

Molecular and granular characteristics of corn starch modified by HCl-methanol at different temperatures

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Abstract

Native corn starch was hydrolyzed with 0.36% HCl in methanol at 25 and 45 °C for periods of time up to 240 h. The action of acid penetration and hydrolysis was investigated by confocal laser scanning microscopy (CLSM), scanning electron microscopy (SEM), high-performance anion-exchange chromatography (HPAEC) and high-performance size-exclusion chromatography (HPSEC) equipped with viscometry, right-angle laser light scattering (RALLS) and refractive index (RI) detectors. Corn starch hydrolyzed at 45 °C for 240 h showed strong intensity of APTS (8-amino-1,3,6-pyrenetrisulfonic acid) fluorescence and sharp growth ring structure. Exocorrosion over the surface of corn starch was only observed on the corn starch hydrolyzed at 25 °C for 240 h and observed on all corn starch hydrolyzed at 45 °C. The M_w and R_h of acid-hydrolyzed corn starch decreased with increasing the degree of hydrolysis. The acid hydrolysis rate in methanol of corn starch was mainly dependent on the temperature, which dominated the penetration efficiency of acid.

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Keywords: Corn starch; Acid-methanol treatment; Starch granule; Confocal laser scanning microscopy

1. Introduction

Starch, the dominant carbohydrate reserve material of higher plants, mainly consists of two types of α -D-glucose homopolymers, that is, amylose and amylopectin. Amylose is an essentially linear molecule composed of anhydroglucose units connected through (1 → 4)- α -linkages with a few (1 → 6)- α -linkages (Buleon, Colonna, Planchot, & Ball, 1998) and its weight-average molecular weight (M_w) is approximately 1×10^5 – 1×10^6 (Biliaderis, 1998; Buleon et al., 1998; Mua & Jackson, 1997). Amylopectin is a much larger molecule with M_w of 1×10^7 – 1×10^9 (Yoo & Jane, 2002) and a heavily branched structure built from about 95% (1 → 4)- α - and 5% (1 → 6)- α -linkages (Tester, Karkalas, & Qi, 2004).

Inside starch granules, the assembly of macromolecules with alternating semi-crystalline clusters and amorphous growth rings is not uniform. Fannon and co-workers (Fannon, Huber, & BeMiller, 1992) reported the exist of surface pores of corn, sorghum, and millet starch granules and presented the evidences that the internal cavity at the granule helium was

connected to surface pores by channels (Huber & BeMiller, 1997, 2000). The central region of starch granule surrounding the helium is believed to be less organized (Gray & BeMiller, 2004; Huber & BeMiller, 2001) and the starch molecular arrangement at outer layer of granule also seems to be different from the inner portion (Fannon, Gray, Gunawan, Huber, & BeMiller, 2004; Ziegler, Creek, & Runt, 2005). Since the considerable heterogeneity within starch granules, the chemical modified starches are influenced by the architecture of starch granules including the way of starch molecules packing inside the granule, the exist of central cavity, and the number of the surface pores and channels, which are depended on the plant origins. The optical sections of corn starch granules, which had been subjected to a short-time treatment with aqueous merbromin solution then examined by CLSM (confocal laser scanning microscopy), indicated the possibility of non-uniform reaction with chemicals during modification (Huber & BeMiller, 2000).

The typical procedure to prepare acid-hydrolyzed starch may be divided into two ways, according to the acid used. Treatment of native potato starch in water with 15% H_2SO_4 for 30 days at room temperature produces Nageli amyloextrins (Nageli, 1874), whilst immersion starches in 2.2 N HCl at elevated temperatures (30–40 °C) results in lintnerised starch (Lintner, 1886). Moreover, Small (1919) prepared the soluble starch by refluxing starch granules in 95% ethanol containing

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0.2–1.6% (w/v) HCl for 6–15 min to produce low molecular weight dextrin. The acid modification in four different alcohols (methanol, ethanol, 2-propanol, and 1-butanol) or mixtures of alcohols produced starches with different average DP values. The highest DP value was obtained when methanol was used as the reaction medium (Ma & Robyt, 1987; Robyt, Choe, Fox, Hahn, & Fuchs, 1996a,b). The different alcohols and the mixtures of alcohols resulted in different concentrations of acid inside the granules (Ma & Robyt, 1987) and influenced on the availability or the susceptibility of the ‘various’ α -(1 \rightarrow 4)-glycosidic linkages to the reaction of acid (Robyt et al., 1996b).

