Utilization of α-amylase enzyme from Bacillus stearothermophilus RSAII1B for maltodextrin production from sago starch

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Abstract. The dried sago flour derived from Palopo contains 28.80% amylose and 91.23% total carbohydrate. Based on the data, sago starch has the potential to become an alternative raw material for maltodextrin production. Maltodextrin is one of the starch derivative products produced by hydrolysis process using the α-amylase enzyme with a maximum DE (dextrose equivalent) value of 20. The use of maltodextrin for food and pharmaceutical industries is increasing because of maltodextrin is widely used as thickener, filler, surfactant and sugar substitute in milk powder. The aims of this study are to optimize the addition of enzyme concentration and hydrolysis time of α-amylase enzyme to obtain high quality of maltodextrin. This study also aimed to characterization the obtained maltodextrin. The first step was isolation and purification of α-amylase from the isolate of Bacillus stearothermophilus RSAII1B, followed by determination of the α-amylase concentration (0.05%, 0.07% and 0.09%) in 2.0% starch substrate, and the hydrolysis time of α-amylase (60, 90, 120, 240 minutes). Maltodextrin characters observed were dextrose equivalent (DE), reducing sugar, moisture content, pH changes, color, solubility, viscosity, and total plate count (TPC). The results showed that the value of DE was 12.31, reducing sugar was 11.4%; water content was 10.92%; pH was 4.85; The color of maltodextrin powder was white bone color; solubility was 153.2 g/L; Viscosity was 210-240 cps, TPC was 380 cfu/g. Maltodextrins produced from sago starch using the α-amylase enzyme from B. stearothermophilus RSAII1B met the quality requirements of SNI 7599: 2010.

1.Introduction

Bacillus stearothermophilus RSAII1B is a thermophilic bacteria derived from the hot spring of Lejja South Sulawesi that can produce the thermostable α-amylase enzyme. The α-amylase enzyme derived from B. stearothermophilus RSAII1B has the characteristic that the optimum pH and the stability pH of 5.0; the optimum temperature of 50-60°C and the temperature stability of 60°C for 3 hours, Mr range 47.32 KDa [1]. The α-amylase enzyme can be used to partially hydrolyze starch to maltodextrin. The production process of maltodextrin using high temperature was 95°C for wheat starch [2], 75°C for cassava starch [3], and 75°C for banana starch [4]. Based on these data, it appears that the process of
hydrolysis of starch to maltodextrin requires high temperatures so that the enzyme used has to be a thermostable enzyme which remains active and stable at high temperatures.

Maltodextrin is a mixture of glucose, maltose, oligosaccharide, and dextrin produced from partial hydrolysis of starch using enzymes, acids or irradiated with a maximum DE value of 20. The DE value of 2-5 can be used as a substitute for milk fat in desserts mouth, yogurt, bakery products and ice cream [5]. Maltodextrin with the DE value of 15-20 is used as a high-calorie food product [6] and [7]. Maltodextrin from wheat starch with the DE value of 3-9 values can be used as a source of carbohydrates in isotonic sports drinks [2]. Most of the needs of maltodextrin in Indonesia are still filled with imported products, it is estimated that the import value of modified starch to Indonesia each year reaches the US $ 150 million. Indonesia has the great natural potential to meet these needs with domestic products because Indonesia has many plants that are the source of starch. One potential source of starch to modify into maltodextrin is sago starch.

Sago (Metroxylon sagu Rottb) is native to Southeast Asia. In Papua, the area of sago is more than 1 million ha, while the utilization of sago is still low i.e. 0.1% of the total national sago area [8]. Sago that is managed well can give a dried starch of 25 tons/ha/year [9]. Due to the highproduction of sago and the low price of wet sago (6000 IDR/kg), it is necessary to convert sago into high-value products such as maltodextrin.

The dried starch of sago from Palopo, South Sulawesi contained a total carbohydrate of 91.23% with the water content of 7.42% and the calcium content of 408.39 ppm [1]. Purnawani [10] reported that the dried starch of sago from Palopo contained amylase of 26.80% with the water content of 8.30% and the gelatinization temperature of 70.5°C. Based on the characteristics, sago starch from Palopo can be used as raw material in making maltodextrin using the thermostable α- amylase enzyme.

