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Enzymatic Kinetics of Enzymatically Extruded Degerminated Maize Using Glucamylase

Chengye Ma[1, 2], Shuangshuang Yu[1], Yuyan Fan[1], Shuhua Wu[1], Zhehao Zhang[1], Yuedong Song[1] & Hongjun Li[1, 2]

[1]School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo, 255000, China

[2]Key Laboratory of Shandong Provincial Universities for Technologies in Functional Agricultural Products, Zibo, 255000, China

Correspondence: Chengye Ma, School of Agricultural Engineering and Food Science, Shandong University of Technology, Zibo, China. E-mail: mcycn2002@163.com

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Abstract

In this study, the reaction rates of native degerminated maize, extruded degerminated maize and enzymatically extruded degerminated maize using glucoamylase were evaluated and the extrudate models were investigated. The effects of enzyme concentration, substrate pH, temperature and incubation time on the reaction rates were studied. The Lineweaver–Burk equation was used in order to obtain the parameters of the kinetics equation of catalysed hydrolysis. The results show that NDM's vm is 0.0845g/(mL·min) and km is 0.0032, EDM's vm is 0.6251g/(mL·min) and km is 0.0167, EEDM's vm is 1.897g/(mL·min) and km is 0.0240. The reaction rate of EEDM is quicker than those of NDM and EDM. The kinetics equation of EEDM is in accordance with the Michaelis–Menten equation.

Keywords: extrusion, degerminated maize, glucoamylase, enzymatic kinetics

1. Introduction

Maize is a major grain crop rich in linoleic acid, minerals and vitamins, and has a high nutritional and medicinal value. Maize has been used in many processing sectors including food processing, feed processing and deep processing, and starch syrup is a very important component of deep processing. Starch produced using the wet method of degerminated maize compared with using the dry method has many advantages, including reducing the equipment investment cost, shortening the process flow, and reducing sewage discharge and energy consumption. The combined application of extrusion and enzymatic technology is an effective method of biological and mechanical degradation, accelerating the rate of hydrolysis of amylase and improving the utilization rate of starch (Shen et al. 2010).

Enzymatic kinetics can be described as a science based on enzyme catalysis and the factors which affect it (Román et al. 2016). The parameters and equation of enzymatics kinetics can be obtained by analysing different actors such as substrate concentrations, and varying the pH, temperature and reaction time (Baks et al. 2008; Raphaelides et al. 2012). Literature regarding enzymatic kinetics has been reported (Stephen and Wang 2009) and an enzymatic kinetics model has been built by analysing the influence of the above-mentioned parameters(Gao et al. 2017). The parameters and equation of enzymatic kinetics of maize starch have been obtained by analysing the effects of different concentrations of enzymes, and varying the pH, temperature and time ofthereaction rate of β-amylase (Zhao et al. 2009).

Extrusion technology is a process involving transporting, mixing, smashing, shearing and pumping, which has the advantages of energy saving and enabling high quality and yield (Morales et al. 2015). Enzyme technology combined with extrusion activation is an effective method of the biological and mechanical degradation of starch, accelerating the reaction rate of amylase and increasing the ratio of starch. During the extrusion process, the effect of moisture, heat and mechanical shear ruptures hydrogen bonds, the crystalline structure and starch grains; hence, the enzymatic kinetics equation of extrudates needs to be investigated.

The aim of this study was to compare the reaction rate of enzymatically extruded degerminated maize with degerminated maize and extruded degerminated maize, and to build an enzymatic kinetics model using the

Lineweaver–Burk method, as the rules and a model of enzymatic kinetics can provide support for the production of starch syrup.

2. Material and Methods

2.1Materials

The raw material used in this study was degerminated maize with a 12.58% moisture content, 74.46% starch content, 7.96% protein content and 0.96% fat content, which was purchased from Tianjin Food Co., LTD. Glucoamylase, 20000u/g, was purchased from Beijing AoBoXing Biological Technology Co., LTD. Thermostable α-amylase, 40000u/mL, was purchased from Shandong LongDa Biological Engineering co., Ltd. All other chemicals used were of analytical grade.

2.2 Instruments and Equipment

Figure 1 is the single-screw extruder .It was made by Shandong University of Technology, which consisted of a modular barrel (three pieces) and screw (four pieces), with a productivity of 100 kg/h. The screw rotation speed varies from 0 to 1200 rpm, the barrel is continuously adjustable at a temperature range between 0~300℃and the extruder is equipped with an automatically controlled closed-loop digital instrumentation system. The die diameter of the extruder and clearance between the templates and screw top are adjustable.

