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ORIGINAL RESEARCH PAPER

KINETICS OF THE HYDROLYSIS OF CASSAVA STARCH BY GLUCOAMYLASE AND A GRANULAR STARCH HYDROLYZING ENZYME

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Abstract: Kinetic studies of the enzymatic hydrolysis of cassava starch have been carried out using glucoamylase and granular starch hydrolyzing enzyme (GSHE) as Stargen^{TM} 002. The enzymatic hydrolysis of cassava starch was conducted at *p*H 4 by employing slurry concentrations of 25-300 g·L^{-1}, temperatures 30-60 °C and 0.5-1.5 % (w/w) of enzymes glucoamylase and Stargen^{TM} 002. Higher temperatures accelerated the enzymatic reaction using glucoamylase, while the similar effect levelled of at 40 °C for hydrolysis using Stargen^{TM} 002. The cassava starch concentrations of 200 and 250 g·L^{-1} were found as the critical substrate concentrations for lower (glucoamylase 0.5 and 1 %) and higher (glucoamylase 1.5 %) enzyme concentrations used, respectively. Based on the Lineweaver-Burk plot for cassava starch hydrolysis using glucoamylase, the maximum reaction rates, V_{max} for 0.5, 1.0 and 1.5 % (w/w) glucoamylase were 3.55, 5.78 and 9 g·L^{-1}·h^{-1}, while the Michaelis-Menten constants, K_m were 140.35, 140.75 and 152.10 g·L^{-1}. Accordingly, the maximum reaction rates, V_{max} for 0.5, 1 and 1.5 % (w/w) of Stargen^{TM} 002 were 7.19, 11.6 and 18.18 g·L^{-1}·h^{-1}, respectively. While the Michaelis-Menten constants, K_m obtained at those enzyme concentrations were respectively 160.86, 161.31 and 160.85 g·L^{-1}. It is an indication that the rate of starch hydrolysis using Stargen^{TM} 002 is faster than glucoamylase.

Keywords: *cassava, enzyme kinetics, glucoamylase, GSHE, hydrolysis*

INTRODUCTION

The conventional enzymatic hydrolysis of starch requires two consecutive processes, namely the liquefaction and saccharification. The use of high temperatures (90-100 °C) and *α*-amylase are required to reduce the viscosity of the mash and to facilitate the cooking process to produce shorter carbohydrate polimers, namely dextrins, maltose and maltotriose. The resulting mash is then cooled to 60 °C and glucoamylase is utilized to convert dextrins to fermentable sugars as glucose [1 – 4]. This process is an energy-intensive, which leads to increase the production cost. It has been estimated that about 30 - 40 % of the total energy is demanded for this glucose preparation during ethanol production from starch [5]. The *α*- and *β*-amylase are not strongly adsorbed by the starch and they also exhibit different hydrolysis ability on raw rice, corn, wheat, potato and sago [6, 7]. Hyun and Zekius [8] observed that *α*-amylase does not significantly attack the raw starch granules because they are very resistant to amylolitic digestion. Therefore, the degradation of starch by amylase requires quite long hydrolysis period, commonly within 24 to 72 h. Kimura and Robty reported the formation D-glucose with increased proportion of glucoamylase over 32 h [9]. For that reason, the importance of the enzymatic liquefaction of raw starch without heating to save the energy has been well encouraged in the recent years.

As an alternative to the conventional process, the hydrolysis of starch can be conducted at low temperature (without cooking) using a specific enzyme [10]. Granular starch hydrolyzing enzyme (GSHE) is a mixture of *α*-amylase and glucoamylase, which is able to hydrolyze starch granules directly into fermentable sugars at low temperature, even below the gelatinization temperature of the starch (30-48 °C) [11]. The use of GSHE also presents another advantage, as the GSHE will negate liquefaction and saccharification. Several researchers have conducted enzymatic hydrolysis and fermentation of starch at low temperature. Saha and Huda [12] reported the effectiveness of alcoholic fermentation of sweet potato without cooking (30-35 °C) by using *Endomycopsis fibuligera* as the source of glucoamylase. Uthumporn *et al*. [13] also reported the hydrolysis of maize, sago and sweet potato starches using *α*-amylase and glucoamylase, at 35 to 50 °C. Li *et al*. [14] reported the hydrolysis of corn and triticle starches at 30-50 °C using *α*-amylase and glucoamylase. Shariffa [11] investigated hydrolysis of tapioca and sweet potato starches by *α*-amylase and glucoamylase at 35-65 °C. Sarikaya *et. al*. [6] studied the degradation ability of *α*-amylase from *Bacillus amyloliquefaciens* and *β*-amylase from *Bacillus cereus* to hydrolyze raw starch granules from potato, sweet potato, wheat, rice and corn at 40 °C. As expected, the hydrolysis of potato starch granules was more effective and 45 % conversion to glucose was achieved in 12 h when the temperature was increased to 60 °C. Unfortunately, no report has been found in the literature on the inhibition kinetics studies to determine the type of inhibition during enzymatic hydrolysis of starch. Therefore, no clear comparison of glucoamylase and GSHE at various temperature has been reported in the literature.

