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Hydrolysis of granular starch at sub-gelatinization temperature using a mixture of amylolytic enzymes

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A B S T R A C T

Native granular starches (corn, cassava, mung bean, and sago) were hydrolyzed using a mixture of alpha-amylase and glucoamylase at 35 °C for 24 h. Hydrolyzed starches were analyzed for the degree of hydrolysis and for physicochemical and functional properties. Corn starch showed the highest degree of hydrolysis, as evidenced by the presence of distinct pores penetrating deep into the granules. Enzymatic erosion occurred mainly at the surface for cassava, whereas isolated porous structures were observed in hydrolyzed mung bean and sago starch. The amylose content was significantly lower in all starches except for sago starch. The powder X-ray diffraction of all starches showed no significant changes after hydrolysis, but hydrolyzed starches showed a more crystalline nature. The action of enzymes caused significant changes in some pasting properties and in the swelling/solubility of starches. Evidently, enzymes were able to hydrolyze granular starches to a variable degree at sub-gelatinization temperature, and produced a relatively high degree of conversion.

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1. Introduction

Starch is the most abundant form of storage polysaccharides in higher plants. In starch granules, amylose and amylopectin are densely packed in a semicrystalline state with inter- and intra-molecular bonds, they are insoluble in cold water, and are often resistant to chemicals and enzymes. Starch from any source can be used as an inexpensive source for the production of fermentable sugars containing glucose, fructose or maltose, all of which are widely used in food industries. In addition, these sugars can be fermented to produce bio-ethanol.

In the course of conventional enzymatic liquefaction, slurry containing 15–35% starch is gelatinized, where it is heated to 105 °C to physically disrupt the granule and open the crystalline structure for the enzyme action (Singh and Soni, 2001). This increases the viscosity of the slurry by 20-fold (Robertson et al., 2006), and therefore makes mixing and pumping difficult. The gelatinized starch is liquefied with thermostable alpha-amylase, and is then saccharificated with

glucoamylase at a much lower temperature of 50–60 °C. The whole process requires a high-energy input, which increases the production cost of inverted sugar products.

In view of energy costs, effective utilization of natural resources and viscosity (handling) problems, direct hydrolysis of starch below gelatinization temperature is desirable. In recent years, the importance of the enzymatic liquefaction of raw starch without heating has been well recognized, mainly due to energy savings and the effective utilization of biomass, which reduces the overall cost of starch processing (Robertson et al., 2006). This has generated a worldwide interest in the discovery of amylases that are capable of digesting raw starches and that do not require gelatinization (i.e. amylases that directly hydrolyze raw starch in a single step or that liquefy starch at moderate temperature, much below the gelatinization temperature).

It is more difficult for amylases to act on raw starch granules than on gelatinized starch. Previous studies (Iefuji et al., 1996) indicate that the saccharification of raw starch by

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amylolytic enzymes might be related to the extent of adsorption of enzyme to the starch granules. According to Leloup et al. (1990) there are several steps involved in the enzymatic reaction which are: (1) the diffusion to the solid surface, (2) the adsorption of the enzyme and finally (3) the occurrence of the catalysis. The adsorption step is essential prior to the subsequent catalytic activity. Therefore the enzyme needs to pass across the boundary between the aqueous phases and solid phases before attaching to the granule. The penetration of hydrolyzing enzymes and other large molecules, however, is restricted and only possible through pores or channels (Oates, 1997). Amylases also must functionally bind glucan chains through several glucose units to their subsites (Oates, 1997). These subsites are found at the active centre of the enzyme and are capable of interacting with one glucose residue in the substrate. There are 4–9 subsites at the active centre. The number and the position of the subsites are unique for each type of amylase (Meagher et al., 1989). The hydrolysis occurs layer by layer with an attacked layer of granule being completely hydrolyzed (Wang et al., 1995). Besides that, a structural support which is known as the specialized starch granule-binding domains has been identified to exist in some amylases and glucoamylases. This binding site is separate from the active centre site and it is considered as essential for granule hydrolysis (Hayashida et al., 1990).

Most raw starch digesting enzymes reported to date hardly digest native (raw) starch or cannot produce high yields of fermentable sugars. Therefore, enzymes that can digest raw starch are economically attractive because they would increase the range of starch sources for direct hydrolysis. With technological advancements in biotechnology and enzyme engineering, a new generation of amylolytic enzymes has been discovered. These granular starch-hydrolyzing enzymes have been used in low-energy processes and can effectively hydrolyze starch that has not been cooked. The commercial enzymes are available in a blend of alpha-amylase and glucoamylase, which can hydrolyze insoluble granular (uncooked) starch into fermentable sugars by enabling the depolymerization of starch to glucose in a simultaneous saccharification and fermentation (SSF) process for alcohol production.