Recently, Chang and co-workers (Chang, Lin, & Lii, 2004; Lin, Lee, & Chang, 2003; Lin, Lii, & Chang, 2005) reported the influence of acid-alcohol treatment conditions on the granular and molecular levels of waxy maize and potato starches. In this study, the granular and molecular changes of HCl-methanol modified native corn starch were observed by confocal laser scanning microscopy, scanning electron microscopy (SEM), high-performance size-exclusion chromatography (HPSEC), and high-performance anion-exchange chromatography (HPAEC). The findings were discussed and provided the improved knowledge of starch granular structure models from \sim micrometers to \sim nanometers scale.

2. Materials and methods

2.1. Starch and enzyme

The native corn starch was purchased from Gu Tong Foods Industrial Ltd (Chia-Yi, Taiwan). The corn starch contained 13.52% moisture and 0.05% ash, 2.72% crude fat and trace amount of crude protein on a dry basis, analyzed according to AACC methods 44-15A, 08-01, 30-20, and 46-12 (AACC, 1995). Isoamylase (EC 3.2.1.68) of *Pseudomonas amyloclavata* (59,000 U/mg) was purchased from Hayashibara Biochemical Laboratories, Inc. (Okayama, Japan). All the chemicals used in this study were reagent grade.

2.2. Preparation of acid-methanol modified starch

Native corn starch (250 g/l) was suspended in anhydrous methanol (purity > 99.9%) (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA) containing 0.36% HCl. The starch suspension was kept in water bath (25 or 45 °C) with continuous stirring. To maintain the concentration of HCl, total weight of starch suspensions were recorded daily and re-added the amount of evaporated loss of methanol. Aliquots were withdrawn at different time intervals (6, 12, 24, 72, 120, 240 h) and neutralized with 1 M NaHCO₃ in ice-bath. The suspension were then centrifuged at 4000 \times g for 5 min. The precipitates were washed with 50% ethanol three times and dried at 40 °C air-oven for 1 day.

2.3. Confocal laser scanning microscopy

Starch granules were stained by using the aminofluorophore 8-amino-1,3,6-pyrenetrisulfonic acid (APTS, Molecular

Probes, OR, USA) according to the method described by Blennow et al. (2003). To elucidate the diffusion of methanol, methanol, instead of water, was used to prepared APTS and CH₃BNNa solution to stain starch granules for 2 min, 15 h, and 36 h.

A confocal laser scanning microscopy (Leica TCS SP2 Confocal Spectral Microscope, Wetzlar, Germany) was equipped with an argon laser and an objective (100 \times Plan apo/1.4 oil). The excitation wavelength was 488 nm with 20% capacity and the light detected at the interval from 500 to 600 nm. The format of image was 30 \times 30 μ m and 512 \times 512 pixels. During image acquisition, each line was scanned eight times and averaged to reduce noise.

2.4. Scanning electron microscopy

Starch granules were mounted on circular aluminum stubs with double sided adhesive tape and coated with gold (Hitachi E101 Ion sputter, Tokyo, Japan). Scanning electron micrographs were taken using a microscope (Hitachi S-2400, Tokyo, Japan). The accelerating voltage was 20 kV.

2.5. Molecular weight distribution determined by a HPSEC-Viscometry-RALLS-RI system

Starch granules (10 mg) were dissolved in 90% dimethylsulphoxide (DMSO, 1 ml) with continuous stirring in a boiling water bath for 1 h then kept at room temperature for 8 h. The starch dispersion was mixed with four volumes of absolute ethanol and stored at 4 °C overnight. The ethanol-precipitated starch was separated by centrifugation at 1167 \times g for 5 min and washed with 95% ethanol three times. To obtain sufficient laser-light scattering and viscosity signals for calculating the molecular weight distribution of starch, the starch pellet from different extent of acid hydrolysis was diluted with different amounts of 0.1 M NaNO₃ containing 0.02% NaN₃ and stirred for 30 min in a boiling water bath. The hot sample solution was filtered through a PVDF membrane filter (5.0 μ m) (TITAN, NC, USA), then injected into a HPSEC system.

