Kinetics of medium-temperature $\alpha$-amylase hydrolyzed $Huai$ yam powder

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ABSTRACT

In order to learn the enzymatic characteristics of Huai yam powder with medium-temperature $\alpha$-amylase, effects of substrate concentration, enzyme concentration, pH and temperature were investigated. The Michealis-Menten equation was used to fit the kinetics of the hydrolysis reaction. Experimental results indicate that maximum rate ($V_m$) is 3.1588 mg/mL·min under the condition of 70$^\circ$C, pH 7.0 and 0.0200 mg/mL of enzyme concentration. The Michealis constant ($K_m$) is 6.6641 mg/mL. The kinetic model, including the factors such as substrate concentration, enzyme concentration and temperature, was established for the hydrolysis reaction under the temperature range from 40$^\circ$C - 70$^\circ$C.

Keywords: Huai Yam; Medium-Temperature $\alpha$-Amylase; Hydrolysis; Kinetics

1. INTRODUCTION

Yam is the tuber of dioscoreaceae plant widely planted in China, and is very rich in resources. Jiaozuo city, Henan province, is the traditional genuine producing area of yam, commonly called Huai yam, and Huai Yam is also one of four famous Huai drugs. Content of amylum in fresh yam is up to 20% - 30% [1-3] and more than 70% in dried yam [4,5]. At present, studies on amylum are mainly focused on corn and wheat [6,7], and few research pares are involved in the yam, especially Huai yam. Medium temperature $\alpha$-amylase can hydrolyze amylum with high efficiency. Hydrolysis products include dextrin, oligosaccharide, maltose and glucose with the advantages of mild reaction conditions, cost-effective and just simple equipment involved [8-10]. In this work, parameters affecting the reaction rate of medium-temperature $\alpha$-amylase were systematically investigated. The hydrolysis kinetic model of medium-temperature $\alpha$-amylase was deduced, which provides theoretical reference for the hydrolysis and application of Huai yam.

2. MATERIALS AND INSTRUMENTAL

2.1. Materials

Huai yam (Yuecun Countryside, Wen County, Jiaozuo City, China), medium-temperature $\alpha$-amylase (2000 U/g, Fuyuan Biology Technology Co.,Ltd., Zhengzhou, China), glucose (Sinopharm. Chemical Reagent Co.,Ltd, Shanghai, China) were used as received. Other chemicals are all analytical grade. Deionized water was used throughout experiments.

2.2. Instrumental

pH values were measured by a pH meter (Leici Instrument Co., Ltd., Shanghai, China). The UV-Vis spectra were performed on a UV-1900 spectrophotometer (Purkinje General Instrument Co., Ltd., China).

2.3. Hydrolysis

An appropriate amount of Huai yam powder was weighed and to prepare 200 mL slurry. After adjusting pH, medium-temperature $\alpha$-amylase was added to hydrolyze Huai yam.

2.4. Sample Preparation

An amount of Huai yam were dried and ground to 100 mesh. After hydrolysis for a certain time, the medium-temperature $\alpha$-amylase was inactive. After centrifuging, the supertant was diluted to 250 mL. Content of gross sugar was measured by phenol-sulfuric acid method [11].

3. RESULTS AND DISCUSSION

3.1. Effect of Substrate Concentration on the Reaction Rate

In order to investigate the effect of substrate concentration on the reaction rate, a series of 200 mL of substrate aqueous solutions (2.5, 5, 7.5, 10, 12.5, 15, 20
mg/mL) were hydrolyzed by 0.2 mg/mL at pH 7 and 70°C. The results are shown in Figure 1.

Obvious, reaction rate and substrate concentration show typical hyperbolic relationship. Under lower levels, substrate concentration and reaction rate is linear suggesting first order reaction. Further increase the concentration of substrate, reaction rate reaches maximum, showing zero order reaction. Therefore, the curve of substrate concentration versus reaction rate shows a hyperbola, the typical characteristics of enzymatic reaction [12], suggesting the hydrolysis following the Michaelis-Menten equation.

