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Hydrolysis of Native and Heat-Treated Starches at Sub-Gelatinization Temperature Using Granular Starch Hydrolyzing Enzyme

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Abstract The effect of heat treatment below the gelatinization temperature on the susceptibility of corn, mung bean, sago, and potato starches towards granular starch hydrolysis (35°C) was investigated. Starches were hydrolyzed in granular state and after heat treatment (50°C for 30 min) by using granular starch hydrolyzing enzyme for 24 h. Hydrolyzed heat-treated starches showed a significant increase in the percentage of dextrose equivalent compared to native starches, respectively, with corn 53% to 56%, mung bean 36% to 47%, sago 15% to 26%, and potato 12% to 15%. Scanning electron microscopy micrographs showed the presence of more porous granules and surface erosion in heat-treated starch compared to native starch. X-ray analysis showed no changes but with sharper peaks for all the starches, suggested that hydrolysis occurred on the amorphous region. The amylose content and swelling power of heat-treated starches was markedly altered after hydrolysis. Evidently, this enzyme was able to hydrolyze granular starches and heat treatment before hydrolysis significantly increased the degree of hydrolysis.

Keywords Starch · Enzymes · Hydrolysis · Heat treatment and dextrose equivalent

Introduction

Native starch granules are semi-crystalline and resistant to amylases hydrolysis. According to Wang et al. [1], native granular starch is hydrolyzed very slowly by amylases and amyloglucosidase. The substrate (starch molecules compacted inside the granules) simply cannot be readily accessed by digestive enzymes. Disruption of the starch granular structure by heating in water (gelatinization) could enhance its chemical reactivity towards hydrolytic enzymes [1]. Direct hydrolysis of starch at raw or native state is desirable as it could reduce the costs associated with the high temperatures required for gelatinization (especially in production of fermentable sugars). In order to simplify the process and save the cost of gelatinization, specific raw starch-digesting α-amylases have been isolated and investigated [2]. In recent years, the

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importance of enzymatic saccharification of raw starch without heating has become well recognized, mainly from the viewpoints of energy savings and effective utilization of the biomass thereby reducing the cost of starch processing [3]. This has generated a worldwide interest in the discovery of new enzymes that can directly hydrolyze the raw starch in a single step and that too at moderate temperature much below the gelatinization temperature.

Restricted heating of starch granules is known to result in a phenomenon known as annealing. In this process, the arrangement of the macromolecules undergoes subtle changes resulting from rearrangement of hydrogen bonds which are broken and reform as the granule components attain more ordered crystal formation. The extent of these changes is being influenced by starch composition and by the arrangement of the starch chains within the amorphous and crystalline domains of the native starch granules [4]. Susceptibility of the starch granules to enzyme attack can be modified by preheating the granules in aqueous solution at low pH 3.5 for 2 h. Following this treatment, granules are more sensitive to enzyme hydrolysis than in untreated material, and the pattern of enzyme attack is also found to be highly dependent on the processing history [1].

The process of annealing has been well reported [4, 5]. However, little information on the correlation between heat treatment and granular starch hydrolysis is scarce. A study by Wang et al. [6] reported the comparison of enzymatic (E-Mill) and conventional dry-grind corn processes using a granular starch hydrolyzing enzyme. However, the publication was merely focused on enzymatic hydrolysis of corn starch. In this paper, we tried to compare the action of the granular starch-hydrolyzing enzyme on starches from different botanical sources such as tuber, palm, and legume starch. Besides, in this research, the effect of heat treatment on granular starch hydrolysis (35°C) will be evaluated. According to Campus et al. [7] and Oates [8], better hydrolysis of raw starch can be achieved by increasing the incubation temperature to approximately 60°C. Recently, Nakazawa and Wang [9] demonstrated that heat treatment also created void, porous structure that allowed for more rapid hydrolysis by acid. Therefore, the objective of the present study was to study the effect of heat treatment on the susceptibility of starch to hydrolysis by granular starch-hydrolyzing enzyme (35°C). Starches of different sources were included to better understand their impacts on enzyme hydrolysis after heat treatment.

Materials and Methods

Materials

Corn (*Zea mays*), potato, and sago (*Metroxylon sagu*) starches were obtained from SIM Company Sdn. Bhd. (Penang, Malaysia). Mung bean (*Vigna radiata*) starch was obtained from Pearl Island Packaging Sdn. Bhd. (Penang, Malaysia).

Enzymes

The commercial enzyme used in the present study was procured from Genencor International (Palo Alto, CA). The enzyme contains alpha-amylase from *Aspergillus kawachi* and a glucoamylase from *Aspergillus niger*, with a specific gravity of 1.10–1.15 g/mL. The pH of the enzyme ranged from 4.0 to 4.5, and the recommended temperature is 20–40°C. The enzyme's activity was determined by reaction at 37°C with soluble starch (1%) that was buffered with sodium acetate (pH 4.4). Aliquots were taken after 10 min to determine the amount of D-glucose released. The glucose concentration was determined using the dinitrosalicylic acid method [10]. The enzyme's activity was 3,736 U/g starch.

Determination of Pasting Temperature

The gelatinization temperature of starches was determined in triplicate by using a Rapid ViscoTM Analyzer (Model RVA Series 4, Newport Scientific Pvt. Ltd., Warriewood, Australia) and a Differential Scanning Colorimeter, DSC-Q100 (TA Instruments, Lukens Drive, New Castle, USA), equipped with a refrigerated cooling system (RCS). For the determination by a rapid visco analyzer (RVA), 2 g of starch sample (corrected to 14% moisture basis) and 25 ml of distilled water were combined and stirred in the aluminum RVA sample canister. Temperature was held at 50°C for 1 min and then raised to 95°C in 3.75 min, held for 2.5 min, cooled to 50°C in 3.75 min, and held for 5 min. The paddle speed was set at 960 rpm for the first 10 s to evenly disperse the starch slurry and was reduced to 160 rpm throughout the entire experiment. The units of viscosity were expressed as RVU. For the determination by a differential scanning colorimeter (DSC), 2 mg of starch sample was loaded into an aluminum pan, and distilled water was added to achieve a starch–water suspension containing 75% water. Samples were heated from 30°C to 130°C at a heating rate of 10°C/min under an oxygen-free N2 flow rate of 50 ml/min.

