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Dual Modification of Starch via Partial Enzymatic Hydrolysis in the Granular State and Subsequent Hydroxypropylation

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The effect of enzymatic pretreatment on the degree of corn and mung bean starch derivatization by propylene oxide was investigated. The starch was enzymatically treated in the granular state with a mixture of fungal α-amylase and glucoamylase at 35 °C for 16 h and then chemically modified to produce enzyme-hydrolyzed-hydroxypropyl (HP) starch. Partial enzyme hydrolysis of starch in the granular state appeared to enhance the subsequent hydroxypropylation, as judged from the significant increase in the molar substitution. A variable degree of granule modification was obtained after enzyme hydrolysis, and one of the determinants of the modification degree appeared to be the presence of natural pores in the granules. Enzyme-hydrolyzed-HP starch exhibited significantly different functional properties compared to hydroxypropyl starch prepared from untreated (native) starch. It is evident that the dual modification of starch using this approach provides a range of functional properties that can be customized for specific applications.

KEYWORDS: Starch; enzyme hydrolysis; amylase; modified starch; hydroxypropylation

INTRODUCTION

Native starches from various plant sources have different properties. These inherent characteristics can be exploited to produce various types of starch-based food products. However, native starch lacks the versatility necessary to function adequately under rigorous industrial processing. Thus, industries have developed a variety of ways to modify starch to provide the desired stability and tolerance toward a wide range of processing techniques. Typically, starch modification is achieved through a number of ways, for example, derivatizations such as etherification, esterification and cross-linking of starch; depolymerization (acid or enzymatic hydrolysis and oxidation of starch); or physical treatment using heat or moisture. Chemical modification causes structural alterations, and it introduces new functional groups, thereby affecting the physicochemical properties of the starch and making it appropriate for various industrial uses.

Hydroxypropylated starch is a popular type of chemically modified starch. Hydroxypropyl starches are important in food applications due to their relatively low pasting temperature, high paste clarity, and desirable low-temperature storage stability. Unmodified starch granules generally have a low degree of substitution by chemical reagents, possibly because the granule surface allows only a limited reaction. Previous studies (*1, 2*) have shown that starch granule microstructural features may play a role in reagent accessibility. For example, Whistler et al. (*1*) also observed that corn starch granules pretreated with glucoamylase prior to esterification show esterification levels five times greater than normal corn starch granules. Their dye experiment showed derivatization not only on the surface but also in the granule central interior, suggesting reagent penetration to the granule interior through pores and derivatization of a lower density porous center. Thus, if the substituent reagent (propylene oxide) had more access to the interior or subsurface of the starch granule, a higher degree of substitution could be expected. Thus, the goal of the present study was to explore the possibility of increasing reagent accessibility by treating starch with amylolytic enzymes in the granular state prior to hydroxypropylation. The availability of a new commercial enzyme that can hydrolyze granular starch in a low-energy process and effectively hydrolyze starch without the need for starch gelatinization makes this approach possible.

This study was designed to achieve two objectives: (a) to investigate the effects of pretreating starch in the granular state (below gelatinization temperature) with amylolytic enzymes on the efficiency of subsequent starch derivatization with propylene oxide, and (b) to characterize the physicochemical and functional properties of the modified starch after the amylolytic and hydroxypropylation treatments. Corn and mung bean starch were chosen for this study. Modified corn starch is widely used commercially. Mung bean starch was chosen because of its

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higher amylose content than that of corn starch. It is interesting to examine the effect of enzyme pretreatment on the amorphous region containing mainly amylose on the subsequent hydroxypropylation.

MATERIALS AND METHODS

Materials. Mung bean starch and corn starch were purchased from Pearl Island Packaging Sdn. Bhd. (Pulau Pinang, Malaysia) and SIM Company Sdn. Bhd. (Pulau Pinang, Malaysia), respectively. The commercial enzyme is a product of Genencor International, B.V. (Genencor International, Palo Alto, CA). This enzyme contains *Aspergillus kawachi* α amylase expressed in *Trichoderma reesei* and a glucoamylase from *Aspergillus niger*. The specific gravity of the enzyme solution is 1.10−1.15 g/mL, the optimal pH is 4.0 to 4.5, and the recommended temperature is 20−40 °C. Enzymatic activity was determined by reaction at 37 °C with soluble starch (1%, v/v) buffered with sodium acetate (pH 4.4). Aliquots were taken after 10 min to determine the amount of D-glucose released. Glucose levels were determined using the dinitrosalicylic acid method (*3*). The enzyme activity was 3.74 units/mg starch. Propylene oxide was obtained from BDH Chemical Ltd. (Poole, UK).