The objectives of this research were (1) to compare the action of a blend of amylolytic enzymes for their ability to hydrolyze different starch in its native (raw) granular state (at sub-gelatinization temperature) to fermentable sugars and (2) to examine the effect of hydrolysis on some physicochemical and functional properties.

2. Experimentals

2.1. Materials

Corn (*Zea mays*), cassava (*Mannihot esculenta* Crantz) 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).

2.2. Enzyme

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*. The specific gravity of the enzyme is 1.10–1.15 g/mL and the optimum pH ranged from 4.0 to 4.5. The recommended temperature is 20–40 °C and the minimum activity is ≥ 456 GSHU/g (GSHU is defined as granular starch-hydrolyzing units). 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 (Miller, 1959). The activity of enzyme was 3736 unit/g starch. The enzyme activity units are given as provided by the enzyme manufacturers. The assay protocol for determining enzyme activity can be obtained from the enzyme manufacturers.

2.3. Determination of moisture content

The moisture content of starch samples were determined by using IR-30 Moisture Analyzer (Denver Instrument, Colorado, USA). Starch (5 g) were spread uniformly on the pan and heated at 105 °C.

2.4. Starch hydrolysis

The starch slurry (25%, w/v) was prepared in 400 mL of sodium acetate buffer. The enzyme (3736 unit/g starch) was added (1%, w/v) into the samples. Samples were then incubated in an incubator shaker (JEIO Tech, SI-600R, 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. This step was done quickly to minimize further hydrolysis of the starch. Preliminary experiments have established that the enzyme deactivation method does not appear to cause significant starch hydrolysis. The pH of starch suspensions was adjusted back to a pH of 5–6 by washing and filtering the starch with distilled water. Starch residues were collected and dried at 40 °C for 2 days.

2.5. Dextrose equivalent (DE)

The reducing sugar value was measured using the dinitrosalicylic acid method Miller (1959) 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, Kyoto, Japan). Glucose was used as the standard. Each analysis was performed in duplicate. DE was calculated as follows:

$$DE = \frac{\text{g reducing sugar expressed as glucose}}{\text{g dry solid weight}} \times 100\%$$

2.6. Scanning electron microscopy

The microstructure of starch granules was viewed with a field emission scanning electron microscope (FESEM Leo Supra 50VP, Carl-Zeiss SMT, Oberkochen, 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.

2.7. X-ray diffraction

Crystallinity patterns of starch granules were examined by X-ray diffraction, as described by Lauro et al. (1999). 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 μ m of Ni-foil. Scattered radiation was detected using a proportional detector. Relative crystallinity was calculated following the method described by Cheetham and Tao (1998).

2.8. Amylose content

Amylose content of each sample was determined in triplicate, according to the procedure described by McGrance et al. (1998).

2.9. Swelling and solubility

Swelling power and solubility of starch were determined in triplicate, using a slightly modified version of the method described by Schoch (1964). Starch (100 mg, dry basis) was accurately weighed in a 50 mL ependorf tube and 10 mL of distilled water was added. The tube was placed in a water bath at the peak temperature for that particular starch (corn = 95 °C, cassava = 89 °C, mung bean = 95 °C, sago = 83 °C, as determined separately from pasting profile) for 10 min until the suspension was translucent. The solution was centrifuged (2328 \times g, 15 min), and then, the supernatant was carefully discarded. The swollen starch sediment was weighed. To determine the amount of soluble starch, an aliquot (5 mL) of the supernatant was dried overnight in an oven at 110 °C. Swelling power was the ratio in weight of the wet sediment to the initial weight of dry starch. The solubility was the ratio in weight of the dried supernatant to the initial weight of starch.

2.10. Pasting properties of starch

The pasting properties of starches were determined using a Rapid Visco™ Analyzer (Model RVA Series 4, Newport Scientific Pvt. Ltd., Warriewood, Australia). About 2 g of starch (corrected to 14% moisture basis) and 25 mL of distilled water were combined and stirred in the aluminium RVA sample canister. Temperature was held at 50 °C for 1 min, 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 960 rpm for the first 10 s to evenly disperse the starch slurry, and was then reduced to 160 rpm for the remainder of the experiment. The units of viscosity are expressed as RVU.