Heat Treatment

The starch slurry (25% *w*/*v*) in 400 ml sodium acetate buffer was incubated in water bath at 50°C for 30 min with continuous stirring using an overhead stirrer. The temperature of the slurry was brought down to 35°C by immersing the starch slurry in the water before being subjected to hydrolysis.

Starch Hydrolysis

The starch slurry (25% *w*/*v*) was prepared in 400 mL of sodium acetate buffer. The enzyme (3,736 U/g starch) was added (1% *w*/*v*) into the samples. Samples were then incubated in an incubator shaker (JEIO Tech, SI-600R, and Seoul, Korea) at 35°C at a speed of 150 rpm. After 24 h, hydrolysis was stopped by adjusting the pH to 1.5–1.6 with 2 M HCl. The pH of starch suspensions was adjusted back to a pH of 5–6 by washing the starch with distilled water. Preliminary experiments have established that the enzyme deactivation method does not cause significant starch hydrolysis. Starch residues were dried at 40°C for 2 days.

The reducing sugar value was measured using the dinitrosalicylic acid method [10] to determine its dextrose equivalent (DE). A small aliquot was withdrawn from each batch of starch slurry at various time intervals, up to 24 h hydrolysis time. Absorbance was measured at 504 nm by using a UV/visible spectrophotometer (UV-160A, SHIMADZU, and Kyoto, Japan). Glucose was used as the standard. Each analysis was performed in duplicate. DE was calculated as follows:

$$\mathrm{DE} = (\text{g reducing sugar expressed as glucose})/(\text{ g dry solid weight}) \times 100\%$$

Scanning Electron Microscopy

The microstructure of starch granules was viewed with a field emission scanning electron microscope (FESEM Leo Supra 50VP, Carl-Ziess SMT, Oberkochem, Germany). Starch granules were mounted on aluminum specimen stubs with double-sided adhesive tape and sputter, with a 20–30 nm layer of gold, using Sputter Coater [Polaron (Fisons) SC515, VG Microtech, Sussex, UK]. The accelerating voltage of the SEM is 5 kV.

X-ray Diffraction

Crystallinity patterns of starch granules were examined by X-ray diffraction, as described by Lauro et al. [2]. The dried starches were conditioned overnight at room temperature in 100% relative humidity (RH). The starches were scanned by X-ray diffractometer (Diffractometer D5000, SIEMENS, Karlsruhe, Germany). Diffractograms were recorded in the reflection mode in the angular range of 4–40° (2θ) with a rate of 0.05°/s. The Cu K_α-radiation (λ 1.5406 Å), which was generated at 40 kV and 30 mA, was made monochromatic using 15 μm of Ni foil. Scattered radiation was detected using a proportional detector.

Amylose Content

Amylose content of each sample was determined in triplicate, according to the procedure described by McGrance et al. [11]. The reported values are the means of triplicate measurements.

Swelling Power and Solubility

Swelling power and solubility of starch were determined in triplicate by adopting the method of Schoch [12].

Pasting Properties of Starch

The pasting properties of starches were determined by using Rapid Visco Analyzer (Model RVA Series 4, Newport Scientific Pvt. Ltd., Warriewood, Australia). About 2 g of starch sample (corrected to 8% moisture basis) and a measured amount of (25 ml) distilled water were combined and stirred in the aluminium RVA sample canister. A programmed heating and cooling cycle was used; the temperature profile was taken from the ICC standard method no. 162, profile no.1. The paddle speed was set at 960 rpm for the first 10 s to evenly disperse the starch slurry and was reduced to 160 rpm throughout the entire experiment. The units of viscosity were expressed as centipoise (cP).

Particle Size Distribution

Particle size distributions of the granules were determined with a low angle laser light scattering (Mastersizer S, Malvern Instruments Malvern, UK).

Statistical Analysis

Duncan's least significant test was used to compare means at the 5% significance level. Simple Pearson's correlation and regression analyses were performed using SPSS version 12.0 (SPSS, Inc., Chicago, IL, USA).

Results and Discussion

In the following discussion, the term “control starch” refers to the samples that were incubated at 35°C without enzyme. “Native starch” refers to the starch that was hydrolyzed enzymatically in the granular state. “Control heat treated” refers to the starch that had undergone heat treatment

at 50°C before being incubated in the absence of enzyme. "Heat treated" refers to starch that had undergone heat treatment before being hydrolyzed enzymatically in the granular state.

The specific mode of enzyme attack depends on both the botanic origin of the starch granule and the enzyme(s) involved [13]. The gelatinization temperature of these starches was determined by using a RVA and DSC. Results obtained showed that the gelatinization temperatures of both starches are higher than 50°C (given in Table 1); therefore, the temperature of 50°C has been chosen to treat the starch for mild heat treatment because it is below the gelatinization temperature of those starches. We hypothesized that the mild heat treatment would allow the starch granules to swell more and open up the small pores or crevices on the granule surface, which would facilitate the access of enzyme into the starch granules. Therefore, the mild heat treatment was expected to increase the degree of hydrolysis of the starches. This approach was based on the observation by Haska and Ohta [14] who reported that treatment of sago starch by heating to below gelatinization temperature at lower pH resulted in an increase in the ability of enzyme to digest sago starch granules.

Scanning Electron Microscopy

The representative SEM micrographs of control, hydrolyzed native and treated starches are presented in Figs. 1 and 2. There was no difference with regard to pattern of enzymatic degradation between native and heat-treated starches. SEM micrographs showed that all the control starch granule surface were smooth except for corn and mung bean granules. Corn granules showed small visible pores and pits on the surface, and they are randomly distributed (Fig. 3a). According to Sarikaya et al. [15], these natural pores would facilitate attacks of the enzyme molecules on starch granules during hydrolysis. This can be seen clearly in Figs. 1 and 2 where hydrolyzed corn starch was highly degraded with the appearance of many large pin holes and distinct layered structures. The enzymes appeared to hydrolyzed corn granules via multiple attack of localized digging, resulting in large and deep pits into the granule. It appeared that the pits were initiated from the non-reducing ends of the molecules located on the surface of the granule.