Hydrolysis of Starch in the Granular State. Starch (20%, w/v, dry basis) was suspended in acetate buffer (pH 4.4). The enzyme (1% w/v; 3.74 units/mg starch) was added to the starch slurry. The starch suspensions were incubated in an orbital incubator shaker SI-600R (JEIO Tech, Seoul, Korea) at 35 °C with a shaking speed of 150 rpm for 16 h. The selection of hydrolysis time was based on the preliminary experiment which showed that under the experimental conditions used, maximum degree of hydrolysis was obtained after 16 h. During hydrolysis, a 1-mL aliquot of the hydrolysate was taken to assay for reducing sugars using the 3,5-dinitrosalycylic acid (DNS) method (*3*). The degree of hydrolysis was expressed as dextrose equivalents (DE), which is calculated according to the formula (gram of reducing sugar expressed as glucose)/(g dry solid weight) × 100. After 16 h, the hydrolysis was halted by adding a predetermined amount of 2.0 M HCl until the pH was 1.5−1.6. 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. Then, the pH of the starch suspensions was immediately neutralized by washing the starch with distilled water, and filtering the starch through Whatman paper number 3 until the pH of the starch was 5.6 or higher. After washing, the starch samples were dried at 40 °C in an oven for 2−3 days. The dried samples were sieved to a size of 250 μm. Moisture content of the starch samples was determined from the loss in weight after drying triplicate 10 g samples in an air-oven at 105 °C to constant weight. Duplicate samples and control starch samples lacking enzyme were prepared from each starch type.

Scanning Electron Microscopy (SEM). The starch samples were attached to aluminum specimen stubs with double-sided adhesive tape and coated with a 20−30 nm layer of gold using a sputter coater [Polaron (Fisons) SC 515 VG Microtech, Sussex, U.K.]. The coated starch samples were observed using a Scanning Electron Microscope (FESEM Leo Supra 50VP, Carl-Zeiss SMT, Oberkochen, Germany).

Amylose Content. The amylose content of the starch was determined using the colorimetric method described by McGrance et al. (*4*). The reported values are the means of triplicate measurements.

Hydroxypropylation of Starch. Hydroxypropyl starch was prepared according to the method of Hjermstad (*5*). Sodium sulfate (10% w/w, based on the dry weight of the starch) was added to the starch slurry (20%, w/v) and stirred. The pH was adjusted to 10.5 with 5% NaOH. For the treatment group, propylene oxide was added to 10% the amount of the dry weight of the starch. The flask was capped and stirred for 30 min at room temperature. Then the suspension was incubated at 40 °C for 24 h while being stirred at 200 rpm in an orbital incubator shaker SI-600R (JEIO Tech, Seoul, Korea) to prevent sedimentation. After 24 h, the suspensions were then adjusted to pH 5.5 with 10% HCl. Samples were neutralized and then washed immediately with distilled water and filtered. The samples were dried at 40 °C for 2−3 days and mill-ground and sieved to the size of 250 μm. Hydroxypropyl content of the starch was determined by the method of Johnson (*6*) and expressed as molar substitution. The method involves hydrolysis of the hydroxypropyl group to propylene glycol which in turn is dehydrated to propionaldehyde and the enolic form of allyl alcohol. These products are measured spectrophotometrically at 590 nm after they are reacted with ninhydrin to form a purple color. Hydroxypropyl starch was prepared in duplicate using native starch (untreated) and hydrolyzed starch, and the mean of the values was determined.

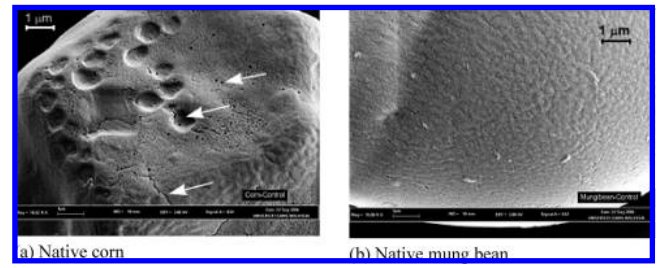

Figure 1. SEM micrographs for native corn and mung bean starch. Arrows show pores, pits and deep cavities on corn starch granule (1000×; scale bar = 1 μm).