A HPSEC system consists of a Hitachi L-6000 isocratic pump (Hitachi Ltd, Tokyo, Japan) equipped with an on-line degasser (Model 3415 α , ERC, Inc., Saitama, Japan), an injection valve (100 μ l sample loop, 7725i, Rheodyne, CA, USA), a viscometer detector combined with a right-angle laser-light-scattering (RALLS) detector (Viscotek Model T-60A Dual Detector, TX, USA) and an Hitachi L-3300RI detector (Hitachi Ltd, Tokyo, Japan). The series interconnection of the viscometer detector and the right-angle laser-light-scattering detector was parallel connected with the RI detector. To monitor the molecular size distribution of acid-methanol modified starch, a TSK gel guard column, and a G5000PW and a G3000PW analytical columns (TOSOH, Tokyo, Japan) were used. The temperature of the columns was maintained at 40 °C using a column oven (Super CO-150, ENSHINE, Taipei, Taiwan). Temperature of RI detector was set at 37 °C. The water used was distilled-deionized water (18.2 m Ω cm) and the eluent was 0.1 M NaNO₃ with 0.02% NaN₃, degassed

and filtered through a Nylon membrane (0.45 μm) (Chrom-Tech, Singapore) before use. The flow rate of mobile phase was 0.5 ml/min. The system was calibrated with a dextran standard (American Polymer Standards Corporation, OH, USA) of known M_w (236,100 Da), intrinsic viscosity (0.467 dl/g), and dn/dc of 0.147 ml/g. The refraction index value of mobile phase was obtained by the reference to the water value of 1.333. The M_w and hydrodynamic radius (R_h) values of the starches was calculated with OminiSEC software 2.0.3 (Viscotek Corp., TX, USA). HPSEC determinations were duplicated.

2.6. Chain length distribution by HPAEC

Native and acid-hydrolyzed corn starch granules were dissolved in DMSO as described above. The starch pellets were diluted with distilled–deionized water to 5 mg/ml and stirred for 30 min in a boiled water bath. After cooling to room temperature, the starch solution (2 ml) was mixed with 2 ml 40 mM acetate buffer (pH 3.5) and 4 μl isoamylase (5.9 U/ μl). The mixture was incubated in a water bath at 37 °C for 3 h and carefully stirred every hour. The enzymatic reaction was stopped by heating the mixture in a boiling water bath for 10 min. The debranched starch solution was filtered through a PVDF membrane filter (0.45 μm) then injected into a HPAEC system. Besides, the acid-hydrolyzed corn starch without isoamylase debranching was also analyzed.

A Dionex Bio-LC system 300 (Sunnyvale, CA, USA) equipped with a BioLC gradient pump and a pulsed amperometric detector (PAD) was used. The filtrated sample was injected (25 μl) into a Dionex CarboPac PA1 column. The pulsed potentials and durations were: $E_1=0.05\text{ V}$ ($t_1=420\text{ ms}$), $E_2=0.75\text{ V}$ ($t_2=180\text{ ms}$), and $E_3=-0.15\text{ V}$ ($t_3=360\text{ ms}$) at range 1 (sampling periods, 16.67 ms). The eluents A and B were 100 mM NaOH and 100 mM NaOH in 500 mM NaOAc, respectively. The solutions were degassed with helium by a Dionex degas module. The flow rate was 1 ml/min and eluent gradient was in a linear gradient of eluent B from 0 to 50% during 0–70 min, then in a linear gradient to 70% of eluent B during 70–120 min. The known DP standards including glucose (ChemService, PA, USA), maltotriose, maltotetraose, maltopentaose, and maltohexaose (Fluka, Buchs, Switzerland) were used to calibrate the elution time vs. DP of sugars. HPAEC determinations were repeated three times.

2.7. Statistical analysis

The data of chain length distributions were analyzed with SAS software (Version 8.2, SAS Institute, Inc., Cary, NC) and Duncan's test was used to analyze the differences at 5% confidence level to compare mean values across the treatments.