The degradation of cassava starch into reducing sugar is expected to increase its economic potential. Cassava starch can be converted into glucose syrup (for food applications) and to ethanol (for fuel application) *via* hydrolysis and fermentation process. To the best of our knowledge, hydrolysis of starch using alpha amylase (liquification) and glucoamylase (saccharification) requires high amount of energy, but

generates low glucose yield. Therefore, intensive studies are required to obtain a better understanding on the many aspects related to enzyme catalyzed starch hydrolysis and fermentation before being implemented in the respective industries, including the kinetics, effect of substrate concentration and enzyme loading on the degradation rate of cassava starch using glucoamylase and GSHE (Stargen^{TM} 002).
The objectives of the study are to present the kinetic expression for the hydrolysis of cassava starch and to investigate the effect of temperature and substrate concentration on cassava starch hydrolysis using glucoamylase and Stargen^{TM} 002.

MATERIALS AND METHODS

Cassava tuber

Ten month old of cassava (*Manihot esculenta* Crantz) tubers were obtained from commercial cassava plantation located in Wonogiri district in Indonesia.

Cassava starch

The cassava (*Manihot esculenta* Crantz) starch used in this study and its physicochemical properties were the same with that used previously by Hargono *et al*. [15].

Chemicals

Potassium sodium tartrate tetrahydrate and 3,5-dinitrosalicylic acid (by Merck), sodium hydroxide (98 %, Merck), sodium sulfite (98.5 %, Merck), sulfuric acid (98.5 %, Merck), sodium acetat buffer (Merck) and glucose (99.5 %, Merck) were purchased from Sigma-Aldrich Indonesia.

Enzyme

The enzymes in the research used for starch saccharification was Dextrozyme GA. *Aspergillus niger* glucoamylase (3.2.1.3) [16], activity 270 AGU g^{-1} (AGU is the amount of enzyme with hydrolyses 1 μmol of maltose per minute under specified condition) was used for starch saccharification. The enzymes were purchased from Novozymes, Denmark. The GSHE as Stargen^{TM} 002 preparation was obtained from Genencor International (USA) [10]. This enzyme contains a mixture of *Aspergillus kawachii* α-amylase expressed in *T. reesei* and glucoamylase from *T. reesei* and optimum *p*H ranged from 4.0-4.5. The recommended temperature is 20-40 °C and the minimum activity is 570 GAU·g^{-1} (GAU is the amount of enzyme that will be liberate on one gram of reducing sugars calculated as glucose per hour from soluble starch substrate under conditions of the assay.

Enzymatic hydrolysis

The concentrations of cassava starch slurry used in hydrolysis experiments to determine effect on temperature on reducing sugars were 100 and 200 g·L^{-1}, respectively. The *p*H was adjusted to 4 by addition of 50 mM sodium acetate buffer. The concentration of glucoamylase was 1.5 % (w/v) and the starch slurry was incubated at (30 ± 1 to 60 ± 1 °C) in a thermostatic water bath under continuous stirring of 100 rpm for 36 h. The samples were collected at 6 h interval and were then subjected to reducing sugar determination. The same procedure was applied for hydrolysis of cassava starch using Stargen ^{TM} 002 (1.5 %, (w/v) at (30 ± 1 to 60 ± 1 °C) with continuous stirring of 100 rpm for 15 h. The samples were periodically withdrawn from the flask at 3 h interval and substantially subjected to reducing sugar analysis.
The cassava starch concentrations used in the substrate inhibition experiment were 100, 150, 200, 250 and 300 g·L^{-1}, while the concentration of glucoamylase and Stargen^{TM} 002 were 0.5, 1 and 1.5 % (w/w), respectively. The experiments were conducted at 40 °C, and *p*H 4 for 12 h.

