Buffer determination for immobilized enzymes in a batch system for glucose syrup production

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Abstract. Glucose is a monosaccharide carbohydrate that is widely used as a sweetener in the food industry. Research on glucose syrup has been carried out. This study focuses on determining the suspension buffer of immobilized enzymes and syrup purification. The purpose of this study was to determine the best-immobilized enzyme suspension buffer solution in the saccharification process, determine the ratio of the use of immobilized enzymes, and determine the effect of heating on glucose syrup purification with various types of activated charcoal. The treatment applied to determining the suspension of immobilized enzyme buffer was enzyme immobilization with acetate and phosphate buffers which was used for saccharification with stirring speed of 200 rpm for 72 hours at 60 °C, and the use of immobilized enzymes until the fifth use. The results obtained in this study indicated that the buffer affects the immobilized enzyme and further affect glucose syrup produced and immobilized enzymes are effective until the fifth use.

1. Introduction

The development of the food industry in Indonesia from time to time also affects the increase in glucose requirements. Glucose syrup is needed continuously while domestic glucose production has not been able to compensate for this increase in demand. This results in an increase in the amount of glucose imports. Glucose can be produced from starch, which is widely found in Indonesia, one of that is starch from tapioca.

Starch is a polysaccharide carbohydrate composed of glucose monomers. Glucose can be produced from starch by cutting the bonds that connect the monomer so that it becomes a simple compound which is mostly glucose. Cutting the chain can be done by the acid and enzymatic method and has already been reported in several studies [1–4]. The enzymatic method is considered better than the acid method because glucose production can be controlled due to the action of specific enzymes. The enzymes needed in glucose products are alpha-amylase, amyloglucosidase, and pullulanase.

The specific activity of the enzyme becomes a positive side. However, the price should also take into account. The enzyme especially the pure one is normally can be purchased with expensive price. Consequently, the method for reusing enzyme through immobilization is necessary. However, in
immobilized enzyme, it is more necessary to maintain the stability of the enzyme.

The main objective to be achieved in this study is to determine the best enzyme suspension buffer in enzyme immobilization that maintain the enzyme stability for saccharification. As well as to determine the re-usage of enzyme immobilized in pumice.

2. **Methods**

2.1 **Materials**

Tapioca used in this study was obtained from a near supermarket (Top Mode), pumice obtained from the traditional market, aquadest was purchased from near chemical store (Sumber Rejeki). This study also used α-amylase, pullulanase, and amylglucosidase, PbO, Pb Acetate, and dinitrosalisilat (DNS) solution, buffer acetate pH 4.5, buffer posfat pH 6, glucose, HCl 0,1 N, CaCl$_2$ 15 ppm, glucose, NaOH, Natrium metabisulfite and Natrium carbonate.

2.2 **Glucose syrup production**

2.2.1 **Matrix preparation.** Pumice stone is washed clean and the clean pumice is inserted in the furnace and then heated at a temperature of 2000C for 2 hours to enlarge the porosity of the matrix. Dry pumice then put in 250 ml Erlenmeyer. Then the 0.048 g pullulanase enzyme and 0.192 g of amylglucosidase were put into Erlenmeyer containing pumice, then added buffer (Acetate or Phosphate) until the pumice was submerged. Erlenmeyer containing the solution is left in the refrigerator for 24 hours. Then the Erlenmeyer buffer is released and pumice is used for saccharification.

2.2.2 **Liquefaction.** Tapioca flour is prepared as much as 4 kg. Put in a stainless steel pan. 8 L of water is added to the pan. Then stir until the tapioca suspension is formed with a ratio of 1: 2. A 0.2% α-amylase enzyme was added to the tapioca suspension. Then added CaCl$_2$ (Calcium Chloride) cofactor 15 ppm. The suspension is heated using a hot plate at a temperature of 70-90°C for 75 to 90 minutes so that tapioca hydrolyzate is obtained. Tapioca hydrolyzate is filtered using filter cloth and discarded the waste so that maltodextrin is obtained as the substrate in glucose syrup production. HCl is added to the maltodextrin until pH 4.5 is obtained.