The objectives of the study were to optimize the addition of α-amylase enzyme concentration and time used for hydrolyzing the α-amylase enzyme to fit the quality requirement of maltodextrin.

2. Research Methods

2.1 Isolation and Purification of the α-amylase enzyme from B. stearothermophilus RSAII1B

2.1.1 Inoculum Preparation. The culture stock of B. stearothermophilus RSAII1B was grown in Luria agar (LA) medium with 1.0% soluble star in order to obtain fresh isolates. Inoculum preparation was then conducted in the production medium with the inoculum medium used by Arfah et al. [11] consisting of 1.5% soluble starch, 0.4% bacto-yeast extract, 1.0% bacto-pepton, 0.005% NaCl, 0.05% MgSO4·7H2O and 0.08% CaCl2. The medium pH was 7.0, incubated at 55°C for 24 h with an agitation speed of 200 rpm.

2.1.2 Production of α-amylase Enzymes. The composition of the production medium was equal to the composition of the inoculum medium. The active inoculum (10%) was incorporated into an Erlenmeyer containing the production medium, incubated in a shaker incubator with an agitation speed of 200 rpm at 55°C for 33 hours [11]. The centrifugation was then conducted at 3500 rpm for 30 minutes. The supernatant obtained was a crude extract α-amylase enzyme. Furthermore, the crude extract enzyme obtained was purified by using ammonium sulfate with saturation of 40-60% and followed by dialysis in the cellophane pouch. Dialysis of the α-amylase enzyme was used to hydrolyze the sago starch partially into maltodextrin.

2.2 Optimization of maltodextrin production

2.2.1 Effect of α-amylase enzyme concentration from B. stearothermophilus RSAII1B on the production of maltodextrin. Sago starch was dissolved in a phosphate buffer pH 5 solution containing 10 ppm CaCl2. Subsequently, the starch solution was heated at 70-80°C for 2 hours on a hotplate stirrer for the gelatinization process. After that, the temperature was lowered to 60°C, then α-amylase with a concentration of 0.05%; 0.07%; and 0.09% was added. The mixture was heated at 60-65°C for 60 min.
The temperature of the mixture was then adjusted to 30 °C. The solution pH was adjusted to 3.7-3.9 with 0.1N HCl. Furthermore, the solution was heated in boiling water to stop the enzyme activity. The resulting liquid (maltodextrin) produced was dried in an oven at 50 °C for 3 day and crushed [12]. The obtained maltodextrin powder was analyzed for the total sugar content by a phenol-H$_2$SO$_4$ method, the reducing sugar content by a DNS method.

2.2.2 Effect of hydrolysis of the α-amylase enzyme from B. stearothermophilus RSAII$_{1B}$ on the production of maltodextrin. The procedure for studying the effect of hydrolysis time of the α-amylase enzyme on the resulting value of DE maltodextrin was similar to the procedure in point 2.2.1 but the concentration of the α-amylase enzyme used was 0.09% and the time used was 60, 120, 180, 240 minutes.

2.3 Maltodextrin production using α-amylase enzyme from B. stearothermophilus RSAII$_{1B}$

The procedure for maltodextrin production was similar to the procedure in point 2.2.1 but the concentration of the α-amylase enzyme used was 0.09% and the hydrolysis time was 120 min.