Analytical methods determination of reducing sugar

The reducing sugar concentration was determined using dinitrosalicylic acid method [17]. Reagent consisting of aqueous solution of 1 % 3,5-dinitrosalicylic acid, 0.05 % sodium sulfite, 20 % sodium-potassium tartrate and 1 % sodium hydroxide solution was added in the ratio of 3:1 to the samples in glass tubes, shaken in an incubated boiling water bath for 8 min. The reacted samples were then cooled in an ice water bath for 5 min, prior to measuring absorbance at 540 nm by using a UV/visible spectrophotometer (UV-160A, SHIMADZU, Kyoto, Japan). Glucose standard solutions (0 to 10 g·L^{-1}) were used as references. Therefore, the reducing sugar concentrations were reported as g·L^{-1}.

Determination of kinetic parameters

In general the Michaelis and Menten [18] framework has proven to be simple yet powerful approach to describe the kinetics most enzyme reaction, as shown in equation (1):

$$v_o = \frac{V_{max}[S]}{K_m + [S]} \quad (1)$$

where v_o is the initial reaction velocity, [S] is the concentration of reactant, V_{max} is the maximum rate of reaction at infinite reactant concentration, K_m is the Michaelis constant of reactant. The typical units for V_{max} and K_m are g·L^{-1}·h^{-1} and g·L^{-1}, respectively. To detemine the kinetic parameters (K_m and V_{max}), we conducted the experiments at *p*H 4 and 40 ± 1 °C using three different substrate concentrations (25 to 300 g·L^{-1}), while the concentration of glucoamylase and Stargen^{TM} 002 enzymes was 1.5 % (w/w). The amount of reducing sugar at different substrate concentration [S_o] is plotted as a function of time. The initial velocity of cassava starch, at concentration (100-300 g·L^{-1}) is determined from the slope of curve the relationship between reducing sugar versus time at beginning of a reaction [19]. The characteristic constant V_{max} and K_m were determined experimentally by incubating with different concentration of enzymes

glucoamylase and Stargen^{TM} 002 (0.5-1.5 %, w/w), respectively for different concentrations of substrates. The results were then plotted as a graph of initial velocity, v_o against initial concentration of substrate [S_o]. When we plot of ($1/v_o$) versus ($1/[S_o]$) a straight line should be obtained and the y and x intercepts were used for determining of V_{max} and K_m, respectively.

RESULTS AND DISCUSSION

Effect of temperatures on reducing sugar by glucoamylase

Effect of temperatures on reducing sugar concentration during enzymatic hydrolysis of cassava starch at concentrations 100 and 200 g·L^{-1}, with addition 1.5 % (w/w) of concentrations glucoamylase and Stargen TM 002 at *p*H 4, are shown in Figure 1 and 2. As shown in Figure 1, the reducing sugar concentrations increased sharply in the first 6 h of the hydrolysis and followed by gradual increased in reducing sugar concentration in the later time up to 30 h. Thereafter, the concentrations of reducing sugar were almost constant for both initial starch concentrations studied.

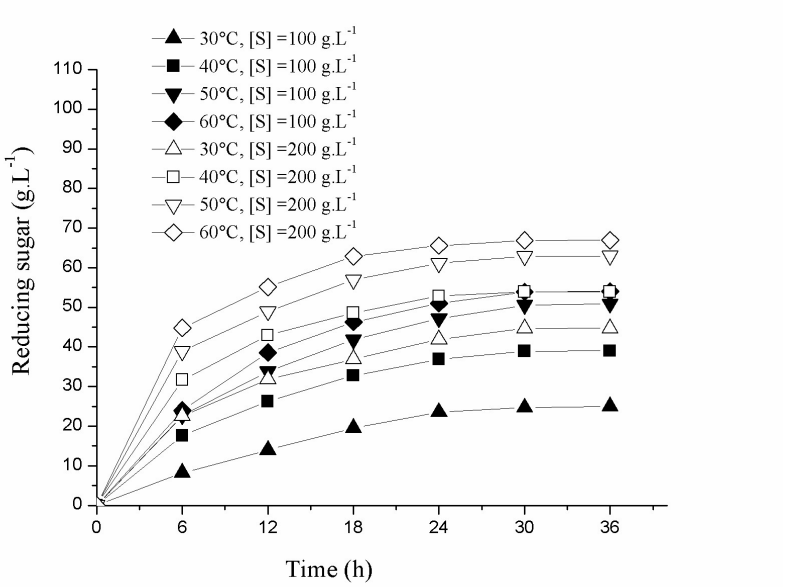


***Figure 1**. Effect of temperatures on the reducing sugar concentration, initial cassava starch concentrations, [S] 100 and 200 g·L^{-1}, glucoamylase 1.5 % (w/w), at pH 4*