2.11. 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).

3. Results and discussion

The specific mode of enzyme attack depends on both the botanic origin of the starch granule and the enzyme(s)

involved (Tester and Morrison, 1990). Therefore, starch granules from different groups, including cereals (corn), roots (cassava), legumes (mung bean) and palms (sago), were chosen to better understand their relative susceptibility/resistance to enzymatic hydrolysis.

3.1. Scanning electron microscopy (SEM)

All four types of starches were observed under SEM for both control and hydrolyzed starch (Fig. 1). Control starches were starch that was hydrolyzed without the addition of enzyme while hydrolyzed starches were hydrolyzed with the addition of enzyme (Stargen 001). The susceptibility of starch granules can be classified by the intensity and the manner by which the granules are eroded and corroded (Gallant et al., 1982). SEM photographs revealed that the hydrolysis of starch granules did not occur uniformly.

From SEM micrographs, the hydrolyzed corn starch exhibited more porous granules compared to hydrolyzed mung bean, cassava and sago starches. After hydrolysis, hydrolyzed corn (Fig. 1) and mung bean starch (Fig. 1f) showed deeper holes and more porous structure as compared to hydrolyzed cassava and sago starch. Meanwhile, hydrolyzed cassava and sago starch showed rough surface with some of the granules are still remained intact (Fig. 1d and h). O'Brien and Wang (2008) found that the porous structure formed during enzymes attack would be larger and deeper into granules as a result of more extensive hydrolysis and also the presence of pores and pinholes on the surface of starch. The relative amounts of porous starch after hydrolysis in each group was as follows: corn > mung bean > cassava > sago.

The granule surface of the control starches appeared smooth except for corn granules (Fig. 1). A magnified view of corn and mung bean granules revealed small pores and pits that were randomly distributed on the surface (Fig. 2a and b). These natural pores would enhance enzyme penetration into starch granules during hydrolysis (Sarikaya et al., 2000). This can be seen clearly in Figs. 1b and 3a, where hydrolyzed corn starch is highly degraded with the appearance of many large pinholes and distinct layered structures. According to Aggarwal and Dollimore (1998), starches that are rapidly digested with enzymes, such as corn and wheat starch, have surfaces that are readily attacked, with the formation of canals. Pinholes were also randomly distributed on the surface of mung bean granules (Fig. 2b), but they were less evident compared to corn starch granules. Similarly to corn starch granules, these structures could provide a larger available surface area for enzyme attack. In contrast, control cassava (Fig. 1e) and sago (Fig. 1g) starch did not have natural pores or deep channels. Limited and isolated porous structures were observed in hydrolyzed mung bean starch, cassava starch and sago starch.

Corn starch granules from different location in the endosperm can have very different appearances. Typically, the hard endosperm has polyhedral shapes where as the soft endosperm has more spherical granules. While mung bean starch granules had irregular shapes, including oval, round and bean-shaped. Enzymatic attack was nevertheless restricted to specific areas of the granule, but was not limited to its surface. This is evident by examining the pinholes in corn and mung bean granules (Fig. 1b and f). According to Sarikaya et al. (2000), the enzyme would penetrate into starch granules through natural pores and disrupted the inside part of the starch granules. This can be seen in Fig. 3a as charac-

