

CHARACTERISTIC OF pH AND TEMPERATURE OF CRUDE EXTRACT OF GLUKOAMILASE FROM *GLIOCLADIUM KE* BY USING SOLID SUBSTRATE OF SAGO HAMPAS

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ABSTRACT

This research was aimed at figuring optimum pH and temperature of crude enzyme extract gluukoamilase from isolate *Gliocladium KE* using substrate of solid sago hampas. The research was conducted by two phases, first was figuring optimum pH and second was optimum temperature. It was based on Complete Random Design. The data were statistically analyzed by using F test. If the result showed significance different, the analysis was carried out by using Duncan's New Multiple Range Test (DNMRT) with 5% tolerance. The result showed that optimum pH of crude gluukoamilase extract from sago hampas was optimum pH of gluukoamilase from *Gliocladium KE* using solid substrate of sago hampas was pH 6,0 with activity of enzyme 76,56 U/ml with final product of soluble starch 0,09%, the level of reducing sugar 62,01%, and total sugar 78,43%. Optimum temperature was 60°C with enzyme activity 78,33 U/ml, final product of soluble starch 0,13%, reducing sugar 63,04%, and total sugar 79,55%

Keywords: *Gliocladium KE*, Glukoamilase, Sago Hampas pH, Temperature

INTRODUCTION

Waste of sago palm, especially waste of sago, was not yet optimally used by people for food. In fact, it had great potential for several regions, particularly in Papua, Sulawesi, and Sumatera. Kiat (2006) reported that waste of sago contains advantage substance such as essence and cellulose. The number of waste from sago timber was about 26% of total product while from waste of sago was about 14% of total product. The waste contains 65,7% essences and the rest were fibers, proteins, fats, and ashes.

Glukoamilase was an enzyme which hidrolize units of polysacaride or oligosacaride. Glucoamilase was usually used in essence industry. Since it had great advantages, many studies had been focuses on gluukoamilase production from various type of mushroom. (Siddhartha, *et al*, 2012). Glukoamilase which was being extracted from *Gliocladium KE* was the new innovation in glocuminase product.

Winarno (2010) stated that enzyme had maximum activity in the pH ambient which was called optimum pH. Ambient with high level of acid or alcaly will denaturate the protein and will decrease the total activity of enzyme. In general, optimal pH wa between netral of week level of acid, around 4,5-8.

Optimal pH was very important to define the characteristic of enzyme. At the different substance, enzyme had different optimum pH as well. The characteristic of certain enzyme had been well known. Glucoaminase from certain isolate had to be figured in order to gain optimal activity (including temperature and pH). Different enzyme was frequently had different level of optimum pH depends on the source of enzyme (Winarno, 2010). This microbe enzyme had wide optimum pH level (Buckle, 1997).

The fastest reaction occurs in optimal temperature (Rodwell, 1988 *cit* Rochmawatin, 2010). Therefore, defining optimum temperature of enzyme activity was important because lower temperature will increase stability of enzyme but decrease its activity while higher temperature will increase the activity but decrease the stability (Winarno, 2010). Optimum temperature and pH of glucomilase from local isolate should be known. According to Naiola (2006), the characteristic of hard glucomilase enzyme from *Saccharomycopsis* sp for optimum temperature activity was 40°C- 50°C for and 6,0-7,0 for optimum pH. Based on the research conducted by Sunaryanto (2003), glucomilase from *Aspergillus niger* BCS had optimum pH in 4,5 and optimum temperature in 60 °C.

EXPERIMENTAL SECTION

A. Material

Microorganism which was used in this research was isolate *Gliocladium* from Microbiology Laboratory and Bioteknologi Faculty of Agriculture Technology collection.

The material used for refreshing the isolate was *Potato Dextrosa Agar* (PDA) (Merck). The cultivation material was waste of sago derived from Pariaman (Subarang-Ciparuh) which had been mashed and pass through sieve with 80 mesh, distilled water, filter paper, buffer phosphate, Ca(NO₃)₂ (Merck), MgSO₄·7H₂O (Merck), Na-acetate, Tween 80, DNS liquid, Pb acetate, Na Phosphate 8 %, phenol 5% and H₂SO₄ (Merck). The materials used in extracting enzyme were acetate buffer with pH 4.6 and solvent isopropanol (Merck). Materials used to determine the optimum pH and optimum temperature of glucoamylase activity was *soluble starch*.