3. Results and discussion

3.1. Effect of starch granular architecture on diffusion pathway of reagents

To trace the pathway of methanol diffusion into starch granules, the starch was stained with a methanolic APTS solution for 2 min, 15 h, and 36 h and optical sections at approximately geometric centers of native corn starches were examined by CLSM (Fig. 1). Fluorophore APTS is a small molecule with M_w 523.39 g/mol and reacts with the reducing end of each starch molecule in a nearly 1:1 stoichiometry (O'Shea, Samuel, Konik, & Morell, 1998). The methanolic APTS solution diffused into outer layer of starch granule and passed through channels into central cavity then diffused from cavity and channels outward the granule matrix (Fig. 1a and b). The diffusion appears preferentially from inside out, i.e. from central cavity toward internal part of granule. The region between granular surface and matrix surrounding interior cavity and channels were the last area for reaction to take place (Fig. 1c; Hamunen, 1995; Huber & BeMiller, 1997). The short-time treatment (15, 30, and 60 s) of native corn starch with aqueous merbromin solution revealed the similar diffusion pattern of dye into the hydrated granule matrix through both central cavity and channels (Huber & BeMiller, 2000).

3.2. Granular characteristics of acid-treated starches

To investigate the internal granular structure of corn starch with HCl-methanol modification, the starch was stained with APTS aqueous solution and reacted at 40 °C for 15 h (Fig. 2). The morphology, including size, shape or distribution of the channels, cavity and growth rings of starch granules, and fluorescence intensity of corn starches showed no significant differences between native corn starches and those hydrolyzed with 0.36% HCl-methanol at 25 °C for 6 and 240 h (Fig. 2a–c). In fact, the pale green region between central cavity and the outer layer is not always observed in all starch granules

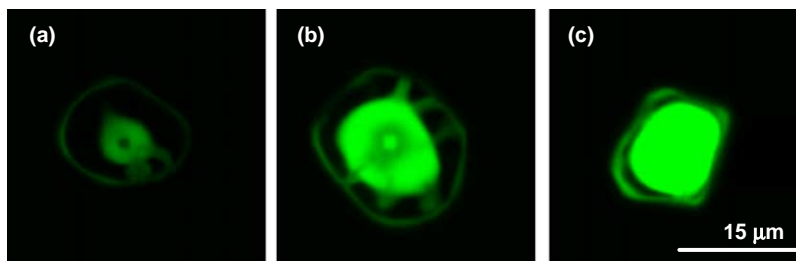


Fig. 1. Confocal laser scanning microscopy optical sections of native corn starch stained with APTS (8-amino-1,3,6-pyrenetrisulfonic acid) in methanol for 2 min (a), 15 h (b), and 36 h (c). The photo-multiplier tube (PMT) of three photos was the same (500).

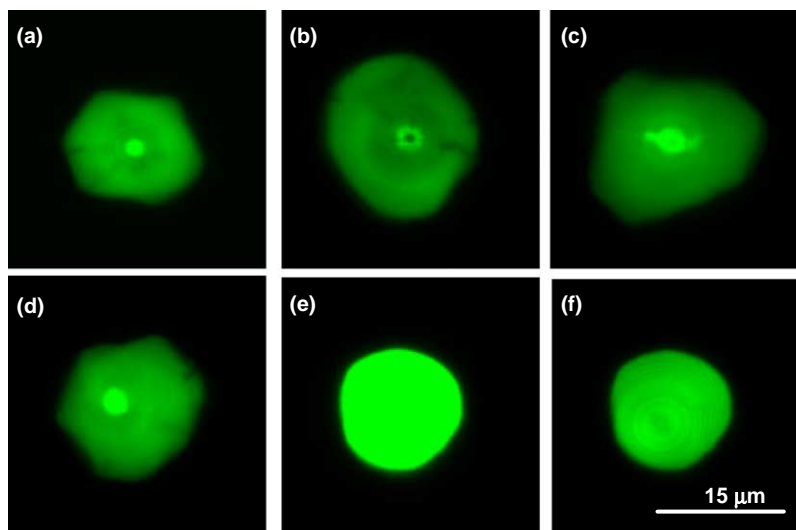


Fig. 2. Confocal laser scanning microscopy optical sections of native corn starch (a), corn starch hydrolyzed with 0.36% HCl in methanol at 25 °C for 6 h (b) and 240 h (c) and at 45 °C for 6 h (d) and 240 h (e and f). The photo-multiplier tube (PMT) of images (a–e) was 500, but image (f) was 450.