In contrast to corn starch granules, distinct pores were not observed in mung bean granules following enzyme hydrolysis (Fig. 1). Control mung bean granules only showed the presence of small pinholes on its surface (Fig. 3b). Most of the mung bean starch granules showed the enzymatic attack of surface erosion and occasional caving. The enzyme hydrolysis did not occur uniformly in mung bean starch granule because some area appeared to be more susceptible than others. This is in accordance with the results reported by Wang et

Table 1 Gelatinization temperature of starches determined by using Rapid Visco Analyzer (RVA) and Differential Scanning Colorimeter (DSC)

Sample	Gelatinization temperature (°C)	
	RVA	DSC
Corn	86.2±1.4	66.70±0.3
Mung bean	75.8±1.3	63.44±0.1
Sago	76.2±1.1	68.90±0.6
Potato	65.1±0.8	62.71±0.4

Mean±SD of triplicate samples

[a] Values followed by the same letter within the same column are not significantly different ($p>0.05$)

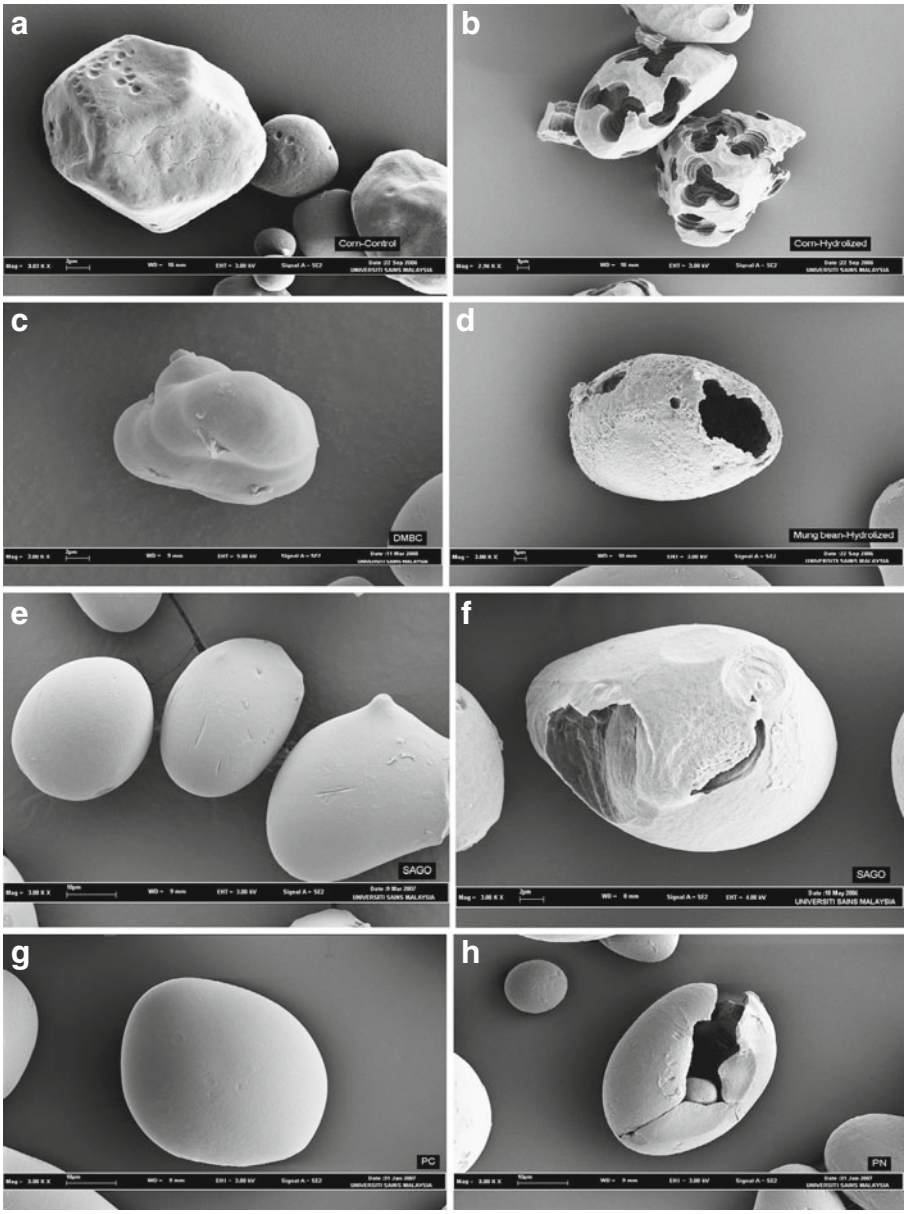

Fig. 1 SEM micrographs (3,000×) for **a** control native corn, **b** hydrolyzed native corn, **c** control native mung bean, **d** hydrolyzed native mung bean, **e** control native sago, **f** hydrolyzed native sago, **g** control native potato, **h** hydrolyzed native potato starches after hydrolysis at sub-gelatinization temperature (35°C) for 24 h (*scale bar*=10 μm)

al. [16]. According to Oates [8], the areas susceptible to enzyme attack are the less-organized amorphous rings, whereas the crystalline lamellae are resistant to enzyme activity.

In the absence of distinct pores, the mode of enzyme action on mung bean starches granules may be different from that on corn starch granules and might behave similarly to other starch that are relatively resistant to enzyme hydrolysis such as sago and potato starch.

