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Effect of Pretreatment Methods on Enzymatic Kinetics of Ungelatinized Cassava Flour Hydrolysis

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Abstract: The energy-saving glucose production process from starchy sources was developed by replacing high-temperature, liquid-phase by low-temperature, solid-phase. Therefore, the enzymatic hydrolysis under gelatinization temperature at very high gravity (≥300 g.L^{-1}) of starchy substrates presents as an emerging technology. This study focused on the hydrolysis kinetics of cassava flour affected by different pretreatment methods. Cassava flour (dried, milled) was prepared in acetate buffer (pH 4.2) with starch concentration ranging from 10–30% (*w*/*w*). The mash was then pre-treated by three different methods for 30 min using heating (30, 40, 50 °C), enzyme (Viscozyme L 0.1% *w*/*w*) and microwave (3 × 20 s at 800 W). The suspension was then hydrolyzed with Stargen 002 (0.2% *w*/*w*) at 30 °C for 48 h. The enzyme adsorption kinetics was described by the Langmuir isotherm equation. The pretreatments at 50 °C and with enzyme resulted in the highest efficiency with the hydrolysis yield ranging from 76–79% after 48 h. The hydrolysis yield decreased to 67% (using microwave), 66% (at 45 °C), 61% (at 40 °C) and 59% (at 30 °C). The linear relationship between enzyme adsorption and produced glucose was demonstrated. The kinetics of glucose production was fitted by an empirical equation (analogy with Michaelis-Menten model) and allowed predicting the maximum hydrolysis yield.

Keywords: cassava flour; enzyme adsorption; granular starch hydrolysis; pretreatment

1. Introduction

In an effort to combat climate change, aid energy independence, and to counteract diminishing supplies of fossil fuels, there has been a resurgence of research on renewable energy sources. Nowadays, bio-ethanol becomes one of the most common and important alternatives to replace fossil fuel resources. It is normally produced by the microbial conversion (common fermentation by yeast) of plant biomass (starchy or/and cellulosic raw materials) [1]. It is important to note that the first generation of biofuel (produced primarily from food crops such as grains, sugar beet, tubers and oilseeds) is still the most produced in the world, especially in tropical regions (e.g., Brazil, South East Asia) [1].

The conventional starch-based sugar production technology presents a high energy demand from fossil sources for two separated steps: liquefaction (95–105 °C) and saccharification (60–65 °C). In corn ethanol technologies, only 5–26% of the energy content is renewable, while the rest is primarily derived from natural gas and coal [2]. The costs due to the high energy demand of starch-based sugar production could be reduced if enzymatic hydrolysis of starch is performed at temperatures below the onset of gelatinization at, for example, 54 °C for wheat, 60 °C for potato, or 65 °C for maize [3]. Due to the insolubility of native starch in aqueous media at sub-gelatinization temperatures, the enzymes must attack the granules in the solid phase. Beside the effect of botanical origin of starch [4], several factors strongly influence the hydrolysis of native starch such as particle size, crystallinity, mass

transfer limitation, etc., thus the no-cook hydrolysis of starch presents hydrolysis rates lower than those observed for gelatinized starch. Several studies have attempted to increase the hydrolysis yield by adding preheating steps. Li et al. [5] studied the effect of adopting a preheating stage below the gelatinization temperature for corn and triticale hydrolysis. They showed that the pre-heating for 30 min at 51–60 °C enabled up to 80% efficiency of hydrolysis in shorter reaction time (48 h). Shariffa et al. [6] reported that a pre-heating step of the cassava and sweet potato starch granules at 60 °C for 30 min resulted in an increase up to 14% in the degree of hydrolysis, after 24 h in comparison with processes without pre-heating. Moreover, the hydrolysis of granular starch was used in ethanol production by simultaneous saccharification and fermentation process, and also some improvement for ethanol yield were investigated. Balcerek and Pielech-Przybylska [7] focused on the effect of thermal pre-hydrolysis of triticale meal using α-amylase and application of protease on the process of raw starch hydrolysis and fermentation. Better efficiency of fermentation was obtained without thermal activation but with an added proteolytic enzyme. Montalbo-Lomboy et al. [8] studied the effect of sonication of cornmeal slurry before direct conversion to ethanol. The results of this research proved that sonification of raw material improved the ethanol yield by 20% in comparison to that of the control sample. Moreover, the ethanol yield in sonicated samples was similar to jet-cooked cornmeal.

To develop practical approaches in optimizing the hydrolysis yield and energy consumption in starch amylolysis process, it requires an understanding of the native starch granule structural features and factors that impact the kinetics of amylolysis. Research has been undertaken on amylolysis of starches using α-amylase and γ-amylase after various pretreatments and addition of natural or synthetic additives. However, few publications have explained these impacts from an enzymatic kinetics standpoint and the kinetics of enzyme adsorption at high dry matter content is still lacking.

In developing countries, especially in Southeast Asia such as in Thailand and Vietnam, the cassava (*Mannihot esculenta* Crantz) is considered an interesting and suitable raw material for biorefinery industry, particularly for first bioethanol generation [9,10].