The instruments that used in this research were *autoclave* (Eyla HVE-50), Incubator (Mettler), pH meter (Hanna-instrument), centrifuge (Sigma 3-18K), Magnetic stirrer (IKA-RH-KT/6), analytic scale (Kern), vacuum filter (Rocker 300), Vortex mixer (VM - 300) , spectrophotometer (V -1100 Mapada D) and other glassware.

B. Method of Research

This research method uses two phases: first determine the proper pH and the second stage determine the right temperature. This study used 3 replication processes:

- a. Research Phase I: Determine the optimal pH of crude glucoamylase enzyme in the substrate from 3 levels of pH.

B1: substrate pH 4.0

B2: substrate pH 5.0

B3: substrate pH 6.0

b. Research Phase II: Determine the optimal temperature of crude glucoamylase enzyme in the substrate from 4 levels of temperature:

C1: substrate temperature 40 ° C

C2: substrate temperature 50 ° C

C3: substrate temperature 60 ° C

C4: substrate temperature 70 ° C

RESEARCH IMPLEMENTATION

Refreshing *Gliocladium* isolates (modified Sunaryanto , 2003)

Refreshing isolates was done by moving the mold culture in a the dish which contain PDA medium. Then, it being incubated for 4 days at a temperature of 27-28 ° C. After the colony grew, each colony was transferred to the slant plan and each colony was coded.

Sago Hampas Drying

Sago hampas drying using solar dryers to 12 % moisture content material. Drying was done by laying out the dregs of sago in a solar dryer.

Sago Dregs Size Decreasing

Sago hampas size reduction was done using belender. After blending, sago dregs was meshed by using 80 mesh sieve. Sago dregs which do not pass 80 mesh sieve were blended again until it pass the 80 mesh sieve.

Substrate Manufacture and Production of Glucoamylase

Substrates used in this research refers to the composition used by Tani *et al* (1986) *cit* Sunaryanto, 2003, which had been modified. Its composition was as follows: 57.82 g Dregs sago, 1.67 g MgSO₄.7H₂O, Ca (NO₃)₂ with specific treatment, and 37 mL of water. All medium of fermentation in 500 mL erlemeyer which was sterilized by autoclave at 121 ° C for 15 minutes. Inoculation *Gliocladium* KE into fermentation medium was done by using *Gliocladium* KE suspense in 0.1 % (v/ v) tween 80 which compose with 9 ml distilled water. After that, the fermentation was done in erlemeyer with aerobic conditions at a temperature of 27-30 ° C for 4 days.

Extraction and Production of Crude Enzyme Glucoamylase (modification Sunaryanto , 2003)

Extraction was done by adding acetate buffer pH wiith 4.6 that 3 times of solid substrate fermentation weight, then stirred for 30 minutes at 4°C. Then, it was centrifuged at 11,000 × g speed for 10 minutes and filtered. The filtrate obtained was called crude enzyme.

The enzyme of extracted filtrate was sedimented by adding isopropanol solvent organic cooled in a freezer at a temperature of -4°C . The filtrate was mixed with an organic solvent, solvent volume of isopropanol was added to the filtrate from the filtrate as much as 1.67 times the concentration of enzyme or 60 % (v / v). The addition of organic solvents was done bit by bit. Once mixed with an organic solvent and then stored in a cold room at 4°C for 24 hours. Once it's done 11,000 xg centrifugation for 10 min at 4°C . Then the precipitate was separated between the filtrate. Deposition of enzyme added acetate buffer pH 4.6 to obtain a crude enzyme extract glucoamylase.

Determination of optimum pH and Temperature of Enzymes Glucoamylase (Pandey et al, 2000 cit Sunaryanto 2003)

The optimum pH of glucoamylase activity was defined by conducting hydrolysis test toward *soluble starch* by applying method by Pandey et al., (2000) cit Sunaryanto (2003). 1.9 ml of 2 % *soluble starch* which had been specified with certain pH by using acetate buffer was added with 0.1 ml of the sample solution. After mixed, it was incubated at specified temperature for 20 minutes. After that, the analysis was conducted toward *starch content*, sugar content, total sugar, and glucoamylase activity by measuring the amount of glucose μmol as the result of hydrolysis of *soluble starch* by enzyme activity per unit (min) and volume (ml).