(Fig. 2b). The heterogeneity within starch granules might inherent the acid hydrolysis effect. Otherwise, corn starch treated with 0.36% HCl-methanol at 45 °C for more than 2 days showed both the increases of fluorescence intensity and the clear growth rings (Fig. 2d and e). The strong fluorescence of corn starch hydrolyzed at 45 °C for 10 days (Fig. 2e) indicated the increase of reducing end after hydrolysis which reacted with more APTS molecules. It is believed that the acid hydrolysis attacked the amorphous region first and the acid etch resulted in a better contrast between amorphous and semi-crystalline layers within starch granules (Fig. 2f).

Native corn starch granules had irregular, polygonal shapes with diameters around 10 μm (Fig. 3a). The surface

of native corn starch granules was smooth. There were no obvious defects or signs of damages on the surface of starch granules even hydrolyzed with HCl-methanol for 72 h at 25 °C (Fig. 3b). However, a slightly rough surface due to corrosion was clearly observed after acid hydrolysis for 240 h at 25 °C (Fig. 3c), whereas slight exocorrosion of starch granular surface was observed on the starch granules hydrolyzed at 45 °C for 6 h (Fig. 3d). After 12 h of hydrolysis at 45 °C, part of starch granular surface became rough (Fig. 3e). Serious exocorrosion of starch granular surface and even the destruction of starch granules were observed on the starch extensively hydrolyzed, for example, hydrolyzed for 240 h (Fig. 3f).

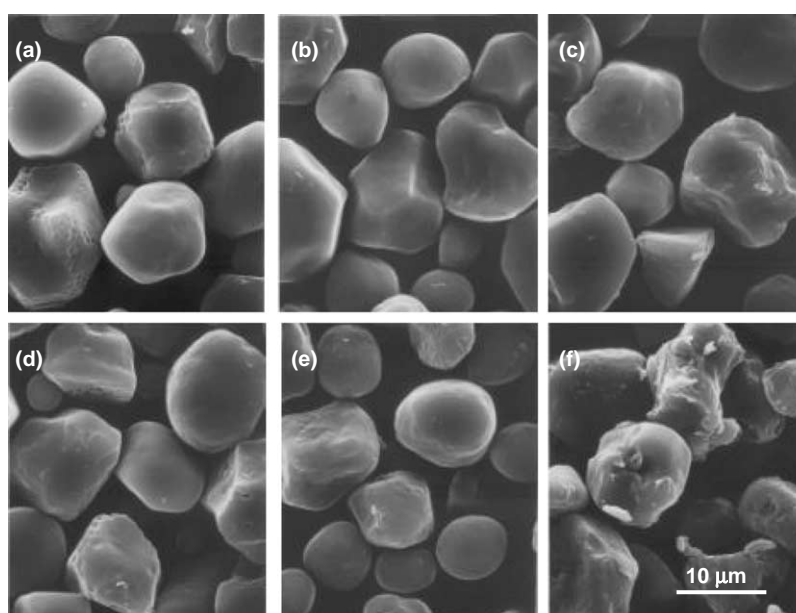


Fig. 3. Scanning electron micrographs of native corn starch (a), corn starch hydrolyzed with 0.36% HCl in methanol for 72 h (b) and 240 h (c) at 25 °C, and hydrolyzed for 6 h (d), 12 h (e), and 240 h (f) at 45 °C.

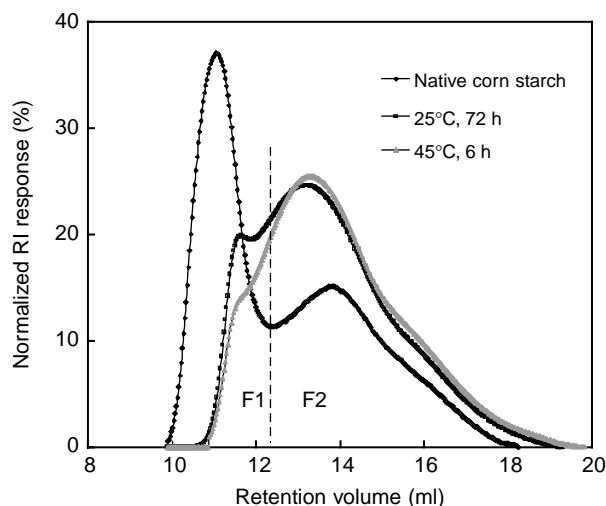


Fig. 4. High-performance size-exclusion chromatograms of native corn starch, corn starch hydrolyzed with 0.36% HCl in methanol for 72 h at 25 °C, and for 6 h at 45 °C.