The objectives of this research were to; (i) investigate the effect of various pretreatment methods on amylolysis of granular starch from cassava flour; (ii) explain these impacts by studying the kinetics of enzyme adsorption onto starch granule; and (iii) model the kinetics of glucose production to predict the maximum yield which can be obtained for different hydrolysis conditions.

2. Results and Discussion

2.1. Impact of Pretreatment Method on Hydrolysis Yield

Three pretreatment methods (heating (30, 40, 50 °C), enzyme (Viscozyme L 0.1% *w*/*w*) and microwave (3 × 20 s at 800 W)) were applied for suspension of cassava flour during 30 min before hydrolysis (except microwave which pretreated flour directly). The hydrolysis yield was calculated based on the quantity of produced glucose. The evolutions of glucose, a function of hydrolysis time, were presented in Figure 1 for 10% *w*/*w* of initial starch concentration (i.e., 14.3% dry matter content). The glucose concentration increased during hydrolysis time as results of amylase attack. The pre-treatment at 50 °C gave the highest glucose concentration after 48 h hydrolysis (87 g.L^{-1}) whereas the lowest glucose concentration was obtained by pretreatment at 30 °C (66 g.L^{-1}). The order of hydrolysis yields decreased from: pretreated with 50 °C or Viscozyme L; microwave or 45 °C; 40 °C or 30 °C, respectively (Table 1). Evidently, treating the starch with different methods before enzyme hydrolysis enhanced significantly the degree of hydrolysis of starch. The enhancement of starch saccharification at an earlier stage (during the first 5 h) of the enzymatic reaction was less significant than at later stages. This observation opposed the results presented by Li et al. [11] who studied the impact of heat-treatment on hydrolysis of corn starch. They reported that the influence of pre-treatment was much more significant for the earlier stage (during first 4 h) than at later stages (beyond 20 h). Keeping the starch at 50 °C for 30 min could cause the irreversible swelling of granules (mostly in the amorphous region) and provide more access for the enzyme to attack starch granule.

Based on previous studies, the thermal pre-treatment also facilitated the enzymatic penetration into starch granules by increasing pinholes sizes and expanding internal cavities, which naturally presented in granules [6].

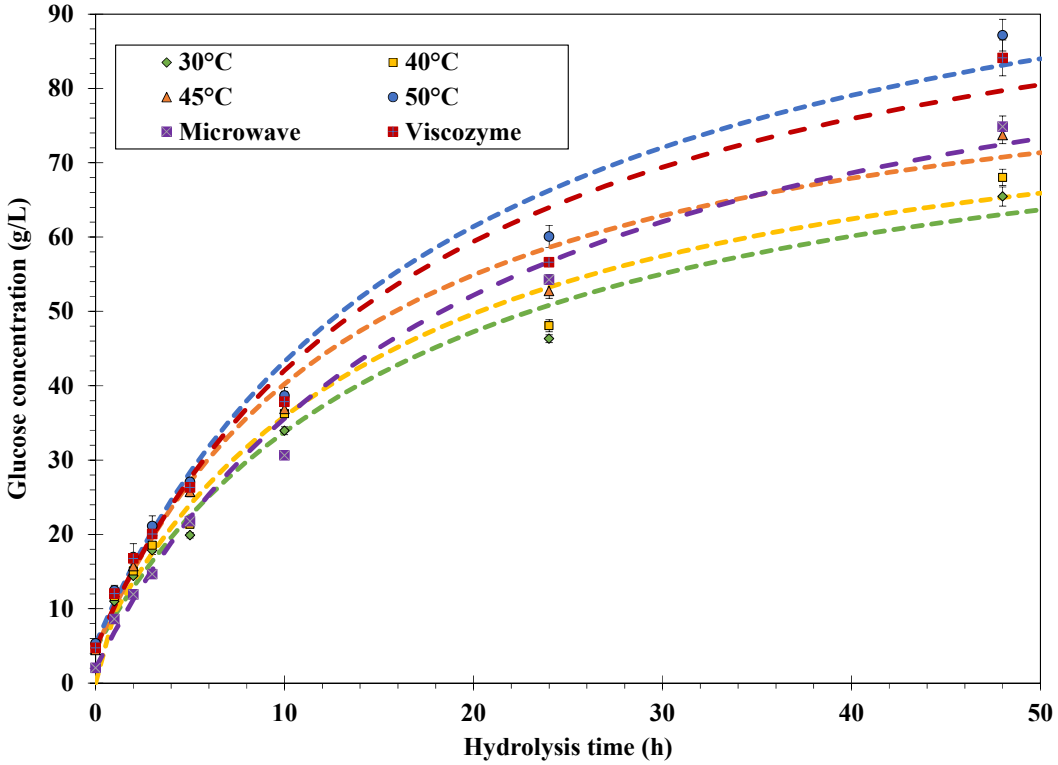

Figure 1. Evolution of produced glucose as a function of hydrolysis time (symbols were experimental data; dotted lines were obtained by regression analysis fitted with Equation (3), 10% *w*/*w* of starch i.e., 14.3% dry matter).

Table 1. Hydrolysis yield after 48 h of hydrolysis.