Glucoamylase Activity Measurement

Crude enzyme extract was filtered with cotton wool and then centrifuged at 1000 rpm at a temperature of 4°C for as many as 1 ml pipetted into a test tube. After the enzyme extract was added 2 ml pipetted acetate buffer pH 4.6. To control the enzyme extract, it had to be incubated first at a temperature of 100°C for 5 minutes. Then take 0.1 ml (V_c) of the mixture and transferred it into another test tube and add 1.9 ml *soluble starch* (V_{sb}) 2 %. Stirred well and then heated at a temperature of 60°C for 20 min (t). After heating process done, the sample will be divided into two parts; at top it will look clear and while cloudy at the bottom. The clear samples were pipetted. The analysis was done by adding 0.5 ml samples with 1.5 ml DNS. After the sample mixed with DNS solution, boil it for 5 minutes. Then get the sample cool by flowing water. The cool sample was pipetted as 0.4 ml and then added distilled water until it becomes homogenous. Then the process continued by conducting reduced sugar measurement (C_{gr}) at 540 nm absorbance. The research procedure was similar to sampling procedure, but in research procedure the sample was change with acetate buffer pH 4.6. Glucoamylase activity can be measured by the following formula:

$$\text{Glucoamylase Activity} = \frac{C_{gr} \times (V_c + V_{sb}) \times F_p}{\text{BM (180)} \times t \times V_c}$$

RESULT AND DISCUSSION

A. Chemical composition of Solid Sago Dregs Substrate

The result of analysis toward sago dregz was reflected in the following table:

Table 1. Chemical composition of solid sago substance.

Component	Content (%)
Extract	54,35 %
Protein	3 %
Dust	3,32 %
Fat	0,13 %

From the Table 1. It can be seen that the starch content in dregs of sago was still high and had high potential for the growth of *Gliocladium* KE to produce glucoamylase. Starch that still high enough which contained in sago dregs can be utilized by *Gliocladium* KE as a carbon source. The starch was broken down into simple sugars by microorganisms into glucose, then glucose was utilized by microorganisms as carbon sources by removing the extracellular enzyme glucoamylase. Kiat (2006) analyzed sago dregs found that starch content also high at 65.7%. The chemical composition of the soluble starch that analyzed was early starch content, that was 2.12%.

B. Optimum pH of Rough Glucoamylase Enzyme Extract

Based on the results of variance done, the pH of the substrate significantly affect the final starch content. Table 2 showed the higher pH used the lower starch content of soluble *starch substrate*.

Table 2. The relationship of starch content result toward pH

Treatment	Starch Content (%)
B.1 (pH 4,0)	0,12 a
B.2 (pH 5,0)	0,11 ab
B.3 (pH 6,0)	0,09 b

The numbers in samiliar row followed by different small letter was significant different with 5% level according to DNMR

Table 2 showed pH was significantly affect the starch content. The lower pH value results the higher substrate starch. The lower starch content was in treatment B.3 treatment using pH 6.0. This happens because at pH 6.0 occurs high glucoamylase activity which made hydrolysis of starch in the substrate become high as well. Glucoamylase was one group of amylolytic enzyme that functions to break down the starch (Maggy, 1989). Winarno (2010) states that glucoamylase break down the starch into glucose.

Based on the results of variance that had been done on the effect of pH levels of reducing sugars and total sugars showed significant effect. Levels of reducing sugars and total sugars showed highest value in treatment B.3 (pH 6.0) that was 62.01% and 78.43%. The effect of pH substrate toward levels of reducing sugars and total sugars were shown in Table 3.

Table 3. The relationship of reducing sugar and total sugars toward ph

Treatment	Reducing Sugar (%)	Amount of Sugar (%)
B.3 (pH 6,0)	62,01 a	78,43 a
B.2 (pH 5,0)	33,58 b	63,82 b
B.1 (pH 4,0)	22,88 c	58,49 c

The numbers in samiliar row followed by different small letter was significant different with 5% level according to DNMRT

The higher reducing sugar result levels indicate higher glucoamylase activity This was because the enzyme glucoamylase to hydrolyze starch into glucose . This statement was supported by the statement of Montenecourt *et al*, (1985) *cit* Sunaryanto (2003) that the end product of starch hydrolysis by glucoamylase produces glucose as the main product . Glucose produced by glucoamylase was a reducing sugar. The existence of glucoamylase in starch suspensions , in particular the enzyme which can catalyze the hydrolysis of amylose, will cause the starch hydrolysed to form reducing sugar (Kusnandar, 2010).