The higher the initial starch concentration used in the enzymatic hydrolysis resulted in the higher reducing sugar concentration in the hydrolyzate. The amount of loaded enzyme (glucoamylase at 1.5 %, w/w) is likely to be sufficient enough to catalyze the hydrolysis of cassava starch with both initial concentrations (100 and 200 g·L^{-1}) at four different temperatures 30, 40, 50 and 60 °C. Shanavas *et al.* [20] studied the hydrolysis of cassava starch at 60-90 °C with Spezyme Xtra (*α*-amylase) at 0.2 % (w/w) using two different initial starch concentrations for 30 min. They found that the reducing sugar obtained at 90 °C were 15.3 and 29.6 g·L^{-1}, respectively for initial cassava starch

concentration of 100 and 200 g·L^{-1}. Matsumura *et al*. [21] reported the hydrolysis of sweet potato starch at 40 °C using 1.5 g·L^{-1} glucoamylase with initial starch concentrations studied were 45.2, 93.0 and 130 g·L^{-1}, respectively. The reducing sugar concentrations obtained from the respective initial starch concentrations were 5.5, 10.6 and 14.8 g·L^{-1}. As the initial starch concentrations were increased 2 times, the concentration of reducing sugar were also increased proportionally to almost 2 times. In constrast to our research, which used cassava starch, the reducing sugar concentration only increased 1.2 times although the initial starch concentrations were doubled. As shown in Figure 1, the reducing sugar concentrations were all higher at higher temperatures indicating a higher enzyme activity at higher temperatures. The extent of substrate degradation was greatly affected by temperature. Increasing of hydrolysis temperature generally results in the production of larger amount of reducing sugar. The increase in reducing power could be ascribed to the enhancement of the activity of the thermostable α-amylase at higher temperature in addition to the extensive swelling of the amorphous regions or starch granule [20]. Konsula and Kyriakides [22] investigated hydrolysis of various starches (rice, potato, corn, oat and chestnut) at different temperatures (38, 50, 60 and 70 °C) by α-amylase from *Bacillus subtilis.* At four different temperatures, potato starch hydrolysis resulted in a higher reducing sugars in comparison to the other starches due to higher starch content. The concentration of reducing sugar liberated from hydrolysis of potato starch at 70 °C for 5.5 h was about 30 g·L^{-1}. Thereafter, the reducing sugars concentration were observed to be constant.

Effect of temperatures on reducing sugar by Stargen^{TM} 002

The effect of temperatures (30 ± 1 to 60 ± 1 °C) on the enzymatic hydrolysis of cassava starch catalyzed by 1.5 % (w/w) Stargen^{TM} 002 with initial concentrations of 100 and 200 g·L^{-1} was studied, and the result are depicted in Figure 2.