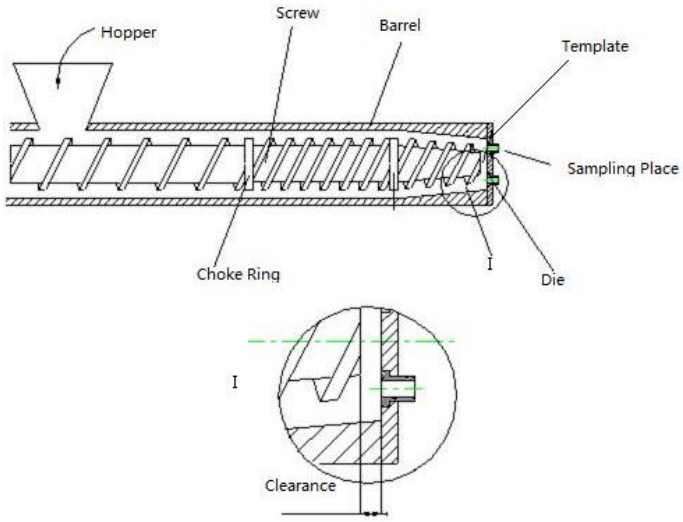

Figure 1. Schematic diagram of single-screw extruder

A UV-2102PCS ultraviolet and visible spectrophotometer (Ke Instrument Co., LTD, Shanghai, China) was used for analysis.

2.3 Preparation of Enzymatically Extruded Degerminated Maize

Degerminated maize with a moisture content was 30.0% was ground to flour and thermos tableα-amylase was added. The system parameters of the single-screw extruder are shown in Table 1.

2.4 Preparation of Liquid Glucose and Determination of Reducing Sugar Content

6.00g samples were added to 38.0mL acetic acid-sodium acetate buffer in tubes. After 10 minutes in a water bath at 55 ℃,a specific amount of glucoamylase was added and the solution was left in the water bath at 55℃ for30min. The reaction was stopped by increasing the water bath temperature to 100℃ for 10min. Finally, the solution was centrifuged at 4000rpm for 20min to separate the liquid supernatant from the reaction slurry.

The consumption volume of the sugar solution was measured and the reducing sugar concentration was determined on the basis of direct titration of GB/T5009.7-2008(Chinese national standards).

Table 1. System parameters of the single extruder

No.		Diameter of die nozzle/amount mm	Temperature at end of discharge ℃	Speed of screw r/min	Moisture content %	Thermostable α-amylase contentu/g	Remarks
1	Native degerminated maize	/	/	/	/	/	NDM
2	Extruded degerminated maize	φ12×3	60.0	110.0	30.0	0	EDM
3	Enzymatically extruded degerminated maize	φ12×3	60.0	110.0	30.0	10.0	EEDM

2.5 The Measurement of the Kinetics

Constant Enzymatic Kinetics was Analysed Using the Michaelis–MentenEquation and the Parameters of Kinetics were Obtained Using Double-Reciprocal Analysis(BP et al. 2006). Different concentrations of the three samples were taken and the reaction rates were measured. The Michaelis–Menten equation is shown below.

$$v = \frac{v_{\max}[s]}{k_m + [s]}$$

The reciprocal of the Michaelis–Menten equation is:

$$\frac{1}{v} = \frac{k_m + k_m[s]}{v_{\max}[s]}$$

The reaction rate was calculated and the reciprocal of the Michaelis–Menten equation was obtained.

2.6 Statistical Analysis All Experiments were Performedin Triplicate and Data are Expressed as Means

Statistical analysis was performed using SAS9.1, and comparisons between the reaction rate and time were performed using ANOVA; statistical significance was considered as $p<0.05$. The figures were processed by origin8.0 and Vmax and Km were calculated according to the Lineweaver–Burk plot.

3. Results and Discussion

3.1 The Solution of the Michaelis Constant and the Maximal Reaction Rate

The Lineweaver–Burk equation was used to determine the kinetic parameters and the plots obtained are presented in Fig. 2; the least square method was used to perform linear fitting(Jukić et al. 2007). The results show a linear relationship between 1/S and 1/V in the control, and the correlation coefficients of equations were above 0.9500 and equations were highly significant.