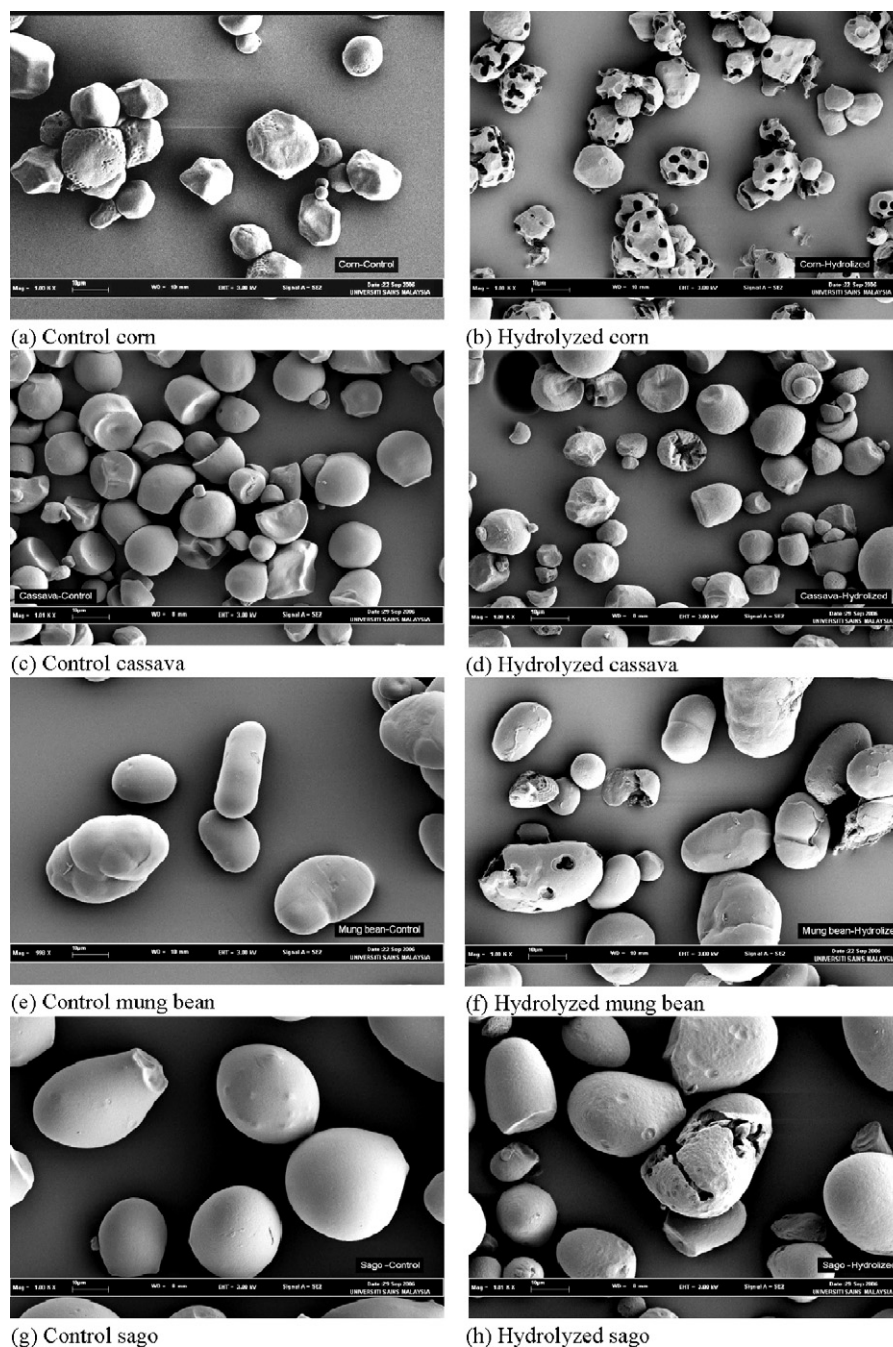


Fig. 1 – SEM micrographs (1000 \times) for control and hydrolyzed starches after hydrolysis at sub-gelatinization temperature (35 $^{\circ}$ C) for 24 h (scale bar = 10 μ m).

teristic lamellar arrangement was displayed in corn starches after enzymatic treatment.

SEM micrographs for cassava (Fig. 1e and f) showed that enzymatic erosion occurred mainly at the surface; for example, the smooth surface of the granules had been roughened and solubilized, which resulted in single, large hole depression (Fig. 3b). This is consistent with a previous report by Franco et al. (2002). Most of the cassava granules were in the truncated form (Fig. 1e). Truncatures are weak points in the granule structure that lead to increased susceptibility (Valetudie et al., 1993). Hydrolysis seems to proceed in a layered manner. Hydrolyzed sago starch observed under the SEM (Fig. 1h) has an oval shape, which is characteristic of surface erosion that consists of occasional crevassing (superficial scratches) to form deep internal cracks. Few differences were detected between the control and hydrolyzed starch granules. There

was heterogeneity in starch granule degradation, with most granules remaining intact or having only slight eroded.

3.2. Hydrolysis of starch

Corn, cassava, sago and mung bean starches were hydrolyzed to determine their relative susceptibilities (or resistances) following reactions with enzymes at 35 $^{\circ}$ C for 24 h. The degree of hydrolysis for these starches was determined by measuring dextrose equivalent at different time intervals (Table 1).

According to the values of dextrose equivalent, the relative order of hydrolysis was as follows: corn starch > mung bean starch > cassava starch > sago starch. Corn starch yielded the highest amount of dextrose equivalent (53%). This is consistent with SEM observations, which showed the presence of numerous pores on hydrolyzed corn starch granules (Fig. 1b).