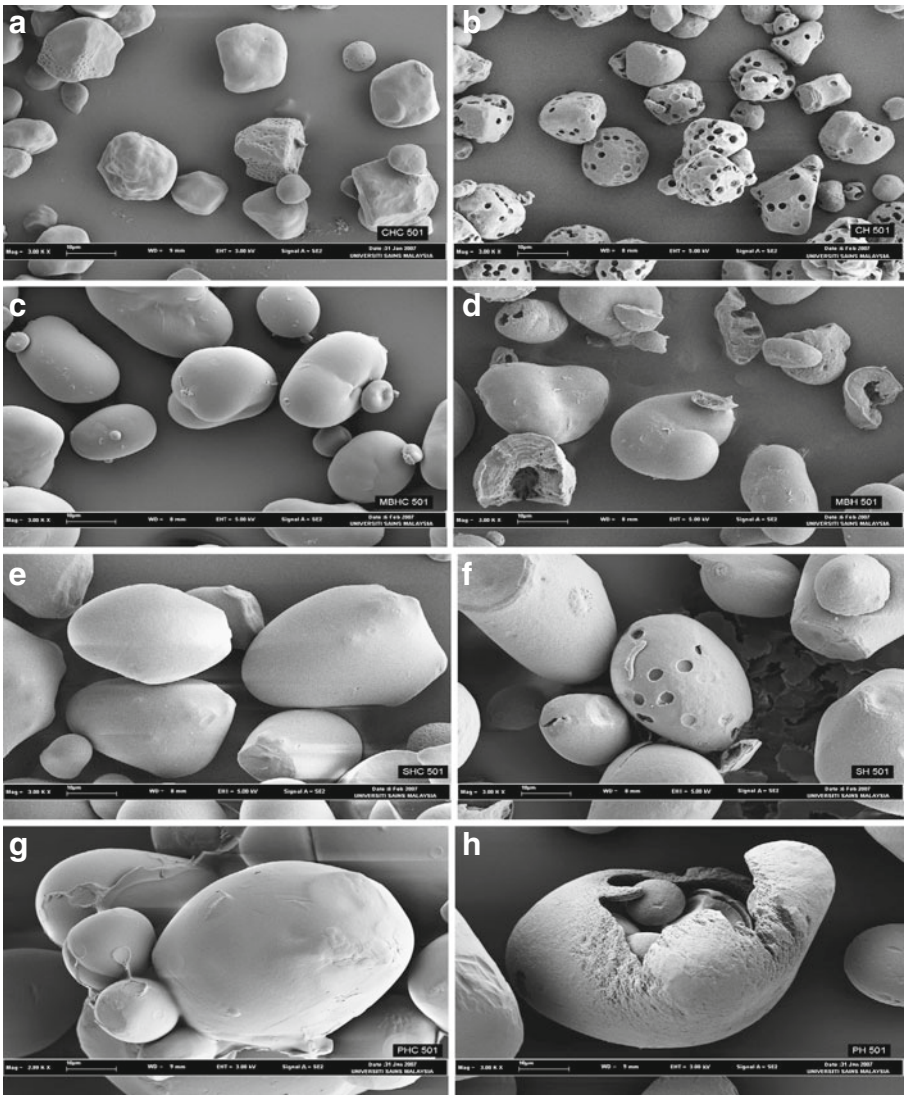

Fig. 2 SEM micrographs (3,000×) for **a** control heat-treated corn, **b** hydrolyzed heat-treated corn, **c** control heat-treated mung bean, **d** hydrolyzed heat-treated mung bean, **e** control heat-treated sago, **f** hydrolyzed heat-treated sago, **g** control heat-treated potato, and **h** hydrolyzed heat-treated potato starches after hydrolysis at sub-gelatinization temperature (35°C) for 24 h (*scale bar*=10 μm)

As shown in Fig. 1, there was only limited and isolated porous structure observed in hydrolyzed sago and potato starches. The degradation pattern of resistant types of starch proposed by Oates [8] occurs in two steps: (1) creation of a superficial microporosity due to uniform adsorption of enzyme molecules, and (2) degradation leading to macroporosity, with deeper grooves where the enzymes encounter a less-organized structure. It seems that the internal structure of sago starch was rapidly digested by α-amylase and glucoamylase, followed by slow surface erosion, while hydrolyzed potato starch showed a single hole on one end of the granule with more extensive hydrolysis of the internal regions of the granule, which agrees with the findings by Wang et al. [1].

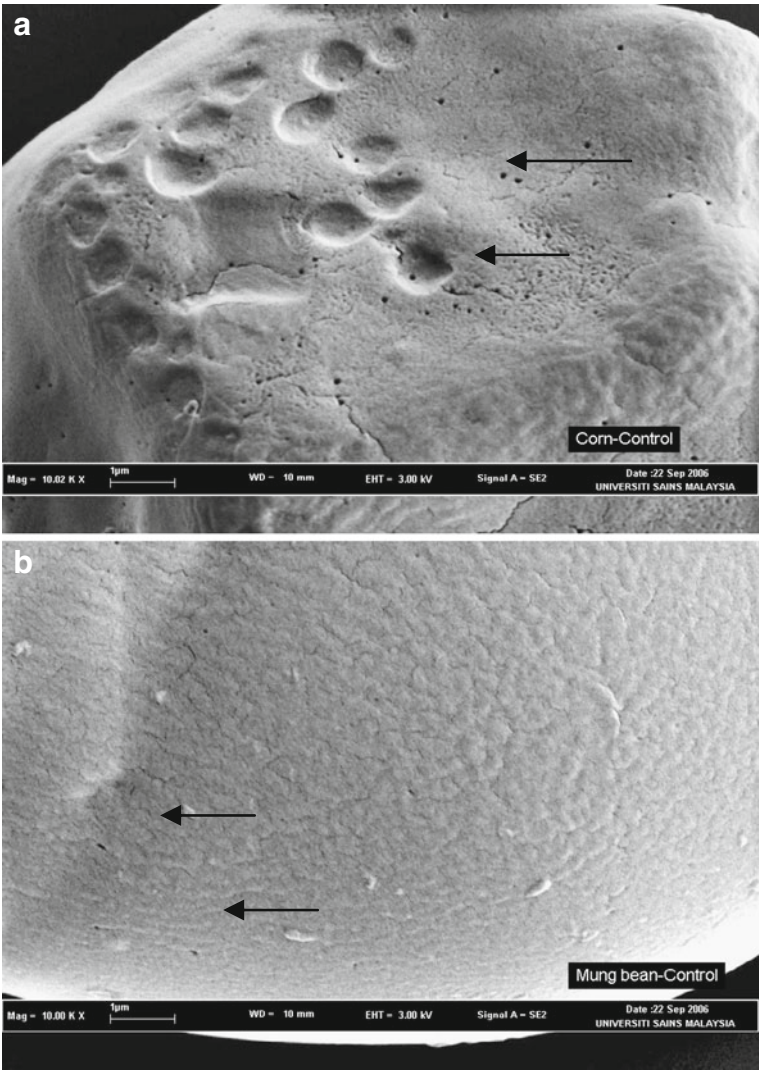

Fig. 3 SEM image showing the presence of pores and pinholes (shown by an *arrow*) on the surface of **a** control corn granule, **b** control mung bean granule (3,000×) (*scale bar*=1 μm)