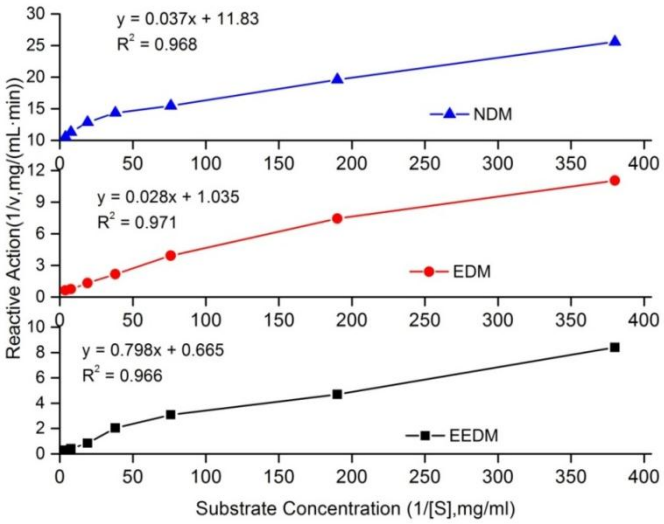

Figure 2. Lineweaver–Burk plot for the glucoamylase catalysed hydrolysis of enzymatically extruded degermed maize

The equation, coefficient of association, Vmax and Km are shown in Table 2. It can be seen that R2 is above 0.96 and there action rules of all three materials follow the Lineweaver–Burk equation and exhibited good correlation. A higher Km value indicates higher affinity and according to the value of Km, EEDM had the strongest affinity with glucoamylase and NDM had a lower affinity than the two other materials used. Enzymatic extrusion can make degerminated maize gelatinized and decrease the degree of polymerization; however, the contact area of degerminated maize and glucoamylase was increased. The advantages of using the Lineweaver–Burk graph method is that it is convenient and fast (Baks et al. 2006b), and the results are accurate. This method is governed by substrate concentration as a low concentration of substrate resulted in a low enzymatic hydrolysis rate and influenced the accurate measurements of Vmax and Km. Generally, the result was accurate when the substrate concentration was 0 to 10 mg/mL or from 0.33 to 2.0 Km (Zhang et al. 2007).

Table2.Kinetics Equation of Catalysed Hydrolysis Using the Lineweaver–Burk Plot

No.	Samples	Equations	R^2	V_{max} mg/(mL·min)	K_m (mg/mL)
1	Degerminated maize	y_1=0.0327x_1+10.4530	0.9680	0.0845	0.0032
2	Extruded degerminated maize	y_2=0.0202x_2+0.8406	0.9768	0.6251	0.0167
3	Enzymaticallyextruded degerminated maize	y_3=0.7980x_3+0.7750	0.9660	1.1897	0.0240

3.2 Effect of the Enzyme Concentration of Feed Materials on Reaction Rate

As shown in Fig. 3, the reaction rates increased with an increase in enzyme concentration. Under the conditions of sufficient substrate, higher enzyme concentrations exhibited faster reaction rates, resulting in more product. This was ascribed to the fact that the increased enzyme concentration provides more active sites, increasing the probability of an enzyme-substrate collision and subsequent reaction, leading to a higher reaction rate (Raphaelides et al. 2012).The reaction rate of NDM increased slowly upon increasing the enzyme concentration, while EEDM increased the quickest. Without squeezing, degerminated maize kept its original crystalline structure and was not easy to hydrolyse; however, extrusion gelatinized the degerminated maize and destroyed the crystalline texture, which led to an accelerated rate of hydrolysis (Zhang et al. 2015). On this basis, glucoamylase hydrolysed the α-1,4 glycosidic and α-1,6 glycosidic linkages, which led to maize producing glucose molecules exhibiting a low degree of polymerization; hence, the reaction rate of EEDM was the fastest (Han 2009). Under the conditions of unchanged temperature and pH and sufficient substrate concentration, the higher the enzyme concentration was, the faster the reaction rate (Zhao et al. 2009).

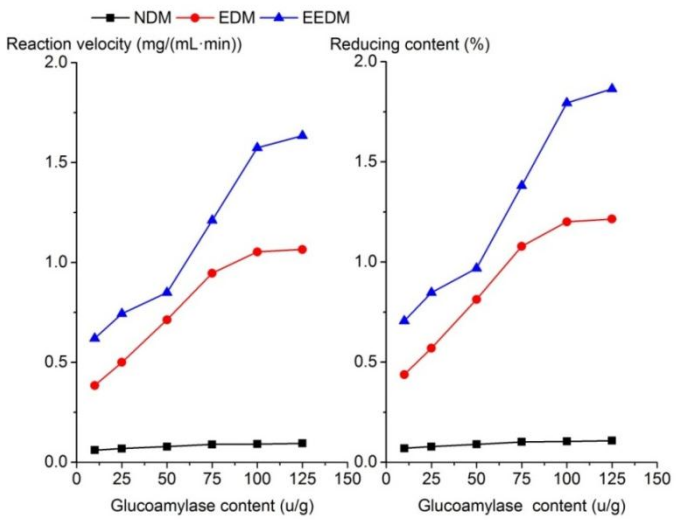

Figure 3. The effects of different substrate enzyme concentrations on reaction rates