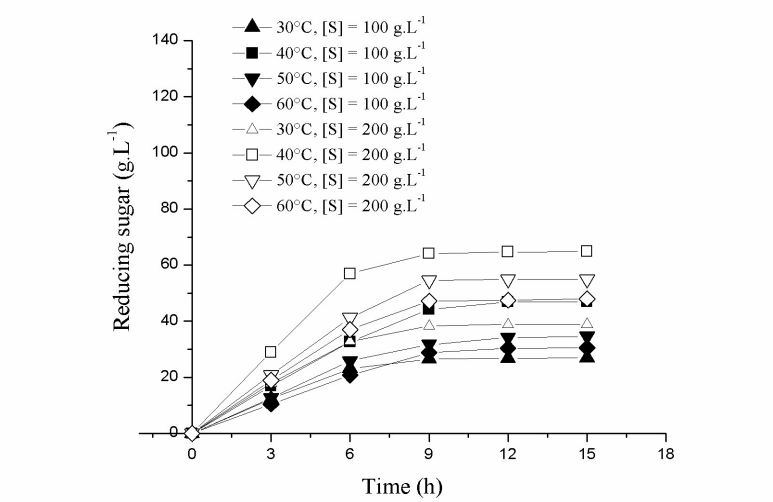


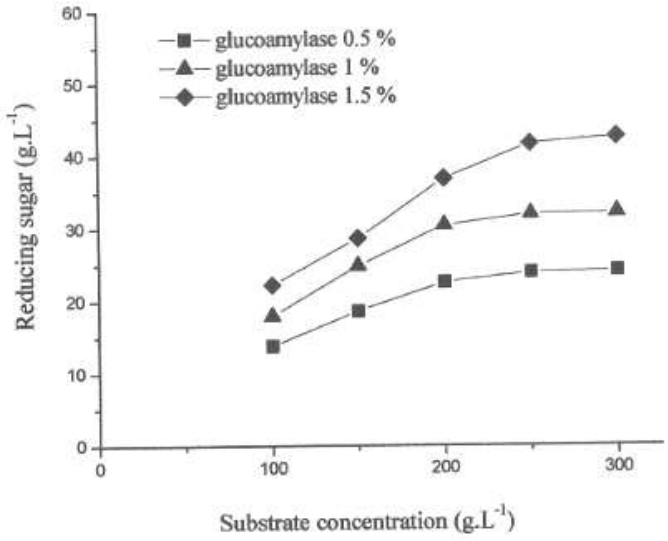
Figure 2. *Effect of temperatures on reducing sugar concentrations, initial cassava starch concentrations, [S] 100 and 200 g·L^{-1}, Stargen^{TM} 002 1.5 % (w/w), at pH 4*

At all of the initial starch concentrations and temperatures studied in this research, the maximum reducing sugars concentration were achieved at approximately 9 h of reaction time. The increase of hydrolysis temperature from 30 to 40 °C accelerated the reaction rates and consequently increased the reducing sugar concentrations. Unfortunately, furthure increase of temperature led to the reduction of the reducing sugar concentrations. The average decrease was approximately 31 % and 16 % for initial starch concentration of 100 $g \cdot L^{-1}$ and 200 $g \cdot L^{-1}$, respectively. This reverse behavior can be ascribed to the rapid decay in the enzyme activity (deactivation), which is in according the fact that Stargen ^{TM} 002 was developed to perfom better in lower temperature [10].
The Stargen^{TM} 002 was found to more efficient than glucoamylase for hydrolysis of cassava starch at low temperature. At 40 °C, the reducing sugar produced by the Stargen^{TM} 002 is 17.63 % higher than that obtained by glucoamylse. This is because Stargen^{TM} 002 is a perfect mixture of synergestic enzymes consisting of α-amylase and glucoamylase. The α-amylase randomly hydrolyzes $\alpha(1\rightarrow4)$ glucosidic bonds in the starch (amylose and amylopectin) molecules [23]. Therefore, a higher formation of dextrin should be achieved by Stargen^{TM} 002 compared with the use glucoamylase alone. As a consequence, the performance of Stargen^{TM} 002 to produce reducing sugar from starch is better than glucoamylase alone. Masiero *et al*. [10] reported the hydrolysis of fresh potato at initial concentration of 200 $g \cdot L^{-1}$ with Stargen^{TM} 002 (45 $GAU \cdot g^{-1}$) for 62 h. They observed that after the first 20 h, 30 $g \cdot L^{-1}$of glucose was liberated. Later, Shanavas *et al*. [20] investigated the hydrolysis of cassava starch at initial concentration of 200 $g \cdot L^{-1}$ by 0.4 % (w/w) of Stargen^{TM} 002 for 24 h at 30 °C. The reducing sugar concentration obtained was 185 $g \cdot L^{-1}$.

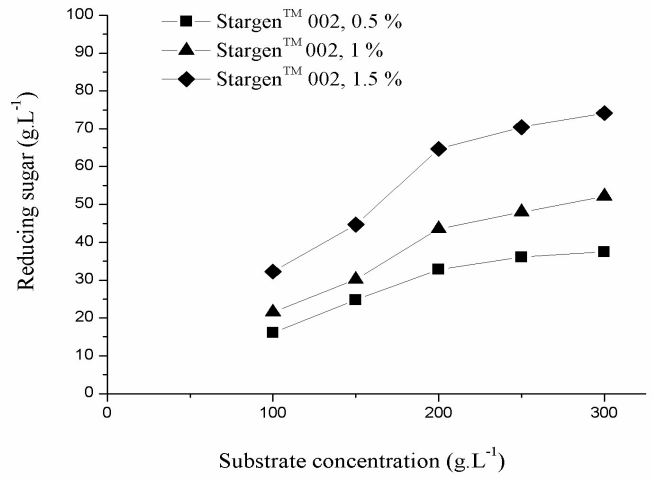
Effect of substrate (starch) concentrations on reducing sugar