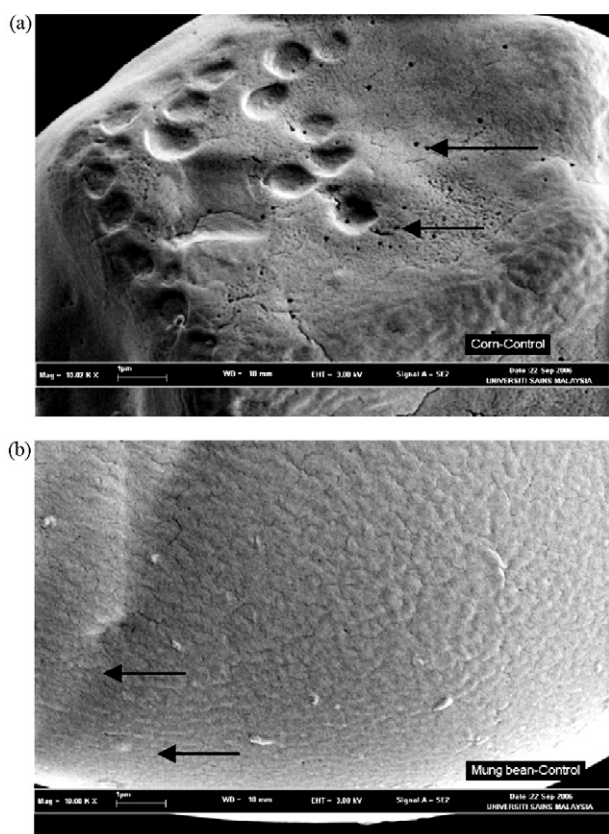


Fig. 2 – SEM image showing the presence of pores and pinholes (shown by an arrow) on the surface of (a) control corn granule and (b) control mung bean granule (3000 \times) (scale bar = 1 μ m).

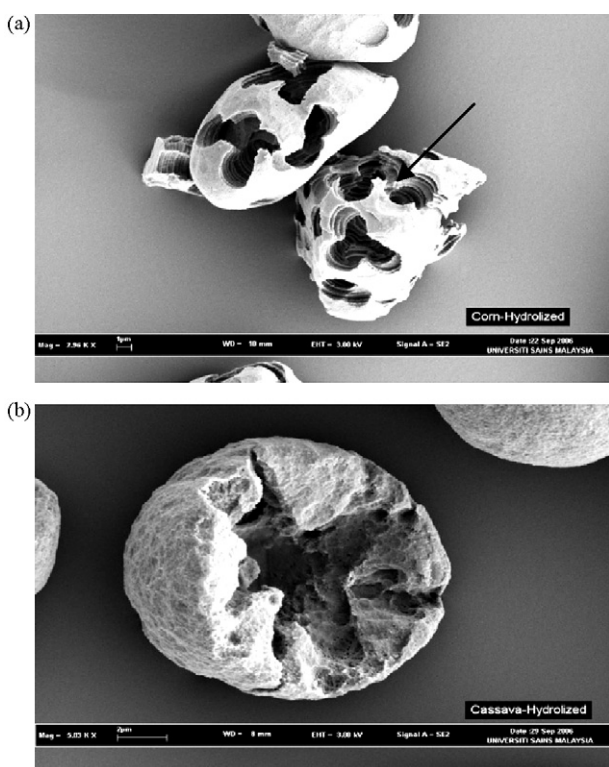


Fig. 3 – (a) Characteristic lamellar arrangement (shown by an arrow) in hydrolyzed corn starch (3000 \times). Scale bar = 1 μ m. (b) Rough surface on hydrolyzed tapioca starch granule (5000 \times). Scale bar = 2 μ m.

Table 1 – Dextrose equivalent of control and hydrolyzed starches^a after 24 h of hydrolysis.

Time (h)	Dextrose equivalent, DE (%)			
	Corn	Mung bean	Cassava	Sago
0	0.9 \pm 0.3	0.4 \pm 0.2	0.3 \pm 0.1	0.3 \pm 0.1
1	11.6 \pm 0.5	5.8 \pm 0.8	6.2 \pm 0.2	2.0 \pm 0.6
2	14.4 \pm 0.5	7.4 \pm 0.1	8.6 \pm 0.1	3.3 \pm 0.1
3	13.6 \pm 0.9	6.2 \pm 0.2	9.8 \pm 0.2	5.0 \pm 0.1
4	18.5 \pm 1.0	9.9 \pm 0.5	13.1 \pm 0.3	5.6 \pm 0.3
8	28.9 \pm 0.9	16.6 \pm 0.2	17.9 \pm 1.0	6.9 \pm 0.1
12	35.5 \pm 0.5	20.9 \pm 0.2	24.1 \pm 0.8	12.0 \pm 0.5
16	44.2 \pm 1.2	28.0 \pm 0.4	24.9 \pm 1.0	13.2 \pm 0.2
24	52.6 \pm 0.7	36.3 \pm 1.5	35.4 \pm 0.9	15.7 \pm 0.5

^a Values are expressed as mean \pm SD of triplicate samples.