3.3 Effect of Temperature on the Reaction Rate

As observed in Fig. 4, the optimum reaction temperature for NDM was 40℃. For the other two materials, the optimum reaction temperature was 50℃. Enzyme activity was enhanced upon increasing the temperature over a range of temperatures; however, when the reaction temperature increased up to 50℃, the reaction rate was lower, due to the fact that high temperatures can prevent gelatinization and lower enzyme activity. Studying the enzymatic kinetics of α-amylase during the saccharification process shows the degree of hydrolysis increased upon increasing the temperature, whereas the enzyme activity decreased; 60℃ was found to be a good temperature for both the degree of hydrolysis and enzyme activity (Baks et al. 2006a).

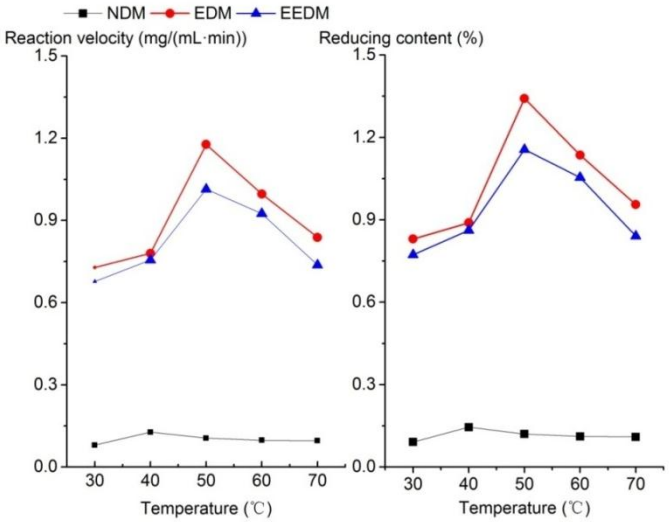

Figure 4. The effects of temperature on reaction rates

3.4 Effect of Reaction Time on Reaction Rate

Figure 5 shows the reaction rate over the reaction time. The initial hydrolysis reaction rate, at the same concentration levels, was highest in EEDM, followed by EDM, and lastly NDM. As the reaction process progressed, the reaction rate gradually slowed down, with the fifth reaction rate of three materials being the fastest. The reaction rate was slowed down by the decrease of raw material concentration and increasing time (Polakovič and Bryjak 2004).

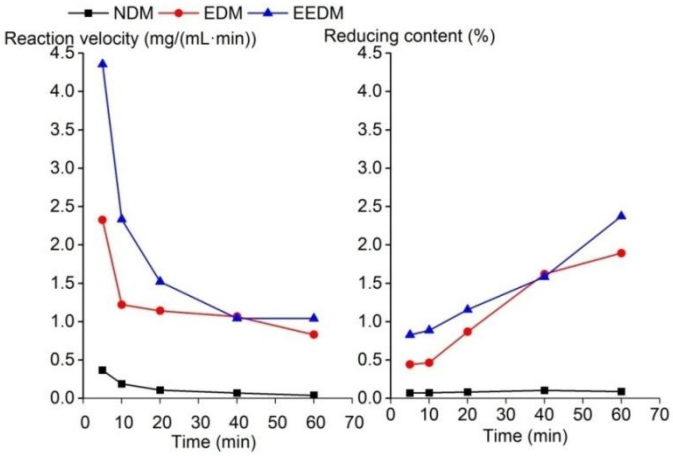

Figure 5. The effect of time on the reaction rate

4. Conclusion

These results show that the degree of hydrolysis and reaction rate of EEDM was higher than EDM and NDM. When the substrate pH was 5.0, the temperature was 50℃ and the reaction time was 5 minutes, the higher the concentration of substrate and enzyme present, the faster the reaction rate was. The Vmax and Km of NDM were 0.0845 and 0.0032, respectively; the Vmax and Km of EDM were 0.6251 and 0.0167, respectively and the Vmax and Km of EEDM were 1.1897 and 0.0240, respectively. Enzymatic kinetics of enzymatically extruded degerminated maize with glucamylase followed the basic rules of the Michaelis-Menten equation, which can be used to perform data fitting.

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