2.2.3 **Saccharification.** 200 ml of maltodextrin was put into Erlenmeyer containing immobilized enzymes with pumice matrix. Saccharification is carried out in a shaker with a temperature of 600C and a speed of 200 rpm. After being clarified for 72 hours, the product is then released into the tube and inserted a new maltodextrin substrate. The products produced are tested based on test parameters.

2.3. **Dextrose equivalent**

To obtain the DE first the reducing sugar was determined. The method used to determine the total reducing sugars in food was spectrophotometry method [5]. In an alkaline atmosphere, reducing sugars will reduce 3.5 dinitrosalicyclic acids (DNS) to form a compound that can be measured absorbance at a wavelength of 550 nm. If the sample is in an acidic atmosphere, it must be neutralized first. The standard curve is made using a standard glucose solution containing 0, 25, 50, 75, 100, 125, and 150 ppm glucose, each pipette of 1 ml and then put in a test tube. Added DNS solution and shake. Then placed in boiling water for 5 minutes then cooled to room temperature. Measured the absorbance at 550 nm. Determination of the sample was done by taking a sample that has been diluted as much as 1 ml and then put in a test tube. Added DNS solution and then shaken. Then placed in boiling water for 5 minutes then cooled to
room temperature. Measured the absorbance at 550 nm. The data obtained was plotted in the standard curve equation dextrose equivalent (DE) can be known by using a formula:

\[
\text{DE} (%) = \frac{\text{Reducing sugar formed} \left( \frac{W}{V} \right)}{\text{Initial substrate} \left( \frac{W}{V} \right)} \times 100\%
\]

2.4. Sweetness Level (°Brix)

The sweetness of the glucose syrup is tested using a "hand-difflectometer" device [5]. How to measure the sweetness of a sample, that is, the sample is pipetted by using a dropper drop and then drops on the surface of the hand refractometer and then looks at the number seen on the device

3. Results and discussion

Glucose syrup is a material that is often used in various confectioneries, preservatives, frozen desserts, and beverages. Syrup is made from glucose, maltose, and dextrin. Glucose syrup can also be used as a sweetener together with sucrose. The glucose syrup can be obtained from starch through acid or enzymatic hydrolysis. The starch generally used to make glucose syrups are corn flour and cassava flour [6].

Making sugar syrup in the enzymatic process is expensive that make it more effective to the immobilized enzyme in a matrix. Nevertheless, in immobilization the enzyme needs to be stable thus it can be used several times. In this study, to maintain the stability of enzyme two kinds of buffer were used namely acetate and phosphate buffer.

Because of the many types of buffers, the selection of buffers to be used becomes a problem. In choosing buffers, what must be considered is the optimum pH and biological properties. The type of buffer solution for immobilization of enzymes can affect the stability of immobilized enzymes in saccharification. Therefore the result present will compare the acetate and phosphate buffer in maintaining the enzyme stability.

3.1. Dextrose Equivalent (DE)

DE is a reducing sugar content which is expressed as percent dextrose against dry solids. DE is determined by dividing the value of reducing sugars obtained with the dry weight of the substrate. The dextrose equivalent (DE) value showed a decrease from the first use to the fifth use both in the use of phosphate buffer and acetate buffer. The DE value of sample (glucose syrup) showed that the highest value was obtained from the first use of phosphate buffer which was 56.645% and the lowest was from the fifth use of acetate buffers which was 33.55%.

The results of the analysis of variance showed that the type of buffer and the use of immobilized enzymes had a very significant effect on the dextrose equivalent (DE) value produced. Whereas buffer type interactions and usage do not show any effect on DE. Consequently, the advanced Duncan test is only carried out on buffer type and the use of immobilized enzymes.