3.3. Molecular characteristics of acid-treated starches

Molecular size distribution of native corn starch analyzed with HPSEC-Viscometry–RALLS-RI was presented in Fig. 4. Two major fractions were obtained on molecular size distribution of native corn starch. Fraction 1 (F1), a higher molecular weight component, was eluted at lower elution volumes and mainly corresponded to amylopectin. Fraction 2 (F2), a lower molecular weight component, was eluted at higher elution volumes and mainly corresponded to amylose. F1 and F2 of corn starch shifted to a longer retention volume after acid hydrolysis and indicated the decreases of their molecular sizes. When weight fraction of F1 was plotted vs. hydrolysis time, the corn starch hydrolyzed at 25 °C showed two different rates (Fig. 5). Two different hydrolysis rates of

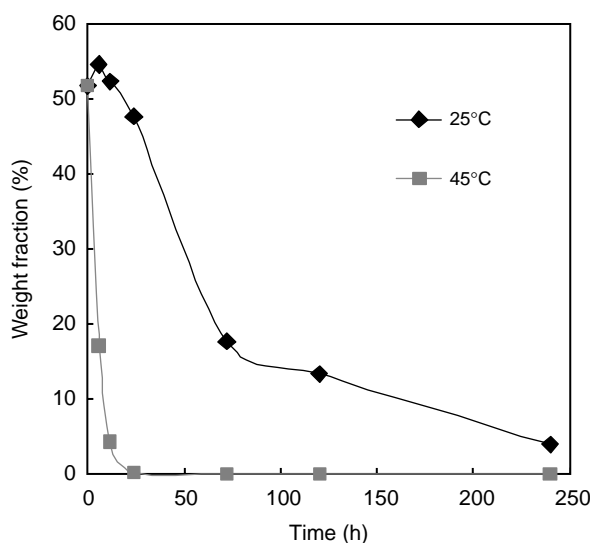


Fig. 5. Comparison of F1 weight fraction (%) of HCl-methanol hydrolyzed corn starch at 25 and 45 °C. F1 weight fraction was calculated as dividing the integration of the reflection index (RI) signals of F1 in high-performance size-exclusion chromatograms (Fig. 4) by the total area of the RI signals.

Table 1
Weight-averaged molecular weight (M_w) and hydrodynamic radius (R_h) of selected corn starches hydrolyzed with 0.36% HCl in methanol at 25 and 45 °C

Hydrolysis	Temperature (°C)	Time (h)	F1		F2	
			M_w ($\times 10^6$ g/mol)	R_h (nm)	M_w ($\times 10^6$ g/mol)	R_h (nm)
Native			513 (102) ^a	219 (16)	9.12 (0.13)	45 (2)
25		6	291 (41)	171 (13)	10.04 (0.82)	46 (2)
		72	44 (21)	76 (11)	3.43 (0.10)	22 (5)
		240	— ^b	—	1.26 (0.07)	16 (1)
45		6	26 (1)	61 (0)	2.54 (0.03)	22 (0)
		12	—	—	1.27 (0.04)	16 (0)
		240	—	—	0.05 (0.00)	4 (0)

^a Standard deviation.

^b Undetermined because of F1 weight fraction was below 10%.