Swelling Power and Solubility. Swelling power and solubility of starch samples were determined in triplicate by adopting the method of Schoch (*7*). Swelling power is the ratio in weight of the wet sediment to the initial weight of dry starch. The solubility is the ratio of the dried supernatant weight to the initial weight of dry starch.

Pasting Properties of Starch. The pasting properties of starch were determined using the Rapid Visco Analyzer (model RVA Series 4, Newport Scientific Pvt. Ltd., Warriewood, Australia). Distilled water (25 mL) was added to each sample in an aluminum canister to achieve a 10%, w/v suspension. The starch suspension was heated from 50 to 95 °C at the rate of 12 °C/min, held at 95 °C for 2.5 min, and then cooled to 50 °C at the same rate. The paddle rotated at 960 rpm for the first 10 s, then at 160 rpm for the remainder of the test. Pasting temperature, peak viscosity, hot paste viscosity, breakdown, setback, and cold paste viscosity were determined from RVA plots. Each sample was run in triplicate to determine a mean value.

Statistical Analysis. Duncan's least significant test was used to compare means at the 5% significance level. Simple Pearson's correlation and regression analysis were evaluated using SPSS 12.0 statistical software for Windows (SPSS, Inc., Chicago, IL).

RESULTS AND DISCUSSION

In the following discussion, the term "native starch" refers to the starch that has not undergone any form of enzyme treatment or chemical modification. "Control starch" (corn or mung bean) refers to starch samples that were incubated at 35 °C without enzyme. "Native-HP" refers to the starch that was hydroxypropylated directly from the native starch. "Enzyme-hydrolyzed-HP" refers to starch that was hydrolyzed enzymatically in the granular state prior to being hydroxypropylated, whereas "control-HP" refers to the control starch that was incubated in the absence of enzyme and subsequently subjected to hydroxypropylation.

Scanning Electron Microscopy (SEM). The microstructure and morphology of the starch granules before and after hydrolysis were examined using scanning electron microscopy. A magnified view of a native corn granule (**Figure 1a**) shows distinct pores and depression (caused by the granule forming next to protein bodies) on the granule surface. This observation is consistent with the results of Fannon et al. (*8*), who reported that some granules of corn have small openings (pores) randomly distributed over their surfaces, often found in clusters and present in different degrees. They suggested that the pores affect the pattern of attack by amylases or chemical reagents. In contrast, the native mung bean granules (**Figure 1b**) did not appear to have distinct pores or cavities on the surface. The

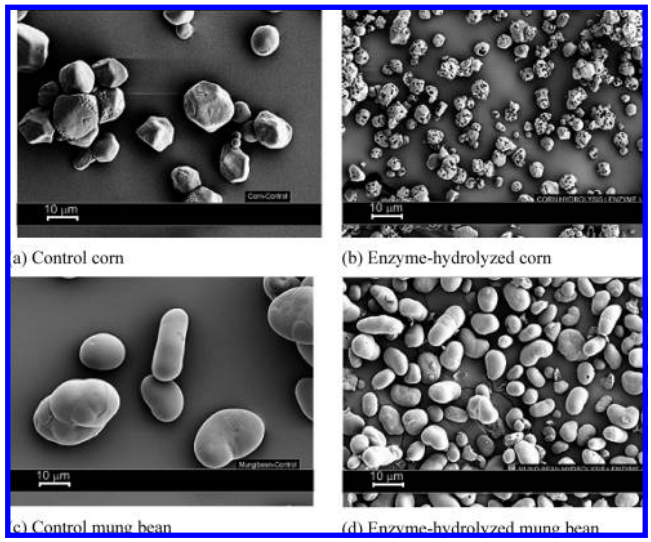

Figure 2. SEM micrographs for control and enzyme-hydrolyzed starches after incubation at subgelatinization temperature (35 °C) for 16 h (1000×; scale bar = 10 μm).