Hydrolyzed heat-treated starches showed the same pattern of enzymatic attack as in hydrolyzed native starch but with higher degradation and more erosion (Fig. 2). Furthermore, there were cracks on the surface of the granules that appeared due to the heat treatment process before the hydrolysis, a similar observation reported by Haska and Ohta [14]. Heating of starch in water causes disruption of hydrogen bond between polymer chain, thereby weakening the granule, so that the enzyme can penetrate and degrade the starch easily. It is also possible that heat treatment process creates pores or fissures which alter the pattern of amylase hydrolysis from surface to internal erosion [1]. Hence, although amorphous and crystalline lamellae become more ordered, accessibility to the amorphous regions by enzymes is facilitated. Therefore, heat-treated starches showed higher degree of degradation to granular starch-hydrolyzing enzyme compared to native starch.

Degree of Hydrolysis

The degree of hydrolysis for native and heat-treated starches was observed by measuring dextrose equivalent at different time intervals (Fig. 4). The results showed that the degree of hydrolysis of native and heat-treated starch granule was affected by the heat treatment and starch type. The relative order of hydrolysis for native starch was corn (53%), mung bean (36%), sago (15%), and potato (12%). This observation is in accordance with the findings of Zhang and Oates [17], who had reported that cereal starches such as corn which also showed A-type X-ray pattern are generally less resistant to enzymatic degradation than non-cereal starches. Furthermore, the presence of pores and pinholes on the surface of mung bean and corn starch also help the penetration of enzyme during hydrolysis and resulted in higher degradation. The absence of pit (pores) and natural pores on native sago and potato starch granule has also been reported by Sopade and Kiaka [18]. Therefore, sago and potato is always less susceptible to enzyme attack.

Hydrolyzed heat-treated starches showed a significant ($p<0.05$) increase in the percentage of equivalent dextrose produced after hydrolysis compared to native starches with heat-treated corn (56%), heat-treated mung bean (47%), heat-treated sago (26%), and heat-treated potato (15%). Evidently, treating the starch with mild heat before enzyme hydrolysis enhanced the degree of hydrolysis of starch significantly. It has been reported by Piacquadio et al. [5] that the enzyme digestible starch percentages will increase with heating time due to changes in the internal structure of the starch granules. Heat treatment could cause partially irreversible swelling of the amorphous region in the granules, consequently allowing greater access for the enzyme to act on the starch. It is also possible that during this time, the slight swelling of the granule caused expansion of the naturally present pinholes and internal cavities in starch granules, allowing the enzyme to penetrate easily into the granules.

Similar trend of hydrolysis with native starches by glucoamylase and α-amylase was reported by Kimura and Robyt [19]. Corn starch was more susceptible compared to legume starch and potato starch. The high resistance to amylolysis of potato starch was ascribed to its

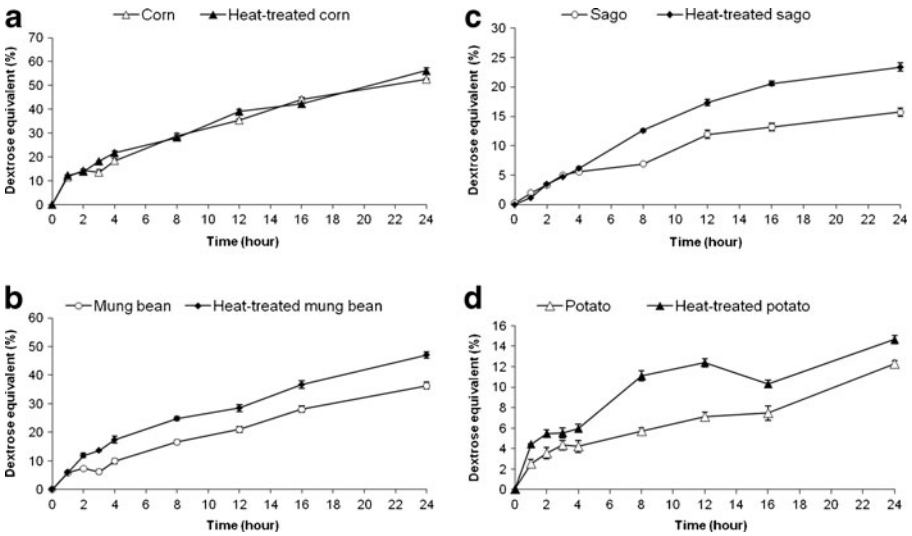

Fig. 4 Hydrolysis profiles of **a** native and heat-treated corn, **b** native and heat-treated mung bean, **c** native and heat-treated sago, and **d** native and heat-treated potato starches hydrolyzed below gelatinization temperature (35°C) for 24 h. Data points are mean±standard deviation ($n=3$)

high percentage of double-helical chains form by amylose and amylopectin. The effect of annealing on the hydrolysis of sago starch was also done by Wang et al. [1]. They reported that annealed sago starch was more susceptible to enzyme hydrolysis compared to native sago which is in accordance with our results. It was proposed that the heat treatment cause disruption of hydrogen bonding between the amorphous and crystalline region and a slight expansion of the amorphous region after heat treatment. The formation of more porous structure as a result of annealing was also proposed by Nakazawa and Wang [9]. These porous structures might enhance enzyme hydrolysis, which possibly depends on starch type and enzyme type.