The high susceptibility of corn starch to enzymatic hydrolysis might be due to the presence of natural pores on the corn starch. This observation is consistent with the findings of Zhang and Oates (1999), who reported that cereal starches are generally less resistant to enzymatic degradation than non-cereal starches. According to Juszczak et al. (2003), pores present on starch surfaces could become centers of enzymatic attack.

In contrast, cassava, mung bean and sago starches showed relatively lower susceptibilities to enzyme hydrolysis compared to corn starch, where this observation is in agreement with the previous results (Zhang and Oates, 1999). After 24 h of hydrolysis, relatively high dextrose equivalent values can be attained for mung bean starch (36%), cassava starch (35%) and sago starch (16%).

SEM observations revealed that a number of small pinholes might be present on the surface of native mung bean starch granules (Fig. 2b). Gallant et al. (1997) have also reported the pinhole structure of mung bean starch. Presumably, these opening enable enzymes to enter granules and therefore increase the rate of hydrolysis but still showed lower DE compared to corn starch. This is due to the presence of smaller pits and pinholes on the surface of mung bean starch (Fig. 2b), in contrast with corn starch which had bigger and more pores on the surface (Fig. 2a). From SEM observation (Fig. 1e), most of the cassava granules were in the truncated form. The presence of truncatures, which are the weak points in the cassava granule, increases susceptibility. This result agrees with those by Valetudie et al. (1993). Thus, the rate of hydrolysis of cassava starches was enhanced.

After 24 h, sago starch yielded the lowest value of dextrose equivalent. This observation is in agreement with Wang et al. (1996), who reported that raw sago starch was a poorer substrate for enzyme action compared to corn and cassava starches. Furthermore, Sopade and Kiaka (2001) reported the absence of pit (pores) on sago native starch granules and the absence of natural pores on the surface of granules. Therefore, sago is less susceptible to enzyme attack.

3.3. X-ray diffraction pattern

Corn starch and cassava starch showed the A-pattern, whereas sago starch and mung bean starch showed the C-pattern (data not presented). Both control and hydrolyzed starch had the same X-ray pattern; however, hydrolyzed starch had sharper peaks and a more crystalline character. This is evident by the increase in the relative crystallinity value after

Table 2 – Amylose contents (%) of starches under different conditions.

Starch samples	Amylose content (%)
Corn	
Raw	23.1 ^c ± 0.4
Control	22.9 ^c ± 0.2
Hydrolyzed	17.2 ^a ± 0.4
Cassava	
Raw	18.8 ^b ± 0.2
Control	18.6 ^b ± 0.2
Hydrolyzed	16.8 ^a ± 0.5
Sago	
Raw	27.9 ^d ± 0.1
Control	27.5 ^d ± 0.7
Hydrolyzed	26.1 ^d ± 1.7
Mungbean	
Raw	52.4 ^f ± 0.4
Control	52.1 ^f ± 1.1
Hydrolyzed	45.8 ^e ± 0.7

Values followed by the same letter in the same column are not significantly different ($P < 0.05$) ($n = 3$).

hydrolysis (except for cassava). These data suggest that the amorphous region of the granule was hydrolyzed more extensively than the crystalline region. This observation agrees with Gallant et al. (1972), who suggested that amylolysis primarily occurs in the amorphous region of starch granules. In comparison to corn starch, mung bean starch and sago starch, hydrolyzed starch of cassava had a decrease in relative crystallinity compared to control starch. It is possible that, apart from amorphous region, a portion of the amylopectin in crystalline regions was also degraded.