The content of reducing sugar was in accordance with total sugar. The higher total sugar, the higher end product reducing sugar. This was because the amount of sugar consist of reducing sugars plus non-reducing sugar. The difference between total sugars and reducing sugars were shown in the Table 3 which tells that the higher pH used in the substrate, the less increment in total sugars with reducing sugars. It showed that the starch contained in *soluble strach* medium was dominantly hydrolyzed into reducing sugars.

Activity of Glucoamylase

The effect of pH toward glucoamylase activity had significance effect. Glucoamylase activity toward of the pH can be seen in Figure 1. The lower pH set in specified substraes, the lower glucoamylase activity was measured

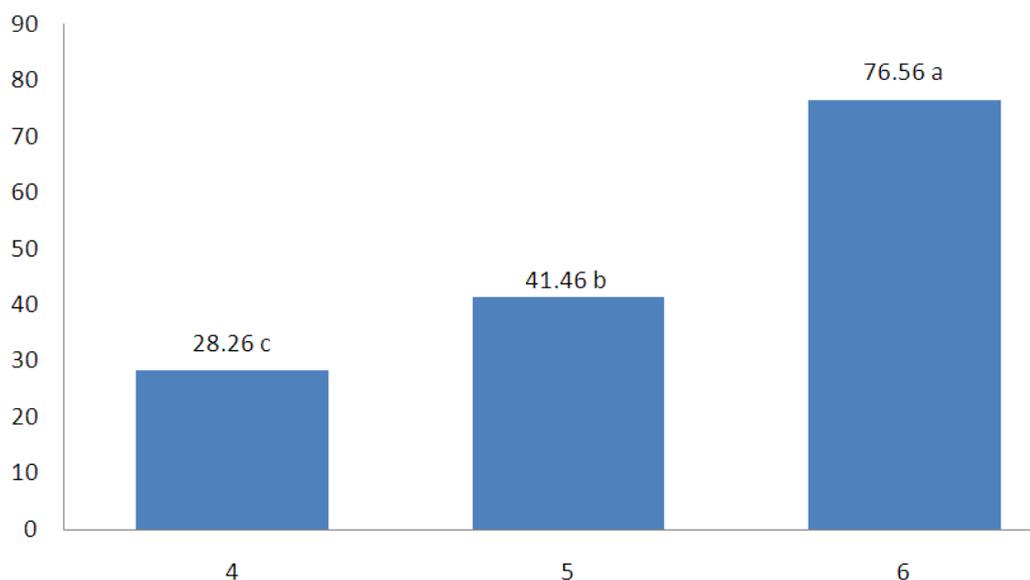


Figure 1. The Relationship of Glucoamylase Activity toward pH Substrate

The enzyme activity at pH 4.0 was not optimal because there were many active side of active glucoamylase which not yet active in binding starch in the substrate. Glucoamylase that not yet active can be activated with the right environment. The right environment for glucoamylase activity was at pH 6.0. The optimum activity of the crude glucoamylase extract at pH 6.0 was at 76.56 U/ml . This was in accordance with the Maggy's opinion (1989) which states , enzymes were proteins which composed of amino acids. Amino acids which located in the active site plays a role in binding reaction between the enzyme to the substrate with a pH neutral or slightly above or below neutral pH.

The enzyme activity was low at pH below or above the optimum pH, due to the denaturation of the enzyme protein. Denaturation of this protein led to the destruction of the molecular structure that leads to inactivation of the enzyme glucoamylase. pH can cause changes to ionized groups contained in the enzyme. This can lead to changes of structure, conformation and active side of enzyme (Creighton, 1984 *cit* Sunaryanto, 2003). High pH can cause protein denaturation. In accordance with Winarno (2010) states that at extreme pH range, both acid and base, occurs irreversible inactivation. Denaturation of proteins occurs due to disruption of hydrogen groups and ionic groups as well as the interaction of the substrate reduced (Campell , 2002) .

Enzymes provide plenty of proton binding. Because enzymes were proteins composed of amino acids that can ionized by binding and releasing protons or hydrogen ions on amine, carboxyl and other functional groups . Amino acid that plays role in binding the enzyme with substrate was amino acid with neutral pH or slightly above or below neutral pH. pH of acid or base can interfere with ionic enzyme (Maggy , 1989). The increase or decrease the pH of optimum pH can break the weak hydrogen bonds that maintain the structure of the enzyme which leads to denaturation of the enzyme and the affect the conformation of substrate and distracted to entering the active side of the enzyme (Anwar , 2008).