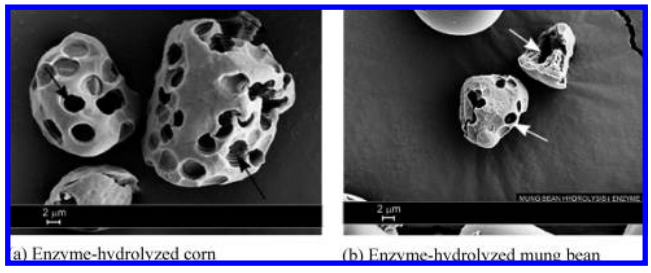

Figure 3. SEM micrographs for enzyme-hydrolyzed starches after hydrolysis at subgelatinization temperature (35 °C) for 16 h. Arrows show deep cavities on starch granule (5000×; scale bar = 2 μm).

absence of natural pores on the mung bean granule surface has also been reported by Hoover and Zhou (*9*).

The SEM micrographs (**Figure 2**) show that the enzyme-hydrolyzed corn and mung bean starch samples showed different patterns of hydrolysis by the amylase enzyme. It is evident that hydrolysis did not occur uniformly among the granules. However, it was noted that corn starch granules were hydrolyzed more uniformly compared to mung bean starch as evidenced by the appearance of many large pinholes in corn starch granules. In contrast, limited and isolated porous structures were observed in hydrolyzed mung bean starch. This was likely because the individual granules were not equally susceptible to enzymatic degradation, since granules probably vary in their ability to adsorb enzyme. A similar observation has been reported by Aggarwal and Dollimore (*10*). Despite the specific mode of attack, starch hydrolysis occurs on a granule-by-granule basis, with an attacked granule being completely or extensively hydrolyzed (*11*).

The exoactivity of glucoamylase and the endoactivity of α-amylase in the commercial enzyme appear to work synergistically to "drill" and widen the pinholes in starch granules. The presence of pores, channels, and cavities in corn starch granules may provide potential sites for initial enzyme attack, which then subsequently widen these openings to allow additional enzyme molecules to enter the granule interior (*2, 8, 12*). Kanenaga et al. (*13*) also reported that corn starch granules containing pores are more susceptible to enzymatic digestion. This concept is supported by **Figure 2b** and **Figure 3a** of our results, where enzyme-hydrolyzed corn starch was observed to be highly degraded and showed many large pinholes and distinct layered structures. The distinct layered structures seen in the bore holes have also been described by Gallant et al. with α-amylase (*14*). **Figure 2b** also reveals uniformly degraded corn starch granules with only a small residual area apparently unaffected by the treatment.

Helbert et al. (*15*) proposed the concept of "centripetal" hydrolysis (surface-to-core directed) and "centrifugal" hydrolysis (involving regions and layers) of native granules by α-amylase. Both forms of hydrolysis were found to occur for corn (*16*). These studies found that the enzyme first randomly diffuse, onto the surface of granules. Hydrolysis then starts at these points, proceeds radially toward the center (centripetal) and results in the formation of a pore and ultimately a channel to the granule core. Finally, the enzyme becomes trapped within the granule and causes diffusion-regulated local hydrolysis, which gradually spreads (centrifugal).

In contrast to corn starch granules, distinct pores were not observed in mung bean granules following enzyme hydrolysis. **Figure 2d** shows that most of the granules were still intact, despite the fact that the granule surface could have been eroded by surface abrasion. The magnified view in **Figure 3b** shows that enzymatic hydrolysis did not occur uniformly in mung bean starch granules because some areas appeared to be more susceptible to attack than others. According to Oates (*11*), 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 starch granules may be different from that on corn starch granules. Thus, the mung bean may behave similarly to other types of starch that are relatively resistant to enzyme hydrolysis, such as potato starch. Oates (*11*) proposed that the degradation pattern of resistant types of starch 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. The central part of the granule erodes rapidly, whereas hydrolysis of the outer shell lasts longer (*17*). In addition, Oates (*11*) also proposed that types of starch that are predominantly hydrolyzed by surface abrasion may contain some type of protective coating around their outer layer, such as blocklets formed by highly structured packing of unit cells.

Dextrose Equivalent. The degree of hydrolysis of corn starch (70.3%) was significantly higher than that of mung bean starch (27.6%) ($P < 0.05$). This suggests that, in the granular state, corn starch is more susceptible to enzyme hydrolysis than mung bean starch. This idea is consistent with the results of Ring et al. (*18*), who reported that the rate and extent of α-amylase hydrolysis of starch from different botanical sources are generally higher for starch from cereals such as wheat and corn than for starch from legumes or tubers.