2.4 Characterization of maltodextrin resulting from partial sago starch hydrolysis using the α-amylase enzyme from B. stearothermophilus RSAII$_{1B}$

2.4.1 Determination of equivalent Dextrose value (DE). The determination of the equivalent Dextrose value begins with the determination of reducing sugar and the determination of total reducing sugar in maltodextrin products, as follows:

2.4.1.1 Determination of reducing sugar by DNS method [13]

Maltodextrin (1000 ppm) was taken as much as 1.5 mL and then put into a test tube containing 1.5 mL of the DNS reagent. After that, the solution was shaken with a vortex for 10 seconds and heated in boiling water for 10 minutes. The equivalent Dextrose was calculated by using equation (1).

\[
DE = \frac{\text{Reduced sugar content}}{\text{Sugar (carbohydrate) total}} \times 100
\]

2.4.1.2 Determination of sugar (carbohydrate) total by phenol-sulfuric acid method

Determination of sugar total using the same maltodextrin solution with the determination of reducing sugar. A 1.5 mL maltodextrin solution was added with 0.75 mL of 5% phenol solution and 3.75 mL concentrated H$_2$SO$_4$ slowly through the tube wall. After that, the solution was allowed to stand for 10 min and shaken in a vortex for 10 sec. After that, the solution was allowed to stand for 20 min at a room temperature until the color change occurred. The standard solution used was glucose solution at various concentrations of 0.04-0.12 mg/mL and distilled water was utilized as blank. Measurement of absorbance was conducted at 490 nm wavelength with a Spectrophotometer. The calculation of carbohydrate total was done by substituting the absorbance of the solution obtained on the determination of glucose concentration into the regression equation of the standard glucose solution of the phenol-sulfate method.

2.4.2 Determination of type and concentration of reducing sugar in maltodextrin by HPLC method.

The resulting maltodextrin was analyzed by high-performance liquid chromatography (HPLC) to determine the type and concentration of reducing sugar of maltodextrin. Determination of the type of reducing sugar in maltodextrin product by HPLC method was by comparing the sample retention time with the standard retention time. HPLC was performed at a flow rate of 2 mL/min, the temperature of 40°C, acetonitrile:double distilled water phase (80:20), carbohydrate column (4.6 mm x 250 mm), particle size 4 μm, 20 μL injection volume and Detector Refractive Index Detector. The sugar content can be calculated by using equation (2).
\[ \text{Sugar content (\%)} = \frac{(\text{peak area} - \text{intercept}) \times \text{factor}}{\text{slope} \times \text{weight of sample}} \] (2)

2.4.3 Determination of water content. A total of 1 g of maltodextrin sample was weighed and put into a container. The sample was dried in an oven at 105 °C. After that, it was cooled and weighed. The work was repeated until achieved a constant weight. The water content was calculated by using equation (3).

\[ \text{Water content} = \frac{A - B}{C} \times 100\% \] (3)

A = weight of container + sample before draining (g)
B = weight of container + sample after drying (g)
C = sample weight (g)

2.4.4 Determination of pH. The determination of pH was performed by dipping the electrode into the maltodextrin suspension placed on a hotplate stirrer so that the suspension is always homogeneous.

2.4.5 Density Measurement. The density was measured by comparing the weight of maltodextrin from a certain amount of powders of the same volume. The maltodextrin powder was put into a 10 mL measuring bottle and weighed. The density maltodextrin was calculated by equation (4).

\[ \text{Density} = \frac{\text{mass}}{\text{volume}} \] (4)

2.4.6 Total Plate Count Test (TPC). TPC testing aims to determine the number of microbial colonies that grow on a material. In the TPC test, Plate Count Agar (PCA) was weighed as many as 2.82 gram added with distilled water until the volume up to 120 mL in an Erlenmeyer. The solution was then heated until dissolved and sterilized in an autoclave at a temperature of 121 °C and a pressure of 2 atmosphere for 15 minutes [14].

3 Result and Discussion

3.1 Effect of α-amylase enzyme concentration from B. stearothermophilus RSAIII B on DE value of maltodextrin

Figure 1 shows the value of DE as a function of the concentration of α-amylase enzyme. It is clear that the DE value increases with increasing the concentration of the α-amylase enzyme. This is due to the increasing concentration of enzyme that can increase the reaction speed. This happens because the more the α-amylase enzyme contacts with the substrate, the higher the enzyme-substrate complex is formed, this contact occurs on the enzyme’s active side.