Pretreatment Method		30 °C	40 °C	45 °C	50 °C	Microwave	Viscozyme L
Yield (%)	10% starch	59.3 ± 1.2 ^{a}	61.6 ± 1.0 ^{a}	65.7 ± 1.0 ^{b}	79.0 ± 1.9 ^{c}	67.4 ± 1.3 ^{b}	76.1 ± 2.3 ^{c}
	20% starch	58.6 ± 0.9	-	-	78.6 ± 3.6	-	-
	30% starch	47.8 ± 2.3	-	-	57.3 ± 1.3	-	-

Values with a different letter (a, b, c) are significantly different ($p < 0.05$) according to Duncan's test.

In this study, the enzymatic pretreatment presented an important effect on hydrolysis yield (76.1%) compared to that of high-temperature pretreatment (79.0%). The Viscozyme L (mainly containing β-glucanase) hydrolyzed β– 1, 3 (4)—glucosides linkage that presented in the vegetal cell wall. This activity reduced the suspension viscosity and also liberated the starch out of their network that promoted the adsorption of enzyme and boosted the reaction speed so increased the hydrolysis yield [12,13].

It is important to note that granular starch hydrolysis is a heterogeneous catalytic reaction, thus, pores present on starch surfaces could become centers of enzymatic attack [14,15]. The important increase in conversion yield of thermal-pretreated starch could also impact weaker areas of the starch granule (e.g., truncated or damaged granules), allowing the enzyme to degrade starch granules more effective [6]. The thermal pretreatment presented as the most effective method to enhance the hydrolysis yield of granular starch, according to previous studies [5]. However, in our case, the pretreatment with carbohydrases (β-glucanase and cellulase) provided a potential advantage for complex substrate. Contrary to the other studies, utilizing starch as a substrate, the cassava flour was used for this research.

This material contained not only starch but also other components (in % dry matter): starch 78.9 ± 3.2; reducing sugar 2.9 ± 0.1; cellulose 5.4 ± 0.2; protein 2.0 ± 0.1 and ash 1.5 ± 0.0), especially lignocellulose that encircled the starch granule inside and formed the natural barrier against enzyme attack [16]. Using auxiliary enzyme could reduce the diffusion limitation of enzyme to the substrate and increased hydrolysis yield [12,13]. This method suggested an emerging approach to develop the uncooked starch hydrolysis process by selecting a suitable enzymatic cocktail (amylase and other auxiliary activities) for each complex biomass.

The impact of substrate concentration on hydrolysis yield was also investigated with thermal pretreatment. The substrate concentration influenced negatively the hydrolysis yield. A decrease in 10–20% of the yield was observed when starch content increased by 20%. When the substrate concentration increases, the hydrodynamic interactions between particles become important. For cassava flour, the space between particles was more limited when the flour concentration increased because of the high water absorption capacity. For the concentrated suspensions, with a lot of contacts between the particles, the viscosity of the suspension increases rapidly with volume fraction, ϕ. When ϕ reaches a critical value ($\phi_G \approx 0.58$ for spherical monodisperse particle), each particle is confined in a cage formed by its nearest neighbors. For volume fractions above this value, only a vibration of the particles inside the cage remains possible, and this possibility completely disappears when ϕ reaches the value of dense packing ($\phi_{RCP} = 0.637$ for monodisperse spheres) [17]. The obtained results also highlighted that the thermal pretreatment could be combined with another method, such as enzymatic pretreatment to increase the hydrolysis yield at very high gravity condition.

2.2. Kinetics of Enzyme Adsorption

The enzymatic action over insoluble substrates, such as starch granules, occurs in several stages involving solid surface diffusion, adsorption and finally catalysis [1,4]. The enzymatic hydrolysis effect was observed in the starch microstructure (Figure 2). The native starch (at 0 h) exhibited a smooth surface and no holes were visible. During hydrolysis, the amylases modified significantly the starch surface. Different holes (with different sizes) were firstly produced in the surface, and from these holes, the attacked zone spread over this surface. The cassava starch was hydrolyzed from the outer to inner layer until total granular degradation. This observation was in line with previous studies [4]. Enzyme adsorption to the substrate surface was determined only at the beginning of the reaction, but not at a higher extent of hydrolysis when the pits become prominent, and the diffusion of enzymes into pores and channels could become rate-limiting [15]. To explain the effect of different pretreatments on enzymatic hydrolysis in more detail, the kinetics constants of enzyme adsorption were determined by fitting the data (for only thermal-pretreatment at 30, 40 and 50 °C) to the Langmuir adsorption model. A very good fit was obtained and the values for maximal adsorbed enzyme concentration, E_{max} and adsorption constant, K_{ad} were listed in Table 2. The thermal-pretreatment (50 °C) increased the adsorption of amylase 5-fold over the untreated substrate (at 30 °C). Increasing the pretreatment temperature involved an important increase of the affinity between enzyme and substrate by reducing the K_{ad} (from 86 to 50 mL.g^{-1} for 50 °C, and 30 °C, respectively), and increasing the maximum absorption (from 0.0085 to 0.0419 mg.g^{-1} corresponding to 30 °C and 50 °C). With high E_{max} values, pretreatment at 50 °C became the most effective method to accelerate the hydrolysis and increase glucose production. For granular starch hydrolysis, amylase is known to exert its catalytic action when adsorbed on starch granule, this finding might be important for the prediction of the effectiveness of amylase action of the pretreated starchy material.