The greater susceptibility of corn starch to enzymatic hydrolysis could be attributed to the presence of natural pores on the surface of corn starch (**Figure 1a**). As mentioned in the preceding discussion, granules containing pores and channels (corn starch) are attacked by enzymes differently and to a greater extent than those without pores and channels (mung bean starch). The lower degree of hydrolysis for mung bean starch could also be attributed to its higher amylose content (**Table 1**). According to Riley et al. (*19*), native starch hydrolysis by amylases was inversely related to amylose content, where high amylose starches are resistant.

Apparent Amylose Content. Table 1 shows the apparent amylose content for starch from corn and mung bean, including

Table 1. Effect of Enzyme Hydrolysis at Subgelatinization Temperature (35 °C) on the Amylose Content of Starches

starch	amylose content (%)[a]
corn	
native	27.9 b ± 0.5
control	27.2 b ± 0.1
enzyme-hydrolyzed	11.0 a ± 0.2
mung bean	
native	56.5 d ± 1.26
control	56.3 cd ± 0.3
enzyme-hydrolyzed	55.0 c ± 1.29

[a] Values are means ± standard deviation ($n = 3$). Means within a column with different letters are significantly different ($p < 0.05$).

the native, control, and enzyme-hydrolyzed samples. The amylose content was 27.9% for native corn starch and 56.5% for native mung bean starch. Sandhu et al. (*20*) reported that the amylose content of starches separated from different corn types ranged between 15.3% and 25.1%. For mung bean starch, Singh et al. (*21*) reported an amylose content of 47%.

Amylose is found mainly in the amorphous regions of the starch granule in the form of single helical structures. Oates (*11*) reported that enzymes degrade the amorphous regions more easily than the crystalline lamellae. Thus, the enzyme preferentially attacks and hydrolyzes amylose in the amorphous region of the granule.

Data in **Table 1** show that enzyme-hydrolyzed corn starch had lower amylose content than that of native or control starch. This indicates that the amylose was extensively degraded after 16 h of hydrolysis. In a separate study (paper submitted), it was reported that the relative crystallinity value of corn and mung bean starch was increased after hydrolysis. These data suggest that the amorphous region of the granule was hydrolyzed more extensively than the crystalline region. Similar observation on crystallinity was also reported by Shariffa et al. (*22*) after hydrolysis of tapioca and sweet potato starch using the same enzymes. The natural pores in the corn starch granule apparently allow the enzyme to work effectively to degrade amylose and produce low molecular weight products. On the other hand, amylose content in enzyme-hydrolyzed mung bean starch was not significantly lower than the content in native or control starch. This observation is consistent with the lower degree of hydrolysis of mung bean starch compared to corn starch. It is possible that, apart from amorphous region, a portion of the amylopectin in crystalline regions was also degraded, hence contributing to the degree of hydrolysis measured. However, further work is warranted to substantiate this statement. Apart from the apparent absence of pores in the granule, the higher amylose content in mung bean starch may also restrict swelling, thus reducing the extent of hydrolysis of the amorphous region. Amylose content has been considered to be a resistant factor that restricts swelling of starch granules (*23*). Pearson correlations also shows a significant negative relationship between % DE and amylose content ($r = -0.6$, $P < 0.05$).

It is evident that the combination of fungal α-amylase and glucoamylase in the enzyme preparation is effective in hydrolyzing the granular starch. Amylase is capable of catalyzing the hydrolysis of the (1→4) glycosidic bonds found between the α-D glucopyranose residues, whereas glucoamylase catalyzes the hydrolysis of both the α-D-(1→4) and α-D-(1→6) linkages from the nonreducing end of the starch chain. Using both of these enzymes together allows more effective hydrolysis of amylose to glucose.

Molar Substitution. Molar substitution (MS) is a measure of the average number of hydroxyl groups on each anhydroglucose unit that are derivatized by substituent groups. **Table 2** presents MS values of hydroxypropylated native and enzyme-treated corn and mung bean starch.

Table 2. Molar Substitution for Native-HP, Control-HP, and Enzyme-Hydrolyzed-HP Starches[a]

starch	molar substitution[b]
corn	
native-HP	0.0204 b ± 0.0003
control-HP	0.0236 c ± 0.0028
enzyme-hydrolyzed-HP	0.0366 d ± 0.0013
mung bean	
native-HP	0.0016 a ± 0.0002
control-HP	0.0023 ab ± 0.0001
enzyme-hydrolyzed-HP	0.0028 b ± 0.0001

[a] The terms native-HP, control-HP, and enzyme-hydrolyzed-HP are defined in the text. [b] Mean ± standard deviation of triplicate samples. For each starch, values followed by the same letter are not significantly different ($P > 0.05$).