The final reducing sugar concentration increased with an increase in the initial starch concentration (100 and 200 $g \cdot L^{-1}$) (Figure 1). Effect of substrate concentration on the reducing sugar concentration by glucoamylase and Stargen^{TM} 002 as shown in Figure 3, when the substrate concentration was 200 $g \cdot L^{-1}$, concentration of glucoamylase were 0.5 to 1.5 % (w/w), time of hydrolysis was 12 h, the product reducing sugar were 22.56, 30.45 $g \cdot L^{-1}$, while the substrate concentration was 250 $g \cdot L^{-1}$ by 1.5 % glucoamylase, the reducing sugar was 41.68 $g \cdot L^{-1}$ (Figure 3a). However, no further significant increase in the final reducing sugar concentrations when the substrate concentrations were higher than 200 $g \cdot L^{-1}$ when the lower amount of glucoamylase used were used (0.5 and 1 %) and 250 $g \cdot L^{-1}$ when a higher amount of glucoamylase was used (1.5 %). These conditions suggest the existence of a critical substrate concentration in the cassava starch hydrolysis using glucoamylase. The use of higher value of initial substrate concentrations may lead to the inhibition of enzyme activity. The long term accumulation of endogeneous reducing sugar and the high substrate concentration may concurently inhibit the enzyme capacity, therefore, the concentration of substrate was seriously restrained. Zhang *et al.* [24] concluded that the critical substrate concentration to ethanol was found 160 $g \cdot L^{-1}$, the high concentration of substrate (glucose) decreased the ethanol yield. Moreover, Akhtar *et al*. [25] reported that pre-treatment of wheat straw, rice straw and bagasse using 2 % NaOH was most effective for increasing the enzymatic saccharification yield by *Baccilus subtilis cellulases.*

In contrast to glucoamylase, hydrolysis of cassava starch catalyzed by Stargen^{TM} 002 cannot be categorized as substrate inhibition. This is because an increase in the initial starch concentration always results in an increase in the reducing sugar concentration, although the increment may be low (see Figure 3b). Clasification of competitive inhibition by Stargen^{TM} 002 was further investigated by a Lineweaver-Burk plot by which the two kinetic parameters, as Michaelis-Menten constant, K_m and maximum velocity, V_{max} can be obtained.



(a)



(b)

***Figure 3**. Effect of substrate (starch) concentrations on reducing sugar production, at 40 °C, 12 h and pH 4, by : **(a)** glucoamylase and **(b)** Stargen^{TM} 002*

Determination kinetic parameters

The effect of initial starch concentrations (25 to 300 g·L^{-1}) and the use of glucoamylase and Stargen^{TM} 002 with concentrations (0.5 to 1.5 %, w/w) during hydrolysis of cassava starch at *p*H 4 and 30 °C on initial velocities of enzyme were investigated and the

results are shown in Figure 4 as the relationship between initial concentration of cassava starch, S_o and initial velocity of enzyme, v_o.

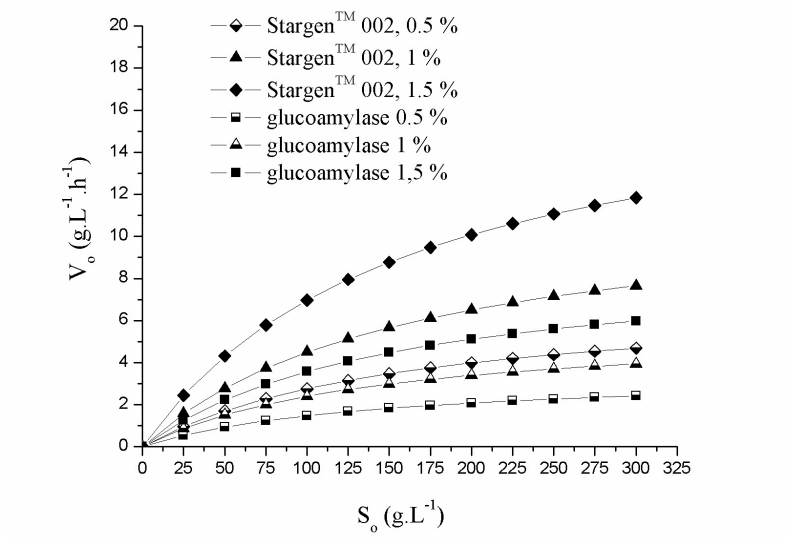
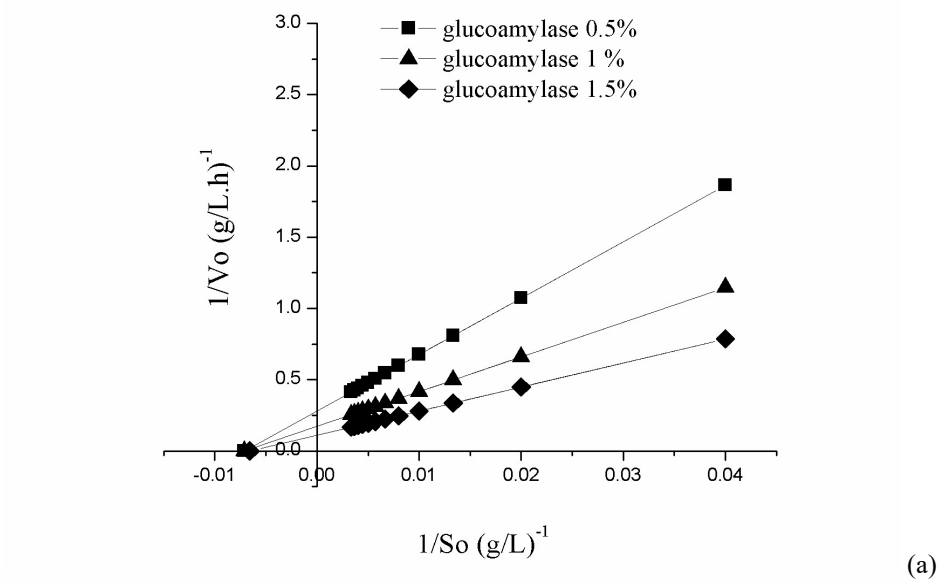