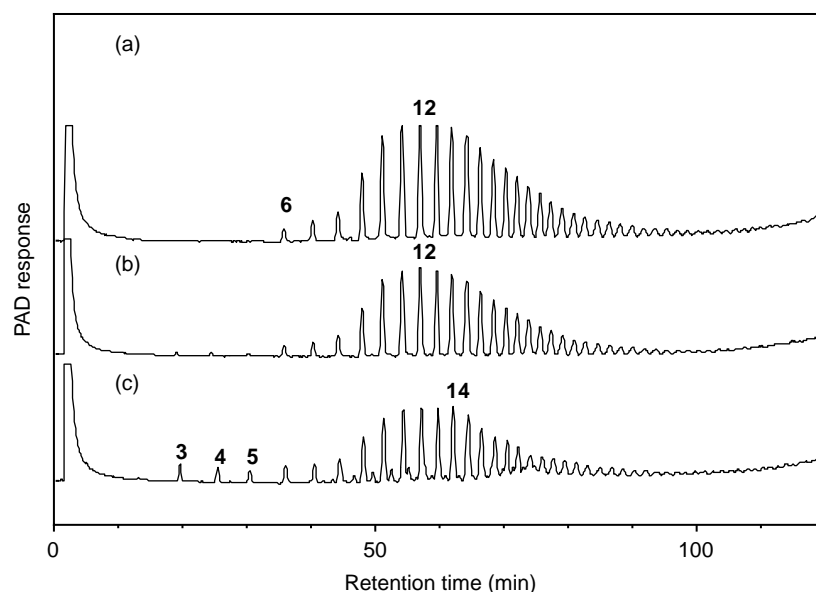


Fig. 6. High-performance anion-exchange chromatograms of isoamylase debranched corn starch (a) and with 0.36% HCl-methanol treatment at 25 °C for 240 h (b) and at 45 °C for 240 h (c).

starch hydrolyzed at 25 °C were mainly due to the diffusion pathway of methanol throughout the starch granule. The first fast rate (0–72 h) was attributed to the hydrolysis of the amorphous regions surrounding the surface of starch granules, near the channels in the granules, and the internal cavity at the granule helium. As indicated in Fig. 1c, the unstained area was probably the hardest part for acid diffusion. The second slow rate (72–250 h) was attributed to the hydrolysis of the amorphous regions in the semicrystalline architecture, and the less order regions between the granular surface and the matrix near the cavity and channels. Furthermore, the increased in temperature facilitated the diffusion rate of methanol, the flexibility of starch molecules and the reaction rate of acid hydrolysis. Thus, the corn starch hydrolyzed at 45 °C showed a very sharp decreasing reaction rate during the first 12 h, so that the F1 was disappeared quickly (Fig. 5).

The reaction of the hydrolysis of the glycosidic linkage inside starch granules are influenced by the following three main factors: (1) the available water molecules, (2) the acid participating in hydrolysis, and (3) the availability and reactivity of the glycosidic linkage. Robyt et al. (1996b) suggested that hydrolysis of the glycosidic linkage in alcoholic solution was occurred with the 10–12% water originally inside starch granule (factor (1)). The diffusion of methanol into starch matrix determined the local acid content inside the starch granules (factor (2)), while CLSM images (Fig. 1) indicated the reagent flow occurs primarily from central cavity or latterly from channels to starch matrix. The crystallinity or organization of starch molecules affect the availability and activity of the glycosidic linkage (factor (3)). It is believed that the ability of acid diffusing into starch granules at 25 and 45 °C was different. At 25 °C, acid might only diffuse into the amorphous regions near the surface, channel and central cavity of the corn starch in the first 72 h, then diffuse into the amorphous regions such as the unstained area in Fig. 1c. While,

the acid diffused almost throughout the amorphous regions of the starch granules at 45 °C during very short time. As a result, the elevated reaction temperature from 25 to 45 °C facilitated the diffusion of acid into the corn starch granules and also increased the reactivity of the glycosidic bond.

Table 1 showed the M_w and R_h of F1 and F2 of starch with different extent of acid hydrolysis. The M_w of amylopectin obtained in this study ($5.13 \pm 1.02 \times 10^8$ g/mol) closed to the reported value ($4.9 \pm 0.8 \times 10^8$ g/mol) of common corn starch obtained by Yoo and Jane (2002), but higher than the value ($2.43 \pm 0.08 \times 10^8$ g/mol) reported by Han and Lim (2004a). Differences between these results can be attributed to the sources of corn starches, the HPSEC system and starch preparation methods. Han and Lim (2004a,b) reported that excess mechanical vortexing, stirring or heating used to prepared starch dispersion significantly affected molecular weight measurement. The use of 90% DMSO as solvent with the proper combination of 1 h boiling and subsequent stirring at room temperature for 8 h (the same preparation in this study) dissolved 98% starch and minimized the chain degradation (Han & Lim, 2004a). Moreover, the R_h of amylopectin of native corn starch was 219 ± 16 nm (Table 1). R_h is considered to be related with the end-to-end size of the molecule in solution (Wyatt, 1993). The weight-average molecular weight of amylose obtained in this study ($9.12 \pm 0.13 \times 10^6$ g/mol) was higher than the value ($3.13 \pm 0.08 \times 10^6$ g/mol) reported by Han and Lim (2004a). The R_h of amylose of native corn starch was 45 ± 2 nm (Table 1). The M_w of F1 and F2 of corn starch hydrolyzed at 25 and 45 °C decreased with increasing in the reaction time of acid hydrolysis (Table 1). The faster M_w decreasing rate of F1 than F2 was attributed to the degraded both amylopectin and amylose that integrated into F2. After hydrolyzed at 45 °C for 240 h, the M_w and R_h of corn starch were 50,000 g/mol and 4 nm, respectively.