The results of the Duncan test in the buffer type indicate that the use of phosphate buffer was different from acetate and both are different from the substrate. While the results of the Duncan test on the use of immobilized enzymes show that the first use was different from all uses. The second use is also different from all uses. Use of the third, fourth, and fifth is not different but different from the substrate. Use of the fifth and substrate is not different. The DE of glucose syrup before and after saccharification with immobilized enzyme used several times is presented in figure 1.

The observation results (figure 1) shows that the dextrose equivalent value was highest in the use of phosphate buffer. These results indicate that immobilized enzymes are more stable with the use of phosphate buffers compared to using acetate buffers. This could be caused by the acidic condition of acetate buffers which has a pH of 4.5. The acids solution accelerate the deterioration of the matrix making
the enzyme no longer usable. While phosphate buffer is more neutral (with a pH range of 7) so that it does not affect the erosion of the matrix.

Figure 1 showed that based on dextrose equivalent, the usage of immobilized enzymes showed high results at first use and decreased until the fifth use. A not too high drop value from the third use up to the fifth use indicates that the immobilized enzyme is effectively used until the fifth use.

3. 2 Sweetness level
The sweetness level is the number of dissolved solids found in glucose syrup. This dissolved solid can show the amount of simple sugars found in the solution. Determination of sweetness level is carried out using a held-hand-refractometer. The level of sweetness for glucose syrup results from saccharification is 30-36°Brix.

The results of the analysis of variance showed that the type of buffer, the use of immobilized enzymes and their interactions had a very significant effect on the value of the sweetness produced. So that the follow-up test is carried out on all factors.

The results of the Duncan test on the buffer type factor to the sweetness level showed that the acetate buffer was the same as the substrate while the phosphate buffer was different from the substrate and acetate buffer. The results of further Duncan test on the factor of use of immobilized enzymes on sweetness level showed that the first use was different from other uses. The fifth and second usage was the same. The fourth and third uses are the same. All usage is different from the substrate. The results of further tests of the interaction of the factor to sweetness indicate that the first phosphate buffer interaction is different from all other interactions. The second and fifth use of phosphate buffer interactions was the same. The first usage buffer acetate interaction is also different from other interactions. The third and fourth phosphate buffer interactions are the same. The fifth and second use of the buffer acetate interaction is the same as the substrate. The second, third, fourth buffer acetate interaction is the same as the substrate. The sweetness level of glucose syrup before and after saccharification with immobilized enzyme used several times is presented in figure 2.
The results of the observation (figure 2) showed that based on the sweetness level, the highest value obtained was in the sample with the use of acetate buffer. Saccharification results, in general, showed a lower in sweetness level compared to the substrate. If the sweetness level is considered as glucose contained in the sample, this result is not in line with the DE value. However, as the sweetness level shows the amount of dissolved solids in glucose syrup which not all dissolved solids are glucose so the sweetness level cannot be used as a determinant of the amount of glucose in the solution.

The results of the observations (figure 2) showed that the highest sweetness level was obtained from the use of the third and fourth immobilized enzymes, then the second and fifth uses, and the lowest on the first use. Generally, saccharified sample with the enzyme showed a lower level of sweetness than the substrate.

The results of the observations (figure 2) show that the value of the sweetness level varies with each interaction. However, both the use of acetate buffer and phosphate buffer both show a pattern of changes in the same sweetness level. The lowest in the first use, then sequentially higher in the second and fifth uses, and the highest in the third and fourth uses. Although all interactions show a decrease in the level of sweetness, all levels of sweetness obtained to meet the standards of above 30° Brix according to Purba (2009) which states that the sweetness level for glucose syrup is 30-36 ° Brix [7].

4. Conclusion
From the result and discussion of this study, we conclude that the results obtained in this study indicated that the buffer affects the immobilized enzyme and further affect glucose syrup produced and immobilized enzymes are effective until the fifth use.

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Figure 2. The sweetness level of glucose syrup before and after saccharification with immobilized enzyme used several times. The number indicated the usage of immobilized enzyme.
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