According to Michaelis-Menten equation, enzymatic reaction can be divided in two steps:

\[ E + S \rightleftharpoons ES \rightleftharpoons E + P \]  

where \( E \) and \( S \) are the gross concentration of enzyme and substrate, respectively. \( k_1, k_2, k_3, k_4 \) are forward and reverse rate constants, respectively.

At the beginning, the rate of \( E + P \rightarrow ES \) is very low and can be neglected due to low level of products. The concentration of intermediate is also much lower than the concentration of substrate so \([S] >> [E]\) and thus can be omitted. Therefore, the reaction rate can be described as follows:

\[ v = \frac{k_3 [ES]}{K_m + [S]} \]  

from Eqs. 2 and 3, it can be deduced:

\[ v = \frac{V_m [S]}{K_m + [S]} \]  

where \( v, V_m \) and reaction rate, maximum reaction rate for totally saturated enzyme and Michaelis-Menten equation constant.

The value of \( K_m \) indicates the substrate concentration when the reaction rate reaches half of the maximum rate. Value of \( V_m \) indicates the maximum rate when the enzyme is saturated by substrate within a certain concentration range. Both \( K_m \) and \( V_m \) are important kinetic parameters of enzyme reaction.

3.2. Lineweaver-Burk Double Reciprocal Plot Method for Solving the Michaelis Constant and the Maximum Reaction Rate [12]

The following equation can be obtained by taking both sides of the Eq.2 to double reciprocal (Figure 2):

\[ \frac{1}{v} = \frac{K_m}{V_m} \cdot \frac{1}{[S]} + \frac{1}{V_m} \]  

After plotting \( 1/v \) versus \( 1/[S] \) and using least square linear method, the maximum reaction rate and \( K_m \) can be calculated: \( V_m = 3.5562 \text{ mg/(mL·min)}, K_m = 8.7219 \text{ mg/mL}, \text{i.e.}, \) Michaelis equation can be expressed:

\[ v = \frac{3.5562 [S]}{8.7219 + [S]} \]  

correlation coefficient is 0.9986 indicating good linearity.

Figure 1. Relationship between reaction rate and substrate concentration.
3.3. Wilkinson Statistical Method for Solving the Michaelis Constant and the Maximum Reaction Rate [13]

Wilkinson statistical method includes two steps: one is estimation of resolutions with nonlinear square method; another is to obtain exact resolutions with Taylor expansion.

a) Estimation: from Table 1, it can be obtained:

\[ \Delta = \alpha \varepsilon - \gamma \delta = 4.3131 \]

\[ V^0_m = \frac{\beta \varepsilon - \delta^2}{\Delta} = 3.1724 \text{ (mg/mL·min)} \]

\[ K^0_m = \frac{\beta \gamma - \alpha \delta}{\Delta} = 6.6641 \text{ (mg/mL)} \]

where \( V^0_m \) and \( K^0_m \) are the estimation value of maximum reaction rate and Michaelis constant, respectively.

b) Precision resolution: from Table 2, it can be deduced:

\[ \Delta' = \alpha' \beta' - \gamma' \delta' = 2.3216 \times 10^3 \]

Figure 2. Lineweaver-Burk plot of medium-temperature \( \alpha \)-amylase hydrolyzing Huai yam.

Table 1. Estimated resolutions by Wilkinson method.