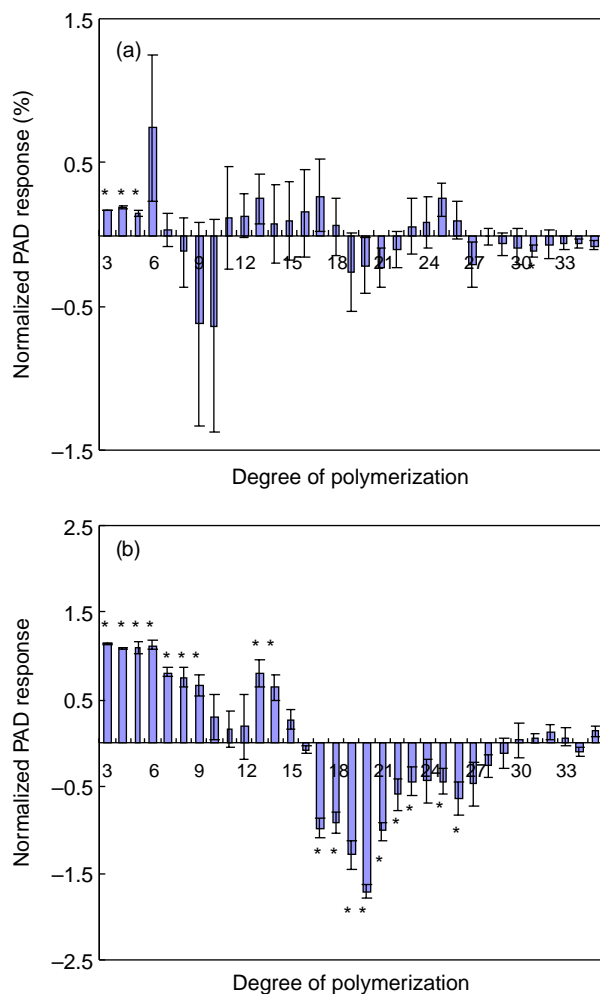


Fig. 7. Differences in percent distribution of high-performance anion-exchange chromatograms of isoamylase debranched corn starch and hydrolyzed with HCl-methanol at 25 °C for 240 h (a) and at 45 °C for 240 h (b). The percent distributions above and below the line represented the increases and decreases of the percentages of distribution after acid hydrolysis, respectively. Symbol (*) above and below the bar meant the differences between the determinations before and after acid hydrolysis were significant.

3.4. Chain length distribution of starch

Fig. 6 showed the chain length distribution of isoamylase debranched corn starch with different degree of acid hydrolysis. The short chains (DP < 6) presented in HPAEC chromatograms (Fig. 6b and c) of corn starch with acid hydrolysis was also found in other studies (Jane, Wong, & McPherson, 1997; Nakazawa & Wang, 2003). These short chains attributed to partially hydrolysis of glycosidic linkage in the amorphous regions. Differences in percentage distribution of HPAEC chromatograms of isoamylase debranched corn starch with acid hydrolyzed at 25 and 45 °C for 240 h were presented in Fig. 7. The DP 3, 4, 5 of corn starch treated at 25 °C for 240 h (Fig. 7a) were significantly increased and the DP 31 was slightly decreased. The populations of short chains with DP 3–9 and DP 13 and 14 increased, whereas the population of DP 17–26 significantly decreased after hydrolyzed at 45 °