Table 3. Effect of Hydroxypropylation on Swelling Power and Solubility for Corn and Mung Bean Starches

starch	swelling power[a] (g/g)	solubility[a] (%)
corn		
native	11.6 bc ± 0.5	1.1 a ± 0.1
native-HP	15.3 f ± 0.1	1.3 a ± 0.1
control-HP	15.0 f ± 0.2	2.1 b ± 0.2
enzyme-hydrolyzed-HP	13.1 e ± 0.7	3.2 d ± 0.2
mung bean		
native	10.6 a ± 0.1	3.7 d ± 0.5
native-HP	12.1 cd ± 0.0	4.0 d ± 0.1
control-HP	11.3 b ± 0.1	4.0 d ± 0.5
enzyme-hydrolyzed-HP	12.7 de ± 0.2	7.3 e ± 0.1

[a] Values are means ± standard deviation ($n = 3$). Means within a column with different letters are significantly different ($p < 0.05$).

The enzyme-hydrolyzed-HP starch from both corn and mung bean showed a significant increase in molar substitution compared to native-HP and control-HP starch ($P < 0.05$; **Table 2**). This result suggests that alteration of the granular structure (internal structure) by enzymatic action increases contact with the modifying reagent, resulting in increased molar substitution.

Enzyme-hydrolyzed-HP corn starch showed a higher molar substitution than enzyme-hydrolyzed-HP mung bean starch (**Table 2**). A high degree of hydrolysis for corn starch correlates with a higher value of molar substitution ($r = 0.609$, $P < 0.05$). This could be attributed to the substantial degradation of the starch by the enzyme, as shown for corn starch in **Figure 3a**. It is apparent that enzyme-hydrolyzed corn starch granules contained deep channels as a result of the enzyme action thus allowing access of reagents into the granule interior. According to Kim et al. (*24*), hydroxypropylation takes place at the relatively less organized central core region of the starch granule. Huber and BeMiller (*25*) also reported that the material within the inner regions of potato starch granules was more susceptible to reaction with propylene oxide than the outer granule layers. Thus, the modifying reagent can penetrate more easily into the core of the granules of enzyme-hydrolyzed corn starch than into the core of the granules of enzyme-hydrolyzed mung bean starch, because the latter lack such channels. Thus, in the case of the mung bean sample, the reagent was limited to diffusing inward through the exterior granule surface, resulting in a lower molar substitution.

Swelling Power and Solubility. The swelling power and solubility of corn starch and mung bean starch are presented in **Table 3**. Both types of modified starch showed a significant increase in swelling power compared to the corresponding native

Table 4. Pasting Properties[a] for Native and Hydroxypropylation Corn and Mung Bean Starches

starch	pasting temp (°C)	peak viscosity (RVU)	breakdown (RVU)	setback (RVU)
corn				
native	80.4 e ± 0.5	151.9 f ± 0.9	33.4 b ± 2.8	5.2 a ± 0.3
native-HP	74.7 d ± 0.1	164.9 g ± 2.6	73.5 e ± 1.2	55.1 d ± 1.6
control-HP	74.6 d ± 0.2	127.6 d ± 2.5	72.0 e ± 2.0	22.7 b ± 1.6
enzyme-hydrolyzed-HP	50.2 a ± 0.1	87.9 a ± 1.8	49.3 c ± 3.6	82.2 e ± 1.2
mung bean				
native	74.3 d ± 0.4	269.0 h ± 2.6	60.1 d ± 7.9	95.9 f ± 7.4
native-HP	72.0 bc ± 0.3	288.8 e ± 0.8	20.8 a ± 0.8	77.4 e ± 0.3
control-HP	72.3 c ± 0.4	229.9 b ± 0.6	17.2 b ± 2.3	30.7 c ± 1.1
enzyme-hydrolyzed-HP	71.7 b ± 0.2	110.5 c ± 5.3	35.6 b ± 5.0	23.5 b ± 2.0

[a] Values are means ± standard deviation ($n = 3$). Means within a column with different letters are significantly different ($p < 0.05$).

starch. Swelling power is a measure of hydration capacity and the magnitude of interaction between starch chains within the amorphous and crystalline domains (*26*). Incorporation of hydroxypropyl groups into the starch chains caused disruption of inter- and intramolecular hydrogen bonds, thereby weakening the granular structure of starch and increasing hydration capacity of the starch granules. The substituted starch is also more easily hydrated because of the hydrophilic nature of the hydroxypropyl groups (*27*).