<table>
<thead>
<tr>
<th>No.</th>
<th><a href="mg/mL">S</a></th>
<th>( \nu ) (mg/mL·min)</th>
<th>( \nu^2 )</th>
<th>( 1/\nu )</th>
<th>( \theta )</th>
<th>( \theta^2 )</th>
<th>( \nu \theta )</th>
<th>( \theta^2 )</th>
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<td>1</td>
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<td>0.7825</td>
<td>0.6123</td>
<td>0.2449</td>
<td>0.4791</td>
<td>0.3749</td>
<td>0.1916</td>
<td>0.1500</td>
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<tr>
<td>2</td>
<td>5</td>
<td>1.3363</td>
<td>1.7857</td>
<td>0.3571</td>
<td>2.3862</td>
<td>3.1887</td>
<td>0.4772</td>
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<tr>
<td>3</td>
<td>7.5</td>
<td>1.6884</td>
<td>2.8507</td>
<td>0.3801</td>
<td>4.8131</td>
<td>8.1265</td>
<td>0.6418</td>
<td>1.0836</td>
</tr>
<tr>
<td>4</td>
<td>10</td>
<td>1.9195</td>
<td>3.6845</td>
<td>0.3685</td>
<td>7.0724</td>
<td>13.5755</td>
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<td>1.3578</td>
</tr>
<tr>
<td>5</td>
<td>12.5</td>
<td>2.1762</td>
<td>4.7358</td>
<td>0.3789</td>
<td>10.3060</td>
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<td>11.4957</td>
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<td>4.1362</td>
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<tr>
<th>Symbol</th>
<th>( \alpha )</th>
<th>( \beta )</th>
<th>( \gamma )</th>
<th>( \delta )</th>
<th>( \varepsilon )</th>
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Table 2. Accurate resolutions by Wilkinson method.

<table>
<thead>
<tr>
<th>No.</th>
<th>[S] + ( K^0_m )</th>
<th>( V^0_m ) [S]</th>
<th>( f )</th>
<th>( f' )</th>
<th>( f^2 )</th>
<th>( f^2 )</th>
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<td>7.931</td>
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<td>0.7489</td>
<td>62.9008</td>
<td>-0.0817</td>
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<tr>
<td>2</td>
<td>11.6641</td>
<td>15.862</td>
<td>1.3599</td>
<td>-0.1166</td>
<td>1.8493</td>
<td>251.603</td>
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<tr>
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<td>23.793</td>
<td>1.6798</td>
<td>-0.1186</td>
<td>2.8217</td>
<td>566.1068</td>
<td>-0.1992</td>
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<td>1006.4122</td>
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</tr>
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</table>

Note: \( f = V^0_m [S] + K^0_m \); \( f' = -V^0_m [S] + K^0_m \).
where $b_1$ and $b_2$ are the rectified constants of $V_m$ and $K_m$, respectively, during the calculation process, $V_m$ is the precision resolution of maximum reaction rate, $K_m$ is the precision resolution of Michaelis constant.

### 3.4. Comparison of Lineweaver-Burk Double Reciprocal Plot and Wilkinson Statistical Methods

There exists differences in the values of $V_m$ and $K_m$ between Lineweaver-Burk double reciprocal plot and Wilkinson statistical methods (Table 3). For the former, the experimental data excessively focus under the left of the line. Data of lower substrate concentration have larger errors after reciprocal and thus resulting in larger error. Although Wilkinson statistical method is closer to the actual results, but complex calculation, tedious process, limit its application [14-16].

According to results from experiments, Wilkinson method is adopted to calculate $V_m$ and $K_m$ ($V_m = 3.1588$ mg/mL·min, $K_m = 6.6641$ mg/mL).

### 3.5. Effect of Enzyme Concentration on Reaction Rate

A series of enzyme solutions (0.050, 0.100, 0.150, 0.200, 0.250 mg/mL) were used to hydrolyze abundant substrate at pH 7 and 70°C. The results are shown in Figure 3. Obviously, the concentration of enzyme is linear to the reaction rate. In enzyme reaction, enzyme interacts with substrate to form intermediate complex, which converts to products and releases enzyme. Under a certain system, if the substrate is fully excessive, enzyme totally binds with substrate. The more enzyme molecules, the more resulting products, the more the reaction rate is faster. When $[S]$ is more than $[E]$, Eq. 3 can be expressed as [17]:

$$v = K[E]$$ (7)

Therefore, when the substrate concentration is excessive, reaction rate is linear to the concentration of enzyme, which is consistent with the experimental results.

### 3.6. Effect of pH on the Efficiency of Hydrolysis

The effect of pH was investigated with 10 mg/mL of substrate and 0.200 mg/mL of enzyme under 70°C with pH values ranged from 5 to 9. The results are shown in Figure 4.