***Figure 4**. Effect of initial cassava starch at concentrations (25-300 g·L^{-1}) with added glucoamylase and Stargen™ 002 at concentrations of 0.5-1.5 % on the initial reaction velocity, at pH 4 and 40 °C*

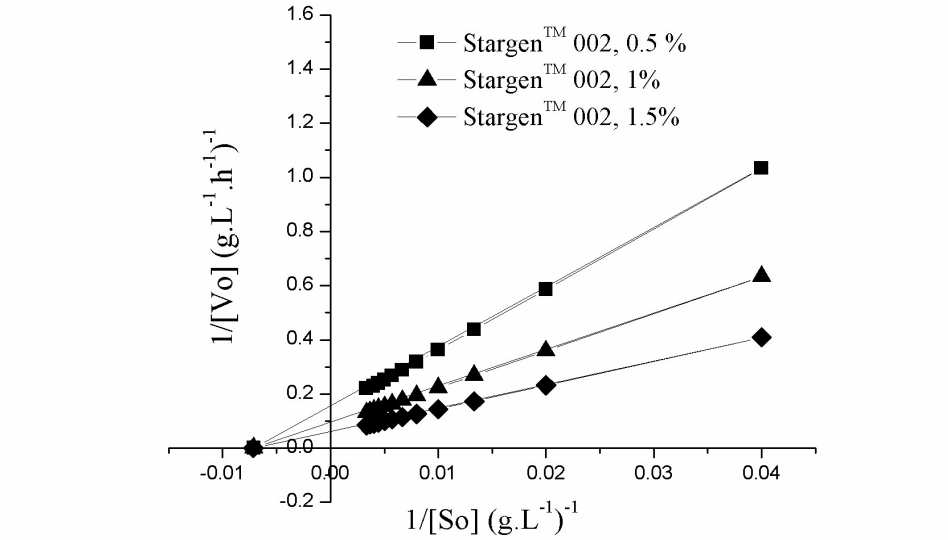
The first region of initial cassava starch at concentration (0-100 g·L^{-1}) was characterized by a linear increase of v_o with enzyme loading, and the extend of this region was dependent on enzyme concentration. Following the first region was a region with almost constant initial reaction rate. In this region, the raw cassava starch particles were saturated with adsorbed enzyme, and therefore any additional of substrate concentration did not result in any increase in v_o. This behaviour is similar to a homogeneous reaction system using soluble starch, where the Michaelis-Menten model would predict that the initial reaction rate is first order with respect to enzyme concentration and zero order with respect to substrate concentration [19]. The enzyme first combines reversibly with the substrate to form an enzyme-substrate (ES) complex in a relatively fast reversible step. The ES complex will then break down in a slower second step to yield the free enzyme and the reaction products. The effect of enzyme types, i.e. glucoamylase and Stargen™ 002 was investigated to determine for kinetic parameters, as Michaelis-Menten constant, K_m and maximum velocity,V_{max}. Several sets of experiments were conducted at various enzyme concentrations to obtain the kinetics data of glucoamylase and Stargen™ 002 catalyzed cassava starch hydrolysis. The glucoamylase and Stargen™ 002 enzymes were used to catalyze the hydrolysis of cassava slurry at 40 °C and *p*H 4 at concentrations of 0.5, 1 and 1.5 % (w/w). The result for kinetic studies are illustrated as a Lineweaver-Burk plot shown in Figure 5a and 5b.