C. Optimal temperature Crude Glucoamylase Extract

Effect of temperature on enzyme extract of crude glucoamylase was analyzed by using parameters starch content product, reducing sugar and total *soluble starch* and glucoamylase activity.

Levels of Starch

Based on the results of variance substrate temperature toward significance affect the starch content product. Substrate temperature affects starch hydrolysis which done by glucoamylase. The lower starch content was in C.3 treatment (using a temperature of 60°C) and the highest starch content was in C.1 treatment (using a temperature of 40°C). The effect of temperature toward starch content can be seen in Table 4.

Table 4. Relationship of starch product toward temperature

Treatment	Strach Content (%)
C.1 (40 °C)	0,27 a
C.4 (70 °C)	0,23 a
C.2 (50 °C)	0,18 b
C.3 (60 °C)	0,13 c

The numbers in samiliar row followed by different small letter was significant different with 5% level according to DNMR

Final product starch content was starch content that can not be hydrolyzed by glucoamylase during the enzymatic reaction. The existence of enzyme in the starch suspension, particularly the enzyme that can catalyze the hydrolysis of the amylose and amylopectin molecules, glucoamylase will cause hydrolysed the starch form saccharide with a shorter chain. The addition of glucoamylase can break the glycosidic bond α (1-4) and β (1-6) (Kusnandar, 2010).

The higher temperatures, the lower starch content , exepct at treatment that use temperature 70°C. The lower starch content of final product, then the glucoamylase activity. This was because the activity of glucoamylase at 70 ° C decreased the hydrolysis of starch which also decreases the starch content. Sudarmadjo, (2008) states in the high heat, the enzyme which was kind of protein will denaturated. Denaturation of this enzyme decreased the activity of the enzyme (Sukandar *et al.* , 2011) .

B. Reducing sugar , total sugar

Based on the results of variance effect of temperature on reducing sugar and total sugar showed significance effect. The highest level of reducing sugars and total sugars was gained at treatment C.3 treatment (using temperature 60°C) they are, 79.55 % and 63.04 % . It can be seen in Table 5 .

Table 5. Relations of reducing sugar and total sugar toward temperature

Treatment	Reducing Sugar (%)	Amount of Sugar (%)
C.3 (60 °C)	63,04 a	79,55 a
C.2 (50 °C)	61,12 a	62,46 b
C.4 (70 °C)	49,80 b	53,82 c
C.1 (40 °C)	47,76 b	52,82 c

The numbers in samiliar row followed by different small letter was significant different with 5% level according to DNMR

The lowest result of reducing sugars and total sugars were found in treatment C.1 (using temperature 40°C), they were 47.76 % and 52.28 % . Siddhartha *et al.* ,(2012) stated that glucoamylase can produce glucose as much as 88.5 to 100 % from hydrolysis of starch to *soluble starch*. Temperature of enzyme affects total sugar that formed during the reaction between the substrate which contain starch using glucoamylase catalyst *Gliocladium* KE using solid hampas sago substrates.

Levels of reducing sugars and total sugars had a relationship with glucoamylase activity. It can be seen in Figure 1. where the higher levels of reducing sugars and total sugars, the higher glucoamylase activity. Levels of reducing sugars formed in final hydrolysis of product of starch using glucoamylase enzyme was glucose. The final product of starch hydrolysis by glucoamylase produces glucose as the main product (Montenecourt *et al* , 1985 *cit* Sunaryanto 2003).

Glucoamylase Activity

Based on the results of variance toward the effect of temperature on enzyme activity glucoamylase showed significance effect. The activity of glucoamylase product was found in treatment C.3 (using temperature 60°C), that was 78.33 U/ml which can be seen in Figure 2.

Temperature was one of the factors that influence the development of glucoamylase. Glucoamylase activity increased from a temperature of 40°C to 60°C. At temperature 70°C the result showed that the activity of glucoamylase was decreased. The highest glucoamylase activity was gained at 60°C which was 78.33 U/ml.

Optimal temperature of glucoamylase derived from *Gliocladium* KE by using solid substrate of sago hampas was temperature 60°C. Each enzyme had an optimum temperature, ie when the enzyme was working properly. The farther the optimum temperature, then enzyme will working properly. Area or range when the working temperature or rate of enzyme reaction was working properly was called optimum temperature (Sudarmadjo, 2008). According to Panesar (2010), this enzyme can withstand a range of temperatures up to 60°C. To see the relationship between the temperature toward glucoamylase activity can be seen in Figure 2 .