As shown in Figure 4, the reaction rate reaches maximum due to extreme acid or base conditions disrupt the conformation of enzyme, which would result in the loss of enzyme, even totally inactivate the enzyme and thus decreases the reaction rate. If pH values do not vary significantly, the dissociative state of substrate is affected by the pH values; enzyme can not bind with substrate or

| Table 3. Comparison of Lineweaver-Burk and Wilkinson methods. |
|---------------------|---------------------|
| Method              | $V_m$/mg/(mL•min)   | $K_m$/mg/mL        |
| Lineweaver-Burk     | 3.5562              | 8.7219             |
| Wilkinson           | 3.1588              | 6.6641             |

![Figure 3. Relationship between α-amylase concentration and reaction rate.](image_url)
Figure 4. Effects of pH on the hydrolysis of Huai yam.

Figure 6 shows the relationship between lnν and 1/T × 10^3 when the values of 1/T × 10^3 range from 2.9 to 3.2, indicating that the hydrolysis rate and temperature follow the Arrhenius equation:

\[ \ln k_2 = -\frac{E_a}{R} \frac{1}{T} + \ln A \]  

where \( A, R \) and \( E_a \) are pre-exponential factor, gas constant (J/mol-K) and activation energy (kJ/mol), respectively.

Values of \( E_a \) and \( A \) are obtained by linear regression equation. It is found that \( E_a = 14.774 \) kJ/mol, \( A = 454 \) mg/mL·min and the correlation coefficient is 0.9907, suggesting that the experimental data is good agreement with Arrhenius equation.

If temperature does not vary significantly, the equilibrium constants and temperature \((T)\) follow Van’t Hoff equation:

\[ \ln K_s = -\frac{\Delta H}{RT} + \ln C \]  

where \( K_s, \Delta H, C \) and \( R \) are equilibrium constant, enthalpy, frequency factor and gas constant, respectively. In enzymatic reaction, \( k^2 >> k^{-1}, K_s \approx K_m \), a linear curve (Figure 7) can be obtained by fitting \( \ln K_m \) versus 1/T × 10^3.

From linear regression equation, it is found that \( \Delta H = 14.641 \) kJ/mol, \( C = 1046 \) and correlation coefficient, \( r \), is 0.9856. Therefore, the kinetic equation of medium-temperature \( \alpha \)-amylase hydrolyzing Huai yam can be expressed as:

\[ \nu = \frac{k_2[E][S]}{K_m + [S]} = \frac{A \cdot \exp(-E_a / RT)}{C \cdot \exp(-\Delta H / RT) + [S]} \cdot [E][S] \]

This equation is suitable in the temperature range of 313.15 to 343.15 K (40°C to 70°C).

**4. CONCLUSIONS**

Because starch includes amylose and amylopectin with different molecular weight sizes, and the hydrolysis products by the medium-temperature \( \alpha \)-amylase include dextrin, oligosaccharides, glucose and maltose [12]. This work adopts phenol-sulfuric acid method for the determination of total sugars after hydrolysis and investigates the parameters affecting reaction rate. It is found that \( K_m \) is 6.6641 mg/mL and maximum reaction rate, \( V_m \), is 3.1588 mg/mL·min. The kinetic model of enzymatic hydrolysis...
Figure 5. Effects of temperature on the hydrolysis of Huai yam.

Figure 6. Relationship between lnν and 1/T × 10³.

Figure 7. Relationship between lnKm and 1/T × 10³.
including the concentrations of enzyme and substrate and temperature is established:

\[ v = \frac{454 \cdot \exp(-14774 / RT)}{1046 \exp(-14641 / RT)} + [S] \cdot [E][S] \]

The suitable temperature for this model ranges from 40°C to 70°C. Every parameter is fitted with high significance suggesting that it is effective with Michaelis-Menten equation simulating the kinetic process of medium-temperature α-amylase hydrolyzing Huai yam.

REFERENCES