3.4. Apparent amylose content

Amylose content was determined in raw (native) starch, control starch and hydrolyzed starch. The analyses reveal that the amylose content of raw and control starches showed no significant changes in each of the four types of starch, as shown in Table 2. This result indicates that the enzyme deactivation method used in this study did not cause significant starch hydrolysis. After 24 h of hydrolysis, hydrolyzed corn starch exhibited the highest reduction (24%) in amylose content compared to control corn starch, followed by mung bean (12%), cassava (10%) and sago (6%). This trend is consistent with the rate of hydrolysis discussed earlier. Manelius and Bertoft (1996) reported that both amylose and amylopectin fractions are hydrolyzed simultaneously in the amorphous fractions (amylose and intercrystallite regions of amylopectin) of starch granules. Amorphous regions, which could be easily degraded by enzymes, were present throughout starch granules. Evidently, the enzyme preferentially attacks and hydrolyzes amylose in the amorphous region of the granule. Sago starch showed the smallest reduction in amylose content after hydrolysis. This may be explained by the presence of a protective layer in sago starch granules, which enhances its resistance to hydrolysis (Manelius and Bertoft, 1996). Therefore, as a result of this layer, amylose in sago was protected and preserved, which is evident from the insignificant changes in amylose content after hydrolysis.

3.5. Swelling power and solubility

The swelling power and solubility of control and hydrolyzed starches of corn, cassava, mung bean and sago are summarized in Table 3. Only corn starch significantly ($P < 0.05$) decreased in swelling power after enzymatic hydrolysis. This result might be due to fact that the amylose (amorphous region) in hydrolyzed corn starch was extensively degraded. Additionally, the presence of holes and channels inside corn starch granules weakened the structure of granules. Therefore, corn starch granules cannot swell to maximum capacity. Schoch and Maywald (1968) stated that when the amylose content was high, the swelling was restricted and the hot-paste viscosity was stabilized. This statement is in agreement with our results. Control mung bean starch had the lowest swelling power, and it contained the highest amount of amylose (52%). The amylose content in control starches was higher than that in hydrolyzed starches (Table 2). Therefore, the swelling power was higher in hydrolyzed starch than in control starch. Tester and Morrison (1990) also concluded that amylose inhibits swelling, especially in the presence of lipids because amylose-lipids tend to form and retard swelling. Therefore, corn starch, which is high in lipid content, can also lead to low swelling power as shown by our results.

Conversely, hydrolyzed mung bean, cassava and sago starches exhibited less extensive degradation compared to hydrolyzed corn starch. Intact granules were still present and abundant. Therefore, starch granules could absorb water and swell when subjected to heat. Furthermore, hydrolyzed mung bean, cassava and sago starches could swell more than control starches. Hydrolyzed mung bean, cassava and sago starches also had very few pores after enzyme hydrolysis and less structure was disrupted, as observed under SEM. In addition, part of the amylose was degraded; therefore, starch granules could absorb water and swell more easily.

Swelling power and solubility provide evidence of the magnitude of interaction between starch chain within the amorphous and crystalline domains. The extent of this interaction is influenced not only by the amylose content value, but also chain length and molecular weight distribution, degree of branching, difference in morphological structure of granules such as specific surface area and accessible pore volume (Singh and Singh, 2001).

3.6. Pasting properties of starches

Table 4 shows the pasting profiles of control and hydrolyzed corn, mung bean, cassava and sago starch. The pasting temperatures for control and hydrolyzed starches were not significantly ($P > 0.05$) different (Table 4), indicating that the onset temperature of viscosity remained the same after enzymatic hydrolysis. The viscosity of corn starch and cassava starch significantly ($P < 0.05$) decreased after enzymatic hydrolysis. The decrease in peak viscosity might be due to the fact that some of the more fragile granules had disintegrated. The extensive degradation of corn starch by the enzyme was evident from the many pores on the surface of the granules. These holes reduced the ability of the granule to hold water and therefore decreased peak viscosity. Furthermore, the amylose for corn starch was degraded into low molecular weight soluble components. This reduced the integrity of the granular structure, making it more fragile, more easily disrupted and less viscous.

Table 3 – Swelling power and solubility of control and hydrolyzed starches^a.

Starch	Swelling power (g/g) ^b		Solubility (%) ^b	
	Control	Hydrolyzed	Control	Hydrolyzed
Corn	13.9 ^d ± 0.3	11.1 ^b ± 0.5	10.6 ^c ± 0.9	15.7 ^d ± 4.0
Tapioca	15.9 ^a ± 0.1	16.5 ^c ± 0.3	3.2 ^a ± 0.2	5.3 ^{a,b} ± 0.8
Sago	13.3 ^d ± 0.5	16.6 ^f ± 0.4	2.8 ^a ± 0.7	3.7 ^a ± 0.5
Mung bean	11.1 ^b ± 1.1	15.4 ^e ± 0.5	6.5 ^b ± 0.9	11.4 ^c ± 0.7

^a Values are expressed as mean ± SD of triplicate samples; values followed by the same letters in the same column are not significantly different at $P < 0.05$.