As shown in Figure 5a and Figure 5b, when the reciprocal of the initial reaction rate of the enzyme, ($1/v_o$) was plotted against the reciprocal of initial substrate concentration, ($1/S_o$), the intercepts at the y+axis ($1/V_{max}$) and the intercepts at x-axis ($1/K_m$) were obtained. The maximum velocity and the Michaelis-Menten constants obtained in the hydrolysis of cassava starch using glucoamylase and Stargen™ 002 are tabulated in Table 1. The value of K_m can be used as an indicator of the affinity of an enzyme to the

substrates [18]. In addition, when the reciprocal of the initial reaction rates of cassava starch hydrolysis using glucoamylase at concentrations of 0.5 and 1.0 % (w/w) was plotted against the reciprocal of initial substrate concentrations, a noncompetitive substrate inhibition was noticed (Figure 5a). Similarly, the same plot of cassava starch hydrolysis by Stargen^{TM} 002 with concentrations ranging from 0.5 to 1.5 % (w/w), also reveals that the x-intercepts of the 3 double-reciprocal plots are coincidentaly at single point (Figure 5b).



(a)



(b)

***Figure 5**. Lineweaver-Burk plots for hydrolysis of cassava starch, at pH 4 and 40 °C with concentration of : **a)** glucoamylase : 0.5, 1.0 and 1.5 % (w/w), **(b)** Stargen^{TM} 002 : 0.5, 1.0 and 1.5 % (w/w)*

Hargono *et al*. [15] investigated the inhibition of sweet cassava starch, bitter cassava and *gadung* (*Dioscoreae hispida* dennst) flours at concentration of Stargen^{TM} 002 of 1.5 % (w/w), *p*H 4 and 30 °C. The Michaelis-Menten constant obtained for these three

substrates were 139.84, 141.43 and 140.93 $g \cdot L^{-1}$, respectively. Accordingly, the values of V_{max} obtained were respectively 13.70, 22.73 and 34.48 $g \cdot L^{-1} \cdot h^{-1}$, and can be classified as noncompetitive inhibition.

Table 1. *Comparison of the maximum velocity (V_{max}) and the Michaelis-Menten constants (K_m) obtained in the hydrolysis of cassava starch using glucoamylase and Stargen™ 002, at pH 4 and 40 °C*

Substrate	K_m [$g \cdot L^{-1}$]	V_{max} [$g \cdot L^{-1} \cdot h^{-1}$]
Cassava starch with added 0.5 % (w/w) of glucoamylase	140.35	3.55
Cassava starch with added 1.0% (w/w) of glucoamylase	140.75	5.78
Cassava starch with added 1.5% (w/w) of glucoamylase	152.10	9.00
Cassava starch with added 0.5% (w/w) of Stargen™ 002	160.86	7.19
Cassava starch with added 1% (w/w) of Stargen™ 002	161.31	11.76
Cassava starch with added 1.5% (w/w) of Stargen™ 002	160.85	18.18

CONCLUSIONS

Based on the experimental results, it can be concluded that cassava starch hydrolysis rate and the reducing sugar concentration increased proportionally with the amount of glucoamylase and Stargen™ 002 used. As for hydrolysis using glucoamylase, the reducing sugar concentration increased as the reaction temperature increased from 30-60 °C. This trend is likely to continue even when the reaction temperatures above 60 °C (not studied). However, when hydrolysis is catalyzed by Stargen™ 002 this similar phenomenon levelled of at 40 °C.

The increase of substrate concentrations from 100 to 250 $g \cdot L^{-1}$ also increases the concentration of reducing sugar. However, no significant increase in the final reducing sugar concentration when the substrate concentration was higher than 200 $g \cdot L^{-1}$ (glucoamylase 0.5 and 1 %) and 250 $g \cdot L^{-1}$ (glucoamylase 1.5 %). This indicates the highest substrate concentration that could be adopted in reducing sugar because the enzyme would be inhibited by substrate (substrate inhibition). In contrast to glucoamylase, hydrolysis by Stargen™ 002 can not be categorized as substrate inhibition because any increases in Stargen™ 002 concentration are still followed by slight increases in reducing sugar. It is also shown by the higher values of K_m and V_{max} of Stargen™ 002 compared to glucoamylase, which indicates faster starch hydrolysis rate by Stargen™ 002 than glucoamylase.

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