![Figure 1. Effect of α-amylase enzyme concentration on the DE value of maltodextrin.](image)
The data in figure 1 also show that without the use of the α-amylase enzyme there is no reducing sugar (DE = 0), although it was heated above its gelatinization temperature at 75 °C for 2 hours, followed by hydrolysis for 60 min, at 60-65 °C. This phenomenon proves that enzymes play an important role in the process of hydrolysis of starch substrate into reducing sugar products. In addition, the enzyme concentration of 0.09% produced the maximum DE value (4.78%) with the time of hydrolysis of 60 min.

3.2 Effect of hydrolysis of the α-amylase enzyme from B. stearothermophilus RSAII1B on the value of dextrose Equivalent (DE) maltodextrin

The effect of the hydrolysis time on the DE value of maltodextrin is shown in figure 2. There is an increase in the value of DE as the hydrolysis time increases. The more enzymes bind to the substrate the more the product of reducing sugars produced, it follows the theory of hydrolysis, that the longer the hydrolysis time the more hydrolyzed the material. The time of hydrolysis used for the α-amylase enzyme to produce maltodextrin with the DE value of about 5-10% is 60-120 minutes. Ni'maturohmah et al. [15] reported that dextrin produced from sago starch using the β-amylase enzyme had a DE value of 2.1% required the hydrolysis time of 15 hours with the enzyme concentration of 0.1%, the hydrolysis temperature of 60°C, and the starch concentration of 30%. This indicated that the α-amylase enzyme from bacterium thermophile isolate of B. stearothermophilus RSAII1B required relatively faster hydrolysis time to hydrolyze 1,4-glycosidic bond compared with the β-amylase enzyme. It can be explained that the α-amylase enzyme works randomly to break the α-1,4-glycosidic bond on the starch, especially on long chains, to produce maltotriose and maltose. Due to its nature, which can break the glycosidic bond randomly, the enzyme works faster than the β-amylase enzyme.

![Figure 2. Effect of hydrolysis time on DE values.](image)

The β-amylase enzyme acts to hydrolyze starch from the non-reduction portion and hydrolyze the 1,4-glycosidic bond in the second stage of starch hydrolysis to form maltose and glucose [16, 17]. The equivalent dextrose value of 5-12% in this study requires a hydrolysis time of about 60-180 minutes. It is expected that the resulting maltodextrins have properties and characters as margarine additives, and as a substitute for milk fat in desserts, yogurt, bakery products, and ice cream.

3.3 Characteristics of maltodextrin produced from hydrolysis of sago starch by the α-amylase enzyme

The characteristics of maltodextrin obtained in this study are shown in table 1. It is clear that the characteristics of maltodextrin is in parallel with the Indonesian National Standard (SNI) requirement in terms of the DE value, sugar reduction, the moisture content, pH and the color.
Table 1. Characteristics of maltodextrin produced from hydrolysis of sago starch by the α-amylase enzyme.

| Characteristics                  | maltodextrin of sago | Syarat mutu SNI 7599-2010 (18) |
|----------------------------------|----------------------|--------------------------------|
| Dextrose Equivalent (DE)         | 12.31                | Maximum 20                     |
| Sugar reduction (%)              | 11.14                | 11 - 14                        |
| Water content (%)                | 10.92                | Maximum 11                     |
| pH                               | 4.85                 | Maximum 5                      |
| Color                            | White beige          | White yellowish                |
| Solubility (g/L)                 | 153.2                | -                              |
| Density (g/mL)                   | 0.3                  | -                              |
| Viscosity of maltodextrin 10% (cps) | 240                  | -                              |
| Glucose (%)                      | 2.82                 | -                              |
| Maltose (%)                      | 5.54                 | -                              |

4. Conclusion
The results showed that the DE value was 12.31, reducing sugar was 11.4%; water content was 10.92%; pH was 4.85; the color of maltodextrin powder was white bone color; solubility was 153.2 g/L; viscosity was 210-240 cps, and the total plate count (TPC) was 380 cfu/g. Maltodextrins produced from sago starch using the α-amylase enzyme from B. stearothermophilus RSAII1B met the quality requirements of SNI 7599: 2010.

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