Table 4 – Pasting properties of 8% starch pastes by rapid visco analyzer (RVA)^a.

Starch sample	Pasting temperature (°C)	Viscosity (cP)		
		Peak	Breakdown	Setback
Corn				
Control	86.4 ^c ± 0.2	891.6 ^b ± 1.1	171.6 ^c ± 1.2	58.8 ^a ± 7.1
Hydrolyzed	87.8 ^c ± 3.3	544.8 ^a ± 0.9	104.4 ^b ± 1.1	200.4 ^b ± 1.0
Tapioca				
Control	70.6 ^a ± 0.3	1846.8 ^f ± 9.8	690.0 ^e ± 11.2	574.8 ^d ± 7.6
Hydrolyzed	70.6 ^a ± 0.5	1531.2 ^e ± 0.3	613.2 ^d ± 1.1	519.6 ^d ± 3.0
Sago				
Control	75.3 ^b ± 0.6	1202.4 ^{c,d} ± 1.0	717.6 ^e ± 1.7	231.6 ^b ± 3.1
Hydrolyzed	75.6 ^b ± 0.2	1285.2 ^d ± 1.0	742.8 ^e ± 0.5	250.8 ^b ± 1.5
Mung bean				
Control	75.0 ^b ± 0.7	847.2 ^b ± 0.5	28.8 ^a ± 0.3	368.4 ^c ± 1.0
Hydrolyzed	74.1 ^b ± 0.6	1132.8 ^c ± 1.9	124.8 ^{b,c} ± 1.6	324.0 ^c ± 2.0

^a Values are expressed as mean ± SD of triplicate samples; values followed by the same letters in the same column are not significantly different at $P < 0.05$.

Similarly, the decrease in peak viscosity for hydrolyzed cassava starch could be due to the low rigidity of the granules in a close packed system. The granules may have had low peak viscosity because they lost their rigidity. Lii et al. (1996) suggested that the rigidity of the starch granular structure might be directly proportional to its amylose content and inversely proportional to the degree of granular swelling. Therefore, the low rigidity of hydrolyzed cassava starch granules was due both to its low amylose content and to the high swelling power of control cassava starch granules.

These results indicate that the water binding capacity and the ability of these starches to swell decreased after hydrolysis. In contrast, the peak viscosity of mung bean starch significantly ($P < 0.05$) increased, from 847.2 to 1132.8 cP (34% increase relative to control). The increased value of peak viscosity for mung bean starch might be due to the fact that its amylose content was still high after enzymatic hydrolysis. This allows the granules to swell to a high extent and increase the viscosity. Sago had a high resistance towards degradation, as its granular structure remained intact and did not disrupt the water binding capacity, even after hydrolysis; therefore, no significant changes were detected in the peak viscosity.

Breakdown indicated the stability of swollen starch granules to shearing and mixing. According to Pearson correlation, peak viscosity was significantly ($P < 0.01$) correlated with breakdown viscosity ($r = 0.76$). As peak viscosity increased, breakdown viscosity also increased. Thus, the breakdown viscosity of corn, cassava and mung bean starches showed a similar trend with peak viscosity after enzymatic hydrolysis. The enzyme treatment caused the breakdown of corn and cassava starches to decrease by 39% and 11%, respectively. In

contrast, the breakdown of mung bean starch increased more than threefold.

4. Conclusions

Amylolytic enzymes were capable of hydrolyzing granular starches at sub-gelatinization temperature (35 °C). Moreover, these enzymes achieved a relatively high conversion of starch to fermentable sugars. Starch granules exhibited variable responses to enzymatic degradation. SEM micrograph showed that corn starch had been extensively degraded by exhibiting the most porous structure followed by mung bean, cassava and sago starch. Corn starch also showed the highest value of DE produced and could be due to the presence of pores on its surface. Susceptibility was likely affected by the botanical origin, nature of the granule surface (such as the presence of natural pores), amylose content and other compound granules. The relative order in the susceptibility of different types of starches after 24 h of enzyme hydrolysis at sub-gelatinization temperature was as follows: corn > mung bean > cassava > sago. No significant changes observed in X-ray diffraction pattern and all starches showed significant decrease in amylose content, indicating that hydrolysis mainly occurred in the amorphous region.

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