact that the active site of the enzyme has not worked completely, the addition of substrate concentration can accelerate the reaction rate. However, if all the active sites of the enzyme are working, the addition of substrate concentration will not accelerate the reaction rate. This is because the enzyme is saturated or the concentration is very high all the active parts of the enzyme have been occupied by the substrate so that it cannot increase the reaction rate. According to the

opinion [19], the main factors that affect enzyme activity are temperature, pH, enzyme concentration, substrate concentration, inhibitors and activators. The reaction rate initially increases with increasing substrate concentration. However, after further substrate increase the maximum activity will be reached. When the concentration is excessive, it results in the saturation of the formation of the enzyme-substrate complex which results in some of the substrate not being converted into products. According to [17], further addition of substrate does not affect the rate of enzyme reaction. This is because at very high concentrations all the active parts of the enzyme have been occupied by the substrate, so that at that time the reaction rate is at its maximum condition.

3.2 Optimization of alpha and gluco-amylase enzyme concentration

Enzyme concentration is also an important factor that greatly affects the work of enzymes, so that it affects the reaction results. Therefore, it was necessary to optimize the concentration of the enzyme used. The optimization results of alpha-amylase and gluco-amylase concentrations were presented in Fig 2. and Fig.3

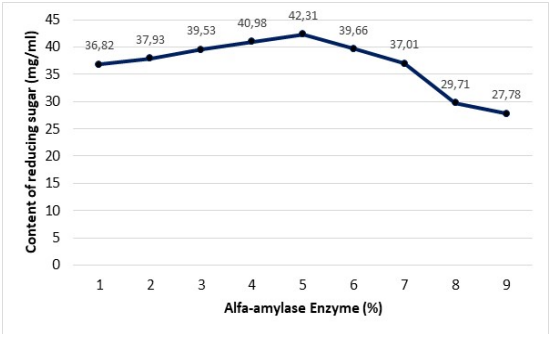

Fig. 2. Effect of alpha-amylase concentration on the reducing sugar content of HFS produced.

In Fig. 2, it can be seen that the higher the concentration of alpha-amylase enzyme (concentration 0.4-0.8%), the reducing sugar resulting from hydrolysis of the lesser yam tubers substrate increased, but if the concentration of the alpha-amylase enzyme was increased to 2.0 %, the reducing sugar content decreases. This is due to the fact that the enzyme is saturated or the concentration is very high, all the active parts of the enzyme have been occupied by the substrate so that the enzyme cannot react anymore. This opinion is in accordance with [20], Suwarso (2015), which states that the enzyme concentration directly affects the speed of the enzymatic reaction, where the reaction rate increases linearly as long as the enzyme concentration is much less than the substrate. From the results of this optimization, it was known that the optimum concentration of alpha-amylase is 0.8%.

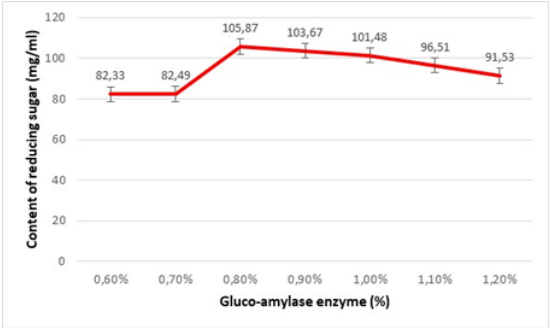

Fig. 3. Effect of gluco-amylase concentration on the reducing sugar content of HFS produced.

In Fig. 3, the higher the concentration of the gluco-amylase enzyme (concentration 0.6-0.8%), the reducing sugar resulting from hydrolysis of the lesser yam tubers substrate increases, but if the concentration of the alpha-amylase enzyme was increased to 1.2%, the reducing sugar content will decreases. This is because the enzyme is saturated with the substrate so that the enzyme is no longer able to form an enzyme-substrate complex so that the product is not formed. This is in accordance with the opinion of [20], which states that the enzyme concentration directly affects the speed of the enzymatic reaction, where the reaction rate increases linearly as long as the enzyme concentration is much less than the substrate. In the optimization of the gluco-amylase enzyme, the optimum concentration is 0.8%.

3.3 Effect concentration of inulinase enzyme and length of saccharification on reducing sugar

Based on the results of analysis of variance, it can be seen that there was a significant interaction between the concentration of the inulinase enzyme and the length of saccharification on the hydrolysis rate of the lesser yam tubers substrate as indicated by the reducing sugar content of the hydrolysis results. Likewise, each treatment has a significant effect. The resulting HFS reducing sugar content. Effect concentration of inulinase enzyme and saccharification time on reducing sugar content of HFS produced from lesser yam tubers can be seen in Fig. 4.