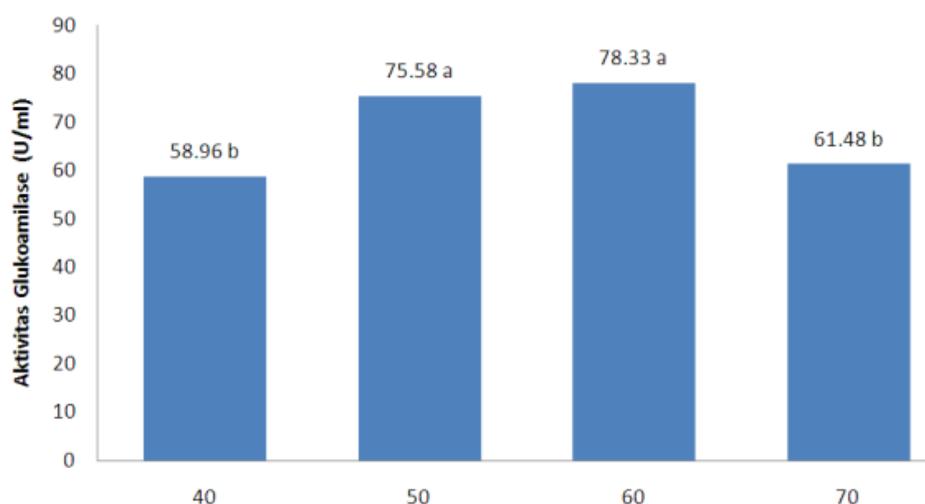


Figure 2. The Relationship of Temperature toward Glucoamylase Activity

The increase of temperature increases the value of glucoamylase activity, but at 70°C glucoamylase activity was decreased. It was caused at 70°C enzymes were denaturated the protein which decrease activity of the enzyme. In general, the higher temperature the faster the rate of chemical reactions catalyzed

by enzymes. However, the enzyme was a protein that will be inactivated when the ambient temperature was too high. Winarno(2010) argues that the higher the temperature of enzyme inactivation process was also increasing due to too high temperatures can accelerate the breakdown or destruction of enzymes that result in denaturation of the protein. Heating above the optimum temperature enzymes can cause damage to the conformation of the enzyme protein binding which may decrease the activity of the enzyme (Scopes , 1982 *cit* Sunaryanto, 2003) .

Suhartono (1992) *cit* Anwar (2008) states that at the beginning, the reaction rate increases along with increase of temperature and the increase of reaction rate was caused by the reaction of molecules which in over high temperatures can affect the conformation of the enzyme and cause distraction in substrate to enter active side of the enzyme. At high temperatures, it will break the hydrogen bonds and hydrophobic which maintain secondary and tertiary structures, for the result restructure of three-dimensional structure of the enzyme which cause denaturation of the enzyme so that the catalytic energy of enzymes was decrease (Robert et al., 2002). Sudarmadjo (2009) states at low temperature enzyme activity was decrease, when the temperature was increased , the speed will up to the optimum temperature. If the temperature continues to increase from the optimum temperature, the enzyme denaturated and will decrease the activities.

The destruction of enzyme bond was disrupted at 70°C which was caused by the increased of thermodynamic movement . As a result of the movement of thermodynamic, the enzyme will be increased which change three- dimensional structure. Sadikin (2002) adds that three- dimensional changes in the structure of this enzyme distract the substrates to occupy the active site of the enzyme, the result the enzyme substrate was difficult to set up which decrease the number of products.

Glucoamylase activity at 40°C was still low due to the lack of collisions between molecules of the enzyme with the substrate. If the contact between the active site of enzyme was low, then the enzyme substrate complex which formed was also low and the product was little as well (Sadikin , 2002).

CONCLUSION

The optimal pH of glucoamylase derived from *Gliocladium* KE solid using sago hampas substrate was pH 6.0. The product of glucoamylase activity at pH 6.0 was 76.56 U/ml with a final concentration of soluble starch was 0.09 % , reducing sugar content was 62.01 % and total sugar content was 78.43 % .

Optimal temperature of glucoamylase derived from *Gliocladium* KE using solid sago hampas substrate was at 60°C. Glucoamylase activity produced at a temperature of 60°C was 78.33 U/ml with starch content of final product was 0.13% , reducing sugar was 63.04 % and total sugar content was 79.55 % .

CONFLICT OF INTEREST

The authors had no conflict of interest

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