Table 2. Adsorption parameters of amylases on cassava flour fitted to the Langmuir model.

Pretreatment Method	E_{max} (g enzyme/kg starch)	K_{ad} (mL/g)	r^2 (/)
30 °C	0.0085 ± 0.0004	85.49 ± 13.55	0.9889
40 °C	0.0098 ± 0.0024	82.55 ± 23.12	0.9044
50 °C	0.0419 ± 0.0014	50.04 ± 11.29	0.9691

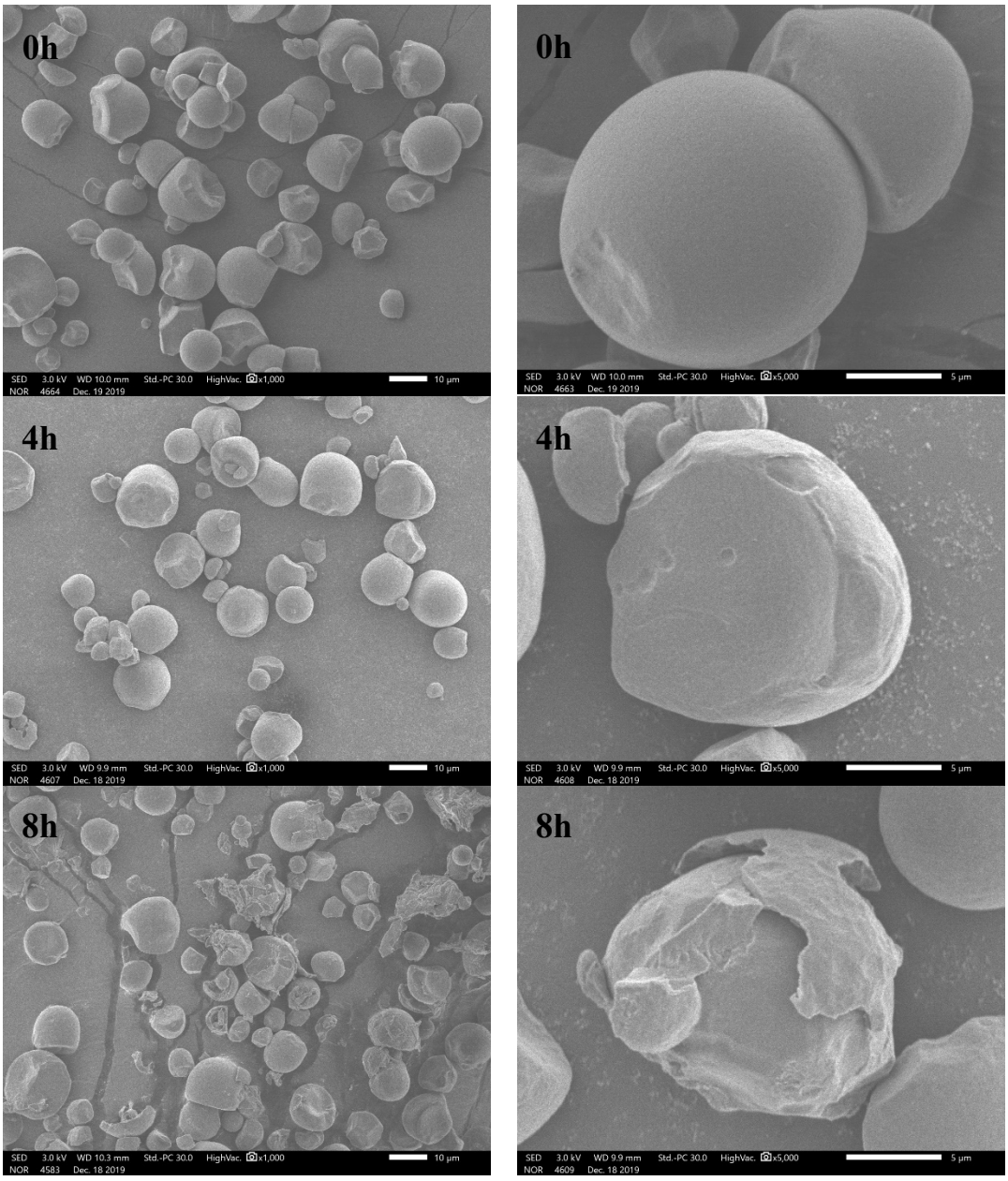

Figure 2. Scanning electron micrograph of cassava starch during hydrolysis (pretreatment at 50 °C, hydrolysis at 30 °C. Magnification 1000× (left) and 5000× (right)).

Substrate concentrations were inversely proportional to the enzyme adsorption. For higher starch content, lower bound enzyme concentrations were obtained (Figure 3a). This could be explained by the increase in particle (cassava flour) interactions and suspension viscosity in the concentrated regime, which limits the mass and heat transfer, as well as the catalyst diffusion in suspension. Consequently, the adsorption of enzyme on starch granule decreased. For pretreatment at 30 °C, the enzyme adsorption reduced from 84.8% to 65.6% when the substrate concentration increased from 10%, to 30% *w*/*w*, respectively. The same tendency was observed for the treated sample at 50 °C. A decrease in 22% of enzyme adsorption corresponded to an increase in 20% of starch content. The impact of substrate concentration exhibited the same at any pretreatment condition (all trend lines in Figure 3a were parallel). However, a higher temperature in pretreatment could reduce this impact, in terms of the absolute value of enzyme adsorption.

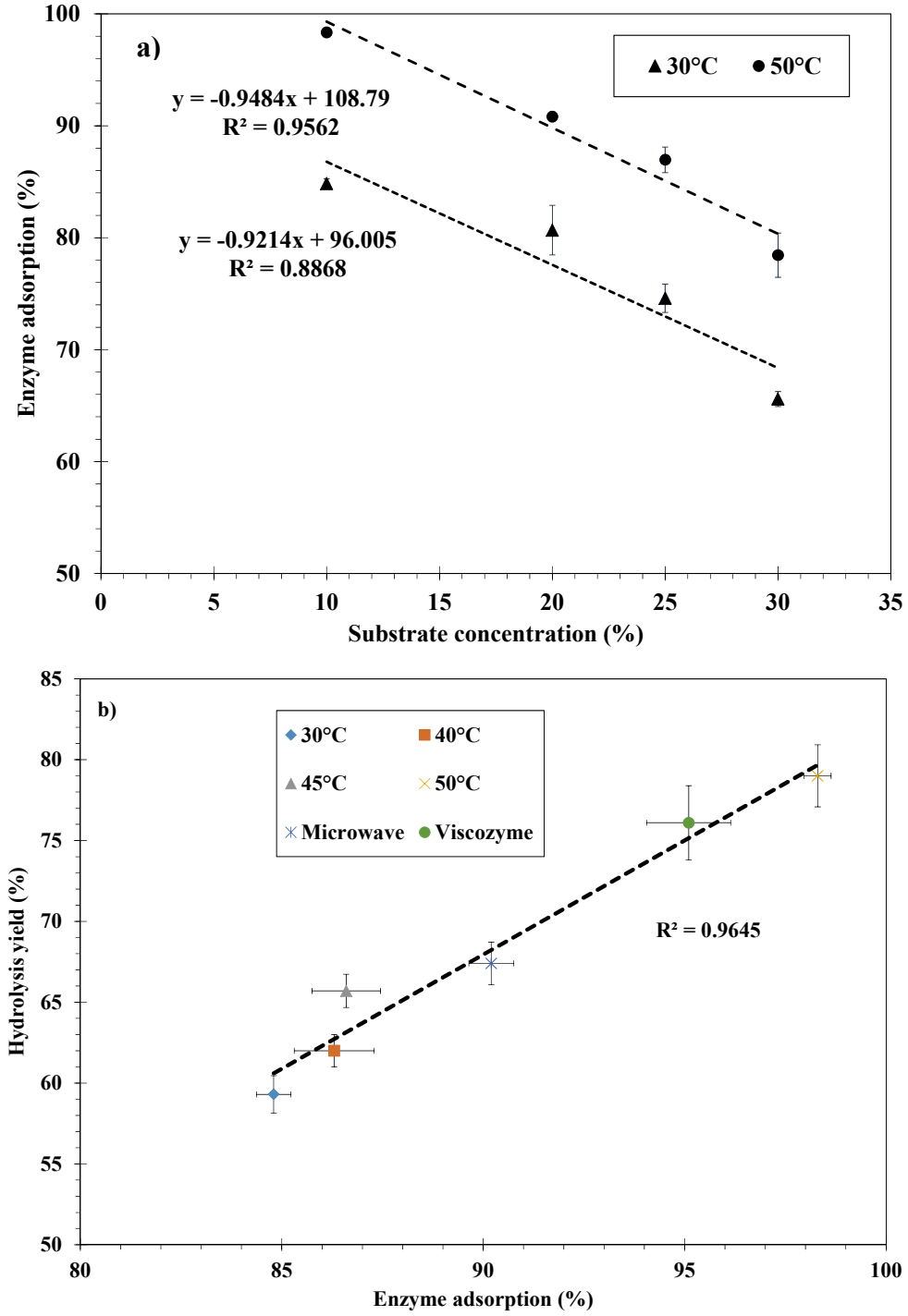

Figure 3. (**a**) Impact of substrate concentration on enzyme adsorption. (**b**) Correlation between enzyme adsorption and hydrolysis yield (10% of starch).

Through microscopy observation, the cassava starch granules appeared mostly with round and truncated shape (circular with a flat surface on one face) with various sizes. In comparing different pretreatment methods, the size and shape of particles were not significantly different (Figure 4). The mean diameters (μm) of starch granules were 15.3 ± 6.8; 15.4 ± 6.7; 16.4 ± 7.2 and 16.6 ± 7.3 for 30 °C; microwave; Viscozyme L and 50 °C pretreatment methods respectively. Therefore, the hydrolysis yield was correlated with the enzyme adsorption and interestingly, a linear correlation was obtained (Figure 3b). Pretreatment at 50 °C for 30 min resulted in the highest adsorption, reaching nearly 100%

and corresponding to a hydrolysis yield of 79% at 48 h. At lower temperatures than the gelatinization temperature, starch does not dissolve in water, so the enzyme must work on solid phase [1]. Previous studies showed that firstly, enzyme adsorbed onto the surface of starch granule and then started to break down the starch into simple sugars. Adsorption rate for amorphous regions was higher than that for crystalline regions [18], so the pretreatment method could expand the amorphous region of starch granule increasing the enzyme adsorption, successively increase the hydrolysis yield. The correlation between enzyme adsorption and hydrolysis yield was also concluded for concentrated and very high gravity regimes. The same hydrolysis yield was obtained, with the same enzyme adsorption (not taking into account the pretreatment methods and substrate concentrations). For example, the enzyme adsorption rate of 20–30 °C was equivalent with that of 30%-50 °C and the yields of two experiments were similar (57–58%) (Figure 3a and Table 1). This result once again confirmed that thermal pretreatment under gelatinization temperatures had an important effect on the efficiency of granular starch hydrolysis. The preheating process made the surface of starch granular more porous, enhanced the adsorption ability of enzyme, which is an important factor determining the velocity and yield of a heterogeneous catalytic reaction.

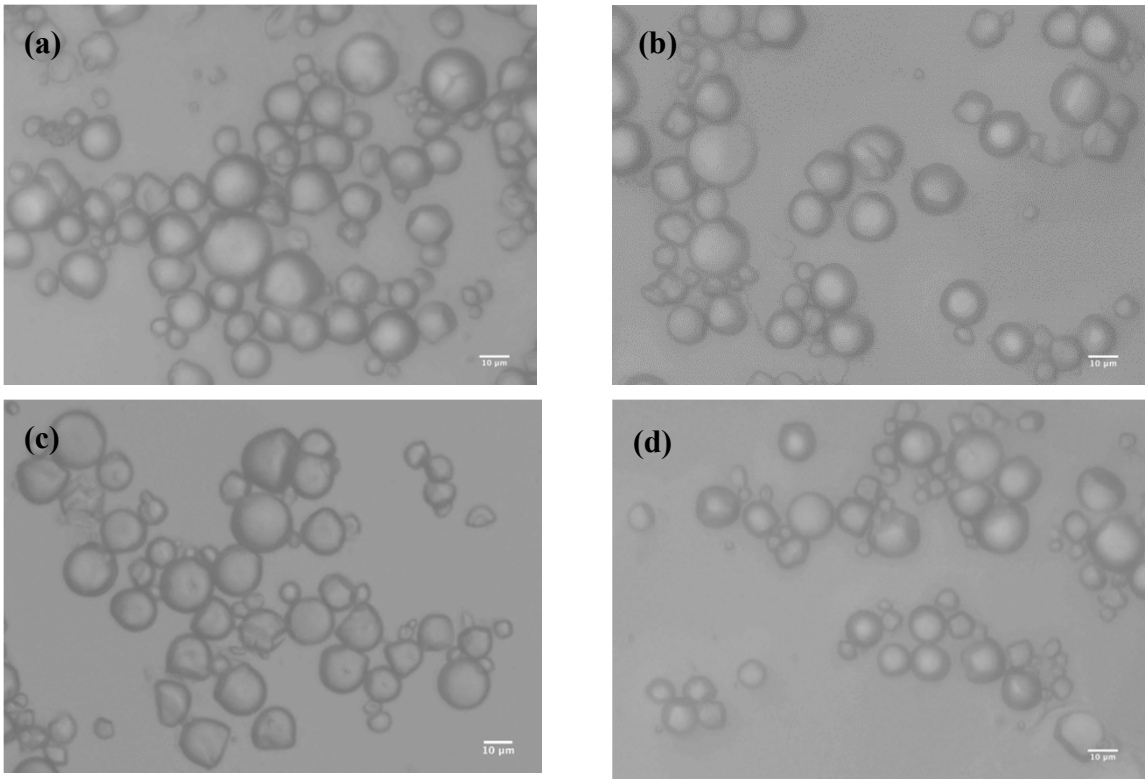

Figure 4. Granular cassava starch observations after pretreatments: (**a**) 30 °C; (**b**) 50 °C; (**c**) Viscozyme L and (**d**) Microwave.

2.3. Hydrolysis Kinetics Modelling

The model used in this study has the same form with the Michaelis and Menten equation, however, in this case, the relationship between hydrolysis time and product (glucose) concentration was established. Whereas, in the Michaelis and Menten equation, the relationship was modelled between reaction rate and initial substrate concentration (at saturating substrate condition). Figure 1 and Table 3 show that Equation (5) satisfactorily fitted the experimental data. Figure 1 indicated that the proposed model seems to well describe the production of glucose over time and the determination coefficient r^2 were near to 1 (Table 3). This model is suitable in the description of curvilinear section

of starch hydrolysis curve. Besides, it is useful in modelling the hydrolysis of starch by enzymatic cocktail (α- and γ-amylase) that means more than one product is released during hydrolysis [19,20].

Table 3. Summary of the results obtained by fitting Equation (5) to the experimental progress curves for the hydrolysis of cassava flour.

Pretreatment Method	Initial Starch Concentration	A (g.L^{-1})	B (min)	r^2 (/)
30 °C	10%	79.4	17.3	0.9514
	20%	180.0	18.9	0.9930
	30%	238.1	13.5	0.9891
40 °C	10%	80.6	15.8	0.9544
45 °C	10%	85.5	13.9	0.9619
50 °C	10%	107.5	18.4	0.9537
	20%	212.8	19.8	0.9939
	30%	285.7	14.8	0.9899
Microwave	10%	99.0	19.5	0.9628
Viscozyme L	10%	102.0	17.3	0.9538

Considering the similarity to the fundamental Michaelis and Menten model, parameter A in Equation (5), represent the maximum glucose concentration which could be reached and parameter B could be the required time for achieving half of this maximum glucose concentration, A/2. Considering different pretreatment methods for 10% of initial starch concentration, parameter A varied between 79.4 to 107.5 g.L^{-1}. And the effect of the pretreatment method was once again highlighted. The 50 °C and enzymatic pretreatments were the most effective and provided the highest maximum glucose productions. Considering the same pretreatment method, by varying the starch concentration, inhibition of initial substrate concentration was observed. The maximum hydrolysis yield decreased (calculated by division of parameter A for theoretical glucose) in raising the starch concentration. For 50 °C and 30 °C pretreatment, parameters A can be regarded as proportional with the initial substrate concentration (or the theoretical glucose concentration, Glu_{theo}) by Equations (1) and (2) respectively:

$$A = 0.898 \times Glu_{theo} \quad (1)$$

$$A = 0.715 \times Glu_{theo}. \quad (2)$$

According to Equations (1) and (2), approximately 90% and 72% in maximum hydrolysis yield may be achieved for the hydrolysis of cassava granular starch with 50 °C and 30 °C pretreatments respectively.

The parameter A, from an industrial angle, played an important role: A reasonable prediction of A guides when to stop starch hydrolysis for economic processes [21].

3. Materials and Methods

3.1. Substrate and Enzymes

Cassava (*Mannihot esculenta* Crantz) chips (moisture under 12%) were purchased directly from the farmer in Son Duong district, Tuyen Quang province, Vietnam. Cassava flour was then produced by grinding cassava chips in a hammer mill with 0.5 mm sieve. They were put in a zip-lock bag and stored at the dry place until use.

An enzyme cocktail (Stargen 002, ref. 3015155108, kindly provided by Dupont) was used for starch hydrolysis containing *Aspergillus kawachi* α-amylase expressed in *Trichoderma reesei* and γ-amylase from *Trichoderma reesei*. The optimum pH ranged from 4.0 to 4.5. The enzyme activity was re-examined in our laboratory and gave the results of 560 GAU.g^{-1} (One Glucoamylase Unit, GAU was the amount

of enzyme that could liberate one gram of reducing sugars calculated as glucose per hour from soluble starch substrate).

Viscozyme L (Novozymes, Denmark, 100 Fungal Beta-Glucanase Units/g) was used for pretreatment step. It was a multi-enzyme complex, containing a wide range of carbohydrases, including arabanase, cellulase, beta-glucanase, hemicellulase and xylanase. Viscozyme L is a special enzyme preparation, used in the breakdown of vegetal cell walls.

3.2. Biochemical Analysis and Hydrolysis Yield

The raw material was characterized biochemically for moisture content, total protein, crude lipid, fiber, starch, reducing sugar and ash. Dry matter content was determined using the AACC Method 44-15A. Total protein, crude lipid were determined by Kjeldahl and Soxhlet method, respectively. Fiber was quantified as sample weight after acid hydrolysis and washing with ethanol and diethyl ether. Ash was the sample weight after calcination at 550 °C for 6 h.

The concentration of protein in solution was measured by Bradford method [22]. Calibration curve for BSA (bovine serum albumin) in a range of 1–25 mg.mL^{-1} was constructed.

Total starch was deduced from reducing sugar content expressed in glucose after acid hydrolysis (HCl 2%) during 2 h. The reducing sugar was determined by DNS (3,5-Dinitrosalicylic acid) method [23] with minor modifications. A sample of 0.5 mL after centrifugation was mixed with 1.5 mL DNS reagent then boiled for 5 min. After cooling to room temperature, the absorbance was recorded at 540 nm. Glucose was used as a standard for the calibration curve. The reducing sugar was expressed in glucose equivalent. Hydrolysis yield was calculated as the ratio between the amount of released glucose and the potential quantity of glucose.

The means and standard deviations were determined for glucose production and hydrolysis yield from at least three replicates. The significant difference of mean values was assessed with one-way analysis of variance (ANOVA) followed by Duncan's test using SPSS software at a significance level of $p < 0.05$.

3.3. Particle Size Determination

After 30 min of pretreatment, cassava starches were viewed using a TE 2000U photomicroscope (Nikon, Japan) equipped with an ORCA-ER C4742-80 camera (Hamamatsu, Japan). Digital photomicrographs (saved as 8-bit tiff format) were then analyzed using Image J software. The particle diameter and standard deviation were deduced from at least 1000 starch particles.

3.4. Scanning Electron Microscopy (SEM)

The morphology of native and hydrolyzed cassava starch was observed using a JSM IT200 scanning electron microscope (SEM) (JEOL, Tokyo, Japan). Samples were coated with platinum in a vacuum evaporator (JEE 400, JEOL, Tokyo, Japan) before observation. The obtained samples were examined at an accelerating voltage of 5 kV and magnified 5000× times.

3.5. Determination of Enzyme Adsorption and Kinetics Constants

The enzyme adsorption (only for amylase in Stargen 002) was determined by the method presented by Bommarius et al. [24] with minor modification. This value was defined as the ratio of the bound enzyme and the total initial enzyme concentration. Starch hydrolysis was carried out in a 15-mL glass test tube with 10–30% *w/w* of the substrate (in acetate buffer pH 4.2) and enzyme concentration ranging from 1 to 15 mg.mL^{-1}. The reactions were run for 10 min at 30 °C. The hydrolysate was then centrifuged at 9000 rpm for 10 min. The resulting supernatant was analyzed using the Bradford protein assay to determine free enzyme concentration. The amount of bound enzyme could be determined

from the free enzyme concentration. The enzyme adsorption was assumed to follow a Langmuir-type isotherm, as in Equation (3),

$$E_B = \frac{E_{\max} \cdot K_{ad} \cdot E_F \cdot S}{1 + K_{ad} \cdot E_F} \quad (3)$$

where E_B is the bound enzyme concentration, E_F is the free enzyme concentration, S is the substrate concentration, E_{max} is the maximum enzyme adsorption in g enzyme/g amylase, and K_{ad} is the adsorption coefficient. To determine K_{ad} and E_{max}, a linearized form of Equation (3) was used:

$$\frac{S}{E_B} = \frac{1}{E_{\max} \cdot K_{ad}} \cdot \frac{1}{E_F} + \frac{1}{E_{\max}}. \quad (4)$$

Once the data was plotted, with S/EB on the y-axis and 1/EF on the x-axis, a linear regression was carried out using Microsoft Excel 2010. From the equation for this line, K_{ad} and E_{max} were calculated.

3.6. Enzymatic Assay

Cassava flour was prepared in acetate buffer (pH 4.2) at different concentration (10–30% *w*/*w* starch i.e., 14.3–43.0% dry matter). Three different pretreatment types were separately applied: Thermal, enzymatic and microwave pretreatments. For thermal pretreatment, the substrate suspension was heated at 40, 45 and 50 °C for 30 min before hydrolysis. The enzymatic pretreatment was performed at 30 °C for 30 min with Viscozyme L (0.05% *w*/*w*). The microwave pretreatment (3 × 20 s at 800W, EM-G256W-Sanyo Electric company, Ltd.) was applied for cassava flour before suspending. A pretreatment at 30 °C for 30 min was considered as reference. Pretreated suspensions were then hydrolyzed by adding Stargen 002 (0.2% *w*/*w*) for 48 h at 30 °C with continuous shaking at 60 rpm. Sampling was taken at 0, 1, 2, 3, 5, 10, 24 and 48 h of hydrolysis. The enzyme was then inactivated by adding KOH 4N until pH ≥ 12. The sample was centrifuged at 9000 rpm for 5 min. The supernatant was subjected to glucose determination. All experiments were conducted in triplicate.

3.7. Hydrolysis Kinetics Modelling

Kinetics of starch hydrolysis can be modelled by theoretical, semi-theoretical, and empirical kinetics equations [20]. In this study, an empirical model was used to describe starch digestogram. This model was also used in previous studies [19,25,26],

$$D_t = D_0 + \frac{At}{B + t} \quad (5)$$

$$\text{or } \frac{t}{D_t - D_0} = \left(\frac{1}{A}\right)t + \left(\frac{B}{A}\right) \quad (6)$$

where D_0 and D_t are the initial glucose concentration and the glucose concentration at t time of hydrolysis (g.L^{-1}), respectively A and B are constants, and a plot of (t/[D_t–D_0]) against t gives a straight line of slope (1/A) and intercept (B/A) [21]. The model can yield D_0, and in drawing an analogy with the Michaelis–Menten model. The parameter B indicates the time (a measure of the rate of digestion) to reach A/2 with A indicates the maximum digestible starch. In this study, glucose production would be modelled by Equation (5).

4. Conclusions

This study aimed to evaluate the impact of pretreatment methods on the enzymatic hydrolysis of granular starch from cassava flour. The results showed that thermal and enzymatic pretreatment methods presented as the most efficient method to enhance the hydrolysis yield. This yield was dependent linearly on the enzyme adsorption rate. The kinetics of glucose production was modelled by an empirical equation and allows predicting the maximum hydrolysis yield which may be achieved.

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