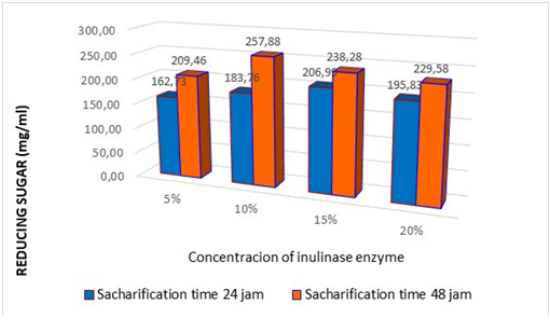

Fig. 4. Effect concentration of inulinase enzyme and saccharification time on reducing sugar content of HFS produced from lesser yam tubers

In Fig. 4. shows that the higher the concentration of the inulinase enzyme and the longer the saccharification time, the lower the reducing sugar content. However, at

enzyme concentrations above 10%, the resulting reducing sugar decreased, but the longer the saccharification the reducing sugar content increased. This can be caused at concentrations above 10% of the enzyme is already saturated by the substrate so that the increase in enzyme concentration has no effect on the speed of hydrolysis. However, the longer the saccharification, the longer the contact time between the enzyme and the substrate, thus giving the enzyme a higher opportunity to hydrolyze the substrate. This is in accordance with the opinion of [19], which states that the reaction rate initially increases with increasing enzyme concentration. However, after a further increase in the enzyme maximum activity is reached. When the concentration is excessive, it results in the saturation of the formation of the enzyme-substrate complex which results in some of the substrate not being converted into products. In Fig. 4, it can be seen that the highest yield of 10% inulinase concentration treatment and 48 hours saccharification resulted in reducing sugars of 257.88 mg/mL or 25.788%. This result is lower than the results of research by [21], which examined the manufacture of glucose syrup using a mixture of alpha-amylase and glucoamylase enzymes, without inulinase, resulting in a glucose concentration of 37.8%. This difference is due to the fact that in [21] study, starch was separated before being reacted with enzymes, while in our research we did not separate the crate first, so there are still other components such as lignin, cellulose that can prevent the formation of enzyme complexes-substrate, thereby reducing the yield of the reaction.

3.4 Fructose and Glucose levels as measured by HPLC

The results of the analysis of fructose and glucose levels using HPLC on HFS liquid from lesser yam tubers with the treatment of inulinase enzyme concentration and saccharification time were presented in Fig. 5. Determination of the retention time of fructose and glucose was carried out by injecting 10 sample or standard solution into HPLC with run time 20 minutes.

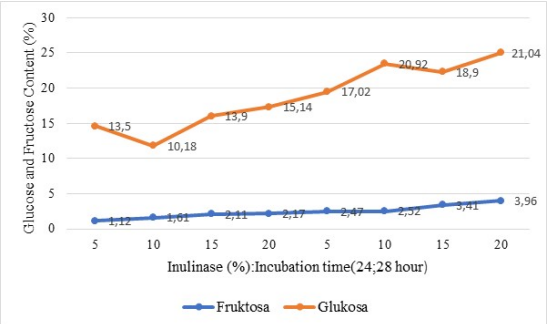

Fig. 5. Effect concentration of inulinase enzyme and saccharification time on fructose and glucose levels in HFS produced from lesser yam tubers.

In Fig. 5, shows that the higher the concentration of inulinase enzyme and the longer the saccharification, the higher the fructose and glucose levels in the HFS liquid sugar produced from the hydrolysis of lesser yam tubers. This can be caused by the higher the concentration of the enzyme inulinase, the more inulin in the lesser yam tubers which is hydrolyzed to form fructose because inulin is an oligosaccharide polymer of fructose. This is in accordance with the opinion [22]; [23], which states that inulin is a fructan-β-(2-1) group consisting of a mixture of oligo- and polysaccharides, where almost every fructose chain is linear, has a GFn structure (with G=glucosyl units, F=fructosyl units and n=number of fructosyl units). The longer saccharification causes the alpha amylase enzyme to be more effective in hydrolyzing starch into glucose, so that glucose levels increase. This is in accordance with the opinion of [23], which states that the alpha-amylase and gluco-amylase enzymes are useful for the production of glucose from cassava starch, liquefaction which cannot be carried out by the gluco-amylase enzyme, the gluco-amylase enzyme works to break the alpha-1,6 glycosidic bond at the saccharification stage which cannot be carried out by the alpha-amylase enzyme.

4 Conclusion

1) The results of optimization of substrate and amylase enzyme showed that the optimum concentration of fresh lesser yam tubers as a substrate was 20%, alpha-amylase was 0.8% and gluco-amylase was 0.8%.
2) The best treatment was inulinase enzyme concentration of 20% and saccharification time of 48 hours which produced HFS liquid sugar with the characteristics: reducing sugar content of 22.958%; fructose content 3.96% and glucose content 23.04%.

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