The Pearson correlation analysis shows a significant positive correlation between molar substitution and swelling power ($r = 0.607$, $P < 0.01$). For corn starch, the control-HP and native-HP showed a higher swelling power than enzyme-hydrolyzed-HP starch. A lower swelling power for enzyme-hydrolyzed-HP corn starch may be due to extensive penetration by the enzyme, which was able to hydrolyze the amorphous and crystalline lamellae deep in the granule (**Figure 3a**). The disruption of these lamellae and the presence of deep channels as a result of enzyme action reduced the swelling power of the granules.

On the other hand, the swelling power was not significantly different ($P > 0.05$) between enzyme-hydrolyzed-HP and native-HP mung bean starch. This could be due to the fact that enzyme-hydrolyzed mung bean starch still showed very few pores even after hydrolysis, as observed under SEM (**Figure 3b**); consequently, less structure was disrupted compared to corn starch. However, all modified mung bean starch samples showed a substantial increase in swelling power compared to native starch. Apparently, enzyme hydrolysis altered the substitution of hydroxypropyl groups, which consequently helped to increase swelling power. 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 starch samples is shown in **Table 3**. It should be noted that the values are rather low compared to values reported in the literature. For example, Sandhu et al. (*28*) reported values ranging from 13% to 20% for several types of normal corn starch. We suspect that some soluble materials have been removed during filtration step after the enzyme hydrolysis. All modified starch samples showed an increase in solubility over native starch, except for control-HP, native-HP mung bean starch and native-HP corn starch (**Table 3**). This solubility was due mainly to the leaching of amylose when the granule swelled. Leaching from swollen starch granules occurred after the granules were immersed in water and heated to 80 °C during the solubility test. When leaching occurs, amylose and low molecular weight components diffuse out from the swollen granule, whereas most of the amylopectin is thought to remain in the granule residue (*29*). The solubility of enzyme-hydrolyzed-HP starch (for both corn and mung bean) was observed to be higher than the control-HP, native-HP, and native starch samples. Control-HP and native-HP starch samples had low solubility presumably because no hydrolysis had occurred in the absence of enzyme pretreatment.

Pasting Properties of Starch. Table 4 shows the RVA parameters for native, native-HP, control-HP, and enzyme-hydrolyzed-HP starch. The pasting temperature for modified starch samples was significantly lower than that of native starch. The Pearson correlation analysis shows the molar substitution to correlate significantly and negatively with pasting temperature ($r = -0.80$, $P < 0.01$). Hari et al. (*30*) attributed this to two factors, both due to extensive substitution with hydrophilic hydroxypropyl groups: (1) progressive weakening of the granular structure of starch, and (2) increased accessibility of starch to water.

Table 4 also shows that the pasting temperature for enzyme-hydrolyzed HP corn starch was significantly lower than for control-HP starch. This can be attributed to the greater number of hydroxypropyl groups in the former, as a result of its higher MS discussed earlier. The pasting temperature of enzyme-hydrolyzed-HP corn starch was significantly lower than that of enzyme-hydrolyzed-HP mung bean starch. Pal et al. (*27*) reported that amylose content and starch granule size influence starch gelatinization temperature. The high amylose content of mung bean starch presumably creates strong associative forces in the granules, which would prevent penetration of water inside the granules and would therefore lead to a higher pasting temperature.

Peak viscosity is an indication of water-absorbing capacity. Water absorption and retention by starch granules increases granule swelling, which results in higher peak viscosity. However, the data in **Table 4** reveal that enzyme-hydrolyzed-HP starch from corn and mung bean had lower peak viscosities than native, native-HP, or control-HP starch samples. This result seems to contradict the expected higher swelling power for these starch samples based on the data in **Table 3**. It should be noted that measurements of swelling power were carried out in a dilute system that allowed maximum swelling of each granule. In contrast, a close-packed system is expected to form during pasting of starch in the RVA. In a close-packed system, the rigidity of the granule and the presence of granule fragments may influence the maximum viscosity that can be achieved. It has been suggested that the formation of pores or crevassing/abrasion on the granule surface reduces the rigidity of the granule, a process that becomes more important in a close-packed, enormously swollen granule network such as starch paste. The end result would therefore be lower peak viscosity, as seen for enzyme-hydrolyzed-HP starch samples compared to the corresponding native starch. Furthermore, the amylose content for enzyme-treated starch samples, particularly that of corn, was degraded into low molecular weight soluble components. This reduced the integrity of the granular structure,

making it more fragile and easily disruptible, and thereby reducing the viscosity.