X-ray Diffraction

The X-ray diffraction patterns of heat-treated starches for hydrolyzed native and heat-treated starches are presented in Fig. 5a, b. Corn starch shows a typical A-type pattern, with strong reflections at 2θ about 15^{o} and 23^{o} and an unresolved doublet at 17^{o} and 18^{o} 2θ. Mung bean starch had a reflection at 2θ about 15^{o} and 23^{o} which gave the characteristics of A type. However, a single diffraction peak at around 17^{o} and a small peak at about 5^{o} 2θ were characteristics of B pattern. This phenomenon indicated that mung bean displays a mix of A- and B-type pattern. Thus, mung bean was classified as a C type. Furthermore, common bean starches were usually the C crystalline type [13]. Similarly for sago starch, which gave the typical A-type reflections at 2θ, about 15^{o} and 23^{o} and an unresolved doublet at 17^{o} and 18^{o} 2θ were observed. An additional small peak appeared at about 5^{o} 2θ which was characteristic of the B pattern. Therefore, sago starch was also classified as a C type. The same X-ray pattern for corn and sago starch was reported by Oates [8]. Meanwhile, potato starch which had strong reflections at 2θ around 17^{o} and 5^{o} gave the characteristic of B-type pattern.

All starches showed a similar X-ray pattern as for native and heat-treated starch after hydrolysis, and this result indicated that the enzymatic hydrolysis did not change the X-ray pattern. However, a sharper X-ray diffraction peak was observed for hydrolyzed heat-treated starch compared with native starches. Sharper X-ray diffraction peaks indicated that the amorphous parts of the starch granules had been disrupted. It is a direct evidence of simultaneous degradation of the amorphous as well as the crystalline structures by α-amylase and glucoamylase. This observation is in agreement with the proposal by Gallant et al. [20] that the amylolysis primarily occurs in the amorphous regions of the starch granules. A study by Planchot et al. [21], who employed transmission electron microscope (TEM) to examine the ultra-structure of corn starch after amylolysis, reported that hydrolysis occurred mainly in the more amorphous zones. Gallant [22] stated that B-type and C-type starches had been shown to be more resistant to enzymatic hydrolysis than A-type starches. This statement was in agreement with our result where A-type starches (corn) were more susceptible to hydrolysis than B-type starch (potato) and C-type starch (sago and mung bean).

Amylose Content

The amylose content of all the starch samples was shown in Table 2. Amylose is found mainly in the amorphous regions of the starch granule in the form of single helical structures. It was reported that enzymes degrade the amorphous regions more easily than the crystalline lamellae [8]. Thus, the enzyme preferentially attacks and hydrolyzes amylose in the amorphous region of the granule. Data in Table 2 show that only hydrolyzed heat-treated corn starch had significantly lower amylose content than that of native or control starch. This indicates that the amylose was extensively degraded after 24 h of hydrolysis. Evidently, the enzyme preferentially attack and

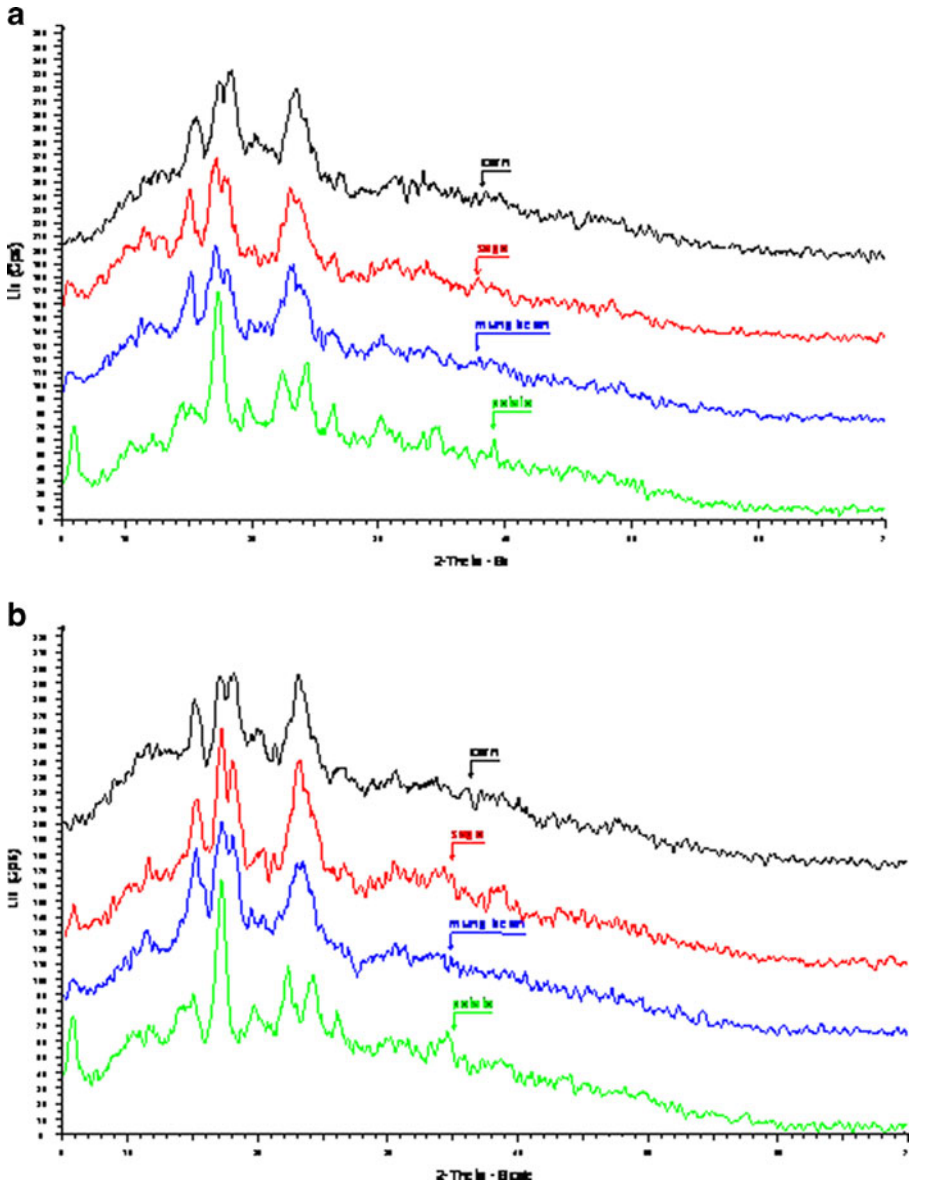

Fig. 5 **a** X-ray chromatograms of hydrolyzed native corn, mung bean, sago, and potato starches after 24 h of hydrolysis. **b** X-ray chromatograms of hydrolyzed heat-treated corn, mung bean, sago, and potato starches after 24 h of hydrolysis