Breakdown is a measurement of the starch granule stability during the pasting process. Extensive breakdown occurs when the starch granule swells to maximum volume but lacks the ability to retain its structure and subsequently collapses. All the samples of modified mung bean starch showed a significant decrease in breakdown compared to the native unmodified starch (**Table 4**). In contrast, all samples of modified corn starch showed significant increases in breakdown compared to the native unmodified starch.

The breakdown of enzyme-hydrolyzed-HP corn starch was higher than that of native corn starch, but lower than that of native-HP and control-HP corn starch (**Table 4**). It has been suggested that introduction of hydroxypropyl groups reduces associative forces within the starch granule (*30*). This reduction in bond strength affects the hydroxypropyl starch, which cannot withstand heating and shear strain conditions. In our study, this translated to a reduced ability of hydroxypropyl starch to retain its swollen structure during the pasting process, resulting in more breakdown. In addition, compared to native corn starch, the granules of enzyme-hydrolyzed-HP corn starch were presumably unable to retain their swollen structure upon reaching peak viscosity, because the granular structure was weakened by the numerous pores and channels.

Based on this reasoning, the enzyme-hydrolyzed-HP corn would be expected to be the least stable, i.e., to show the most breakdown among the samples of hydroxypropylated corn starch. However, this was not the case; in fact, the opposite trend was observed (**Table 4**). A reasonable explanation for this finding is that the greater swelling power of native-HP and control-HP corn starch (**Table 3**) may render the granules more susceptible to mechanical shearing during pasting, thus contributing to the greater breakdown observed for these starch samples.

In contrast to corn starch, enzyme-hydrolyzed-HP mung bean starch showed higher breakdown viscosity than did native-HP or control-HP starch (**Table 4**), suggesting that the former was more sensitive to shear disruption. Data in **Table 3** indicate that the swelling power of these starch samples was comparable, but the solubility for enzyme-hydrolyzed-HP mung bean starch was significantly higher than for the other samples. It is likely that the combined effect of swelling pressure and the weakened structural framework resulting from enzyme degradation reduces the cohesive forces within the granule, leaving it vulnerable to disruption when subjected to shear force.

Setback viscosity is determined by the reassociation of solubilized starch polymers and insoluble granular fragments during cooling. Enzyme-hydrolyzed-HP corn starch showed higher setback viscosity than did native, native-HP, or control-HP starch samples (**Table 4**). This result indicates a higher degree of starch molecule reassociation in the enzyme-hydrolyzed-HP corn starch during cooling. In addition to soluble polymers (e.g., leached amylose or short-chain, low molecular weight components), the higher setback viscosity for enzyme-hydrolyzed-HP corn starch could also be attributed to increased granule fragments or remnants. These fragments would be embedded in the matrix of the associated polymer network thus enhancing the viscosity of the system. It is also reasonable to assume that granule fragments exist in the enzyme-hydrolyzed-HP corn starch paste because the granules show less breakdown than native-HP or control-HP starch.

In mung bean starch samples, in contrast, setback viscosity of the enzyme-hydrolyzed-HP sample was lower than that of native, native-HP, and control-HP samples (**Table 4**). This is a desirable property if a highly stabilized starch is required for specific applications. In this study, a higher degree of substitution for enzyme-hydrolyzed-HP mung bean starch (**Table 2**) inhibited structural ordering in the starch pastes, thereby retarding retrogradation.

In conclusion, this study demonstrated that the extent of starch hydroxypropylation can be increased by pretreating starch granules with amylolytic enzymes capable of hydrolyzing starch in the granular state (below gelatinization temperature). The pretreatment alters the surface and interior properties of the granules but maintaining the overall granular state, allowing the hydroxypropylating agent to react more efficiently with the starch. Depending on the type of starch, a variable degree of granule modification was obtained after enzyme hydrolysis, and one of the important determinants appeared to be the presence or absence of natural pores and cavities in the granules. This study demonstrates the usefulness of a dual treatment of starch granules involving enzymatic pretreatment followed by hydroxypropylation as a method for producing starch with a range of functional properties customizable for specific applications.

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