hydrolyze amylose in the amorphous region of the granule. However, mung bean, sago, and potato starch showed no significant changes in amylose content for all types of samples. This observation is consistent with the lower degree of hydrolysis of other starches as compared to corn starch. It is possible that apart from the amorphous region, a portion of the amylopectin in crystalline regions was also degraded, hence contributing to the degree of hydrolysis measured.

Table 2 Amylose content of raw, control, and hydrolyzed starches after 24 h of hydrolysis in granular state (35°C)

Sample		Amylose content (%)
Sago	Control	$29.8^{a}\pm1.8$
	Native	$30.0^{a}\pm0.3$
	Control heat-treated	$31.4^{a}\pm0.5$
	Heat-treated	$31.2^{a}\pm0.5$
Corn	Control	$22.4^{b}\pm0.1$
	Native	$14.6^{c}\pm0.2$
	Control heat-treated	$14.2^{c}\pm0.1$
	Heat-treated	$10.8^{d}\pm2.1$
Mung bean	Control	$49.8^{e}\pm0.1$
	Native	$47.4^{ef}\pm1.7$
	Control heat-treated	$42.4^{fg}\pm1.5$
	Heat treated	$42.7^{fg}\pm0.9$
Potato	Control	$25.7^{h}\pm1.7$
	Native	$24.4^{h}\pm1.5$
	Control heat-treated	$25.1^{h}\pm0.9$
	Heat-treated	$24.5^{h}\pm1.7$

Mean±SD of triplicate samples

Values followed by the same letter within the same column are not significantly different ($p>0.05$)

Swelling Power and Solubility

The swelling power and solubility of control, hydrolyzed native and heat-treated starches are summarized in Table 3. From the results, only the swelling power and solubility of hydrolyzed native corn starch were higher compared to hydrolyzed heat treated starch. This might be due to the amylose (amorphous region) in hydrolyzed corn starch has been extensively degraded. In addition, the presence of holes and channels inside the corn starch granules weakened the structure of granules and therefore, the granule could not swell to the maximum capacity. On the other hand, the swelling power of hydrolyzed native mung bean, sago, and potato starch increased compared to hydrolyzed heat-treated starch, respectively. This could be due to the fact that heat-treated starch still showed very few pores even after hydrolysis, as observed under SEM (Fig. 1); consequently, less structure was disrupted compared to corn starch. In addition, part of the amylose had presumably been degraded, thereby allowing the starch granule to absorb water more easily and swell more easily when subjected to heat. The solubility of starches showed that all hydrolyzed starches exhibited increment in solubility after 24 h of hydrolysis. After hydrolysis, amylose had been degraded into low molecular weight components such as glucose and dextrin. Those components could contribute to solubility when granules swell as shown in Table 1.

Pasting Properties

The pasting profiles of native and heat-treated starches after 24 h of hydrolysis are presented in Table 4. The pasting temperature of heat-treated starches increased slightly compared to native starches, as the results of heat treatment before hydrolysis. This could be due to the

Table 3 Swelling power and solubility of control and hydrolyzed starches after 24 h of hydrolysis in granular state (35°C)

Sample		Swelling power (g/g)	Solubility (%)
Sago	Control	12.8^{efg}±3.4	5.3^{cd}±0.4
	Native	13.3^{efg}±1.8	7.3^{de}±0.9
	Control heat-treated	11.6^{cde}±0.3	2.8^{abc}±0.2
	Heat-treated	14.0^{fg}±0.5	4.5^{bc}±0.6
Corn	Control	11.1^{bcde}±0.5	2.0^{ab}±0.3
	Native	9.1^{ab}±0.4	2.3^{ab}±0.5
	Control heat-treated	9.8^{abc}±0.1	1.1^{a}±0.3
	Heat-treated	8.5^{a}±0.2	1.7^{a}±0.2
Mung bean	Control	10.3^{abcd}±0.1	4.6^{bc}±0.4
	Native	13.4^{ef}±2.1	7.2^{de}±1.4
	Control heat-treated	9.0^{ab}±0.1	2.2^{ab}±1.2
	Heat-treated	12.2^{def}±0.1	7.2^{de}±0.9
Potato	Control	15.0^{g}±1.7	2.8^{abc}±0.6
	Native	19.5^{h}±1.5	8.8^{e}±0.9
	Control heat-treated	26.7^{i}±0.9	9.3^{e}±0.6
	Heat-treated	28.5^{i}±1.7	13.2^{f}±0.8

Mean±SD of triplicate samples

Values followed by the same letter within the same column are not significantly different ($p>0.05$)

Table 4 Pasting properties of 8% native and heat-treated starch by Rapid Visco Analyzer (RVA) after 24 h of hydrolysis

Sample		Pasting temperature (°C)	Viscosity (cP)		
			Peak	Breakdown	Setback
Corn	Control	86.0^{e}±1.6	913.2^{g}±0.9	297.6^{g}±0.5	142.8^{e}±1.3
	Hydrolyzed native	86.2^{e}±0.9	536.4^{a}±0.6	108.0^{c}±5.5	140.4^{e}±0.8
	Control heat-treated	87.1^{e}±0.8	878.4^{f}±1.2	229.2^{f}±0.1	169.2^{g}±2.3
	Hydrolyzed heat-treated	87.4^{e}±1.8	638.4^{b}±0.8	150.0^{d}±0.7	285.6^{k}±1.4
Mung bean	Control	75.8^{cd}±1.3	698.4^{c}±1.2	28.8^{a}±2.1	268.8^{j}±1.3
	Hydrolyzed native	75.1^{c}±0.5	913.2^{g}±2.5	166.8^{e}±1.6	253.2^{i}±0.6
	Control heat-treated	76.9^{cd}±0.4	946.8^{h}±0.4	43.2^{b}±0.5	286.8^{k}±0.9
	Hydrolyzed heat-treated	76.7^{cd}±0.9	$1{,}042.8^{j}$±1.9	102.0^{c}±1.2	240.0^{h}±2.1
Sago	Control	76.0^{cd}±0.9	794.4^{d}±0.5	550.8^{h}±0.5	91.2^{b}±1.7
	Hydrolyzed native	75.9^{cd}±1.4	820.8^{e}±1.5	570.0^{i}±1.5	102.0^{c}±1.4
	Control heat-treated	76.7^{cd}±0.2	$1{,}104.0^{l}$±0.8	759.6^{k}±0.5	162.0^{f}±1.2
	Hydrolyzed heat-treated	77.1^{d}±0.2	987.6^{i}±1.9	746.4^{j}±3.8	129.6^{d}±0.8
Potato	Control	65.7^{a}±0.2	$1{,}326.0^{m}$±0.3	969.6^{l}±0.3	102.0^{c}±0.9
	Hydrolyzed native	66.4^{ab}±1.2	$1{,}076.4^{k}$±3.9	736.8^{j}±5.0	87.6^{a}±1.1
	Control heat-treated	67.8^{b}±0.2	$6{,}229.2^{o}$±1.8	$4{,}149.6^{n}$±6.5	421.7^{l}±0.7
	Hydrolyzed heat-treated	67.5^{b}±0.2	$3{,}889.2^{n}$±1.7	$2{,}455.2^{m}$±8.5	607.2^{m}±1.2

Mean±SD of triplicate samples

Values followed by the same letter within the same column are not significantly different ($p>0.05$)

annealing treatment, which causes better packing structure in heat-treated starch, thus increasing the pasting temperature.

Heat-treated starch showed an increased value in peak viscosity compared to native starch. As the starch granules were undergoing heat treatment, the granules would swell and thus lead to swollen granules that are larger in volume compared to native starch, resulting in high viscosity. The ability of starch granules to swell during heat treatment would also facilitate the action of enzyme during hydrolysis. Therefore, heat treatment affected the hydrolysis profile and pasting properties of starch granules.

Heat-treated corn and potato starch showed a significant increased in setback viscosity compared to the respective control starches. It shows that corn and potato starch had greater tendency to retrograde compared to other starches. After hydrolysis, amylose was degraded into shorter chain and solutes. The presence of shorter chain, various oligosaccharides (maltose, dextrin, maltodextrin, and glucose) would increase the solubility of starches. Besides, these shorter chains are much easier to re-associate compared to longer chain of starch before hydrolysis. Therefore, the setback values of hydrolyzed starch increase after hydrolysis. Setback value also showed positive correlation with the solubility.

Particle Size Distribution

The particle size distribution and mean diameter of the native and heat-treated starches are shown in Table 5. From the results, the mean diameter of control heat-treated potato showed a drastic increment compared to control potato starch. This result indicates that heat treatment had caused some irreversible swelling of potato starch, resulting in a larger diameter of potato. This is in accordance with our swelling results (Table 3), where control heat-treated potato showed higher swelling power compared to control potato.

Table 5 Mean diameter of the native and heat-treated starches after 24 h of hydrolysis in granular state (35°C)

Sample		Mean diameter (μm)
Corn	Control	25.00^{d}±0.2
	Native	11.94^{a}±0.1
	Control heat-treated	24.92^{d}±0.1
	Hydrolyzed heat-treated	11.97^{a}±0.1
Mung bean	Control	22.02^{c}±0.2
	Native	19.75^{b}±0.6
	Control heat-treated	21.37^{c}±0.6
	Hydrolyzed heat-treated	20.56^{b}±0.1
Sago	Control	29.52^{f}±0.1
	Native	28.90^{e}±0.1
	Control heat-treated	29.48^{g}±0.1
	Hydrolyzed heat-treated	28.59^{e}±0.4
Potato	Control	46.42^{h}±0.1
	Native	42.12^{g}±0.2
	Control heat-treated	68.62^{i}±1.0
	Hydrolyzed heat-treated	42.16^{g}±0.4

Mean±SD of triplicate samples

Values followed by the same letter within the same column are not significantly different ($p>0.05$)

The mean diameter of all the hydrolyzed heat-treated starches showed a significant decrease compared to control heat-treated starch. According to Lauro et al. [2], during hydrolysis, the granule would be attacked extensively. The formation of small particles or porous structure observed in particle size analysis indicated granule fragmentation due to the hydrolysis. Our result is in agreement with Tukomane et al. [23] who reported that heat treatment did not alter the granule's shape and size. The reduction in the granule's size and shape were only due to the extensive degradation of enzyme during hydrolysis.

Conclusion

The granular starch hydrolyzing enzyme (blending of α-amylase and glucoamylase) was capable of hydrolyzing granular starches at sub-gelatinization temperature (35°C), while heat treatment before hydrolysis significantly increased the degree of hydrolysis. Heat-treated starches exhibited different properties from native ones during hydrolysis; higher degree of hydrolysis, lower amylose content, sharper X-ray peak, and higher swelling power. The relative order in the susceptibility of different starches to granular starch-hydrolyzing enzyme at sub-gelatinization temperature (35°C) was corn > mung bean > sago > potato. This conclusion was made by seeking the consistency in the results of amylose content, dextrose equivalent value, swelling power, and SEM photographs. Heat treatment process promoted the formation of more porous structure to allow for a greater accessibility of enzymes to the amorphous as well as the crystalline regions to enhance enzyme hydrolysis, which significantly change some physicochemical properties, but the extent of change was affected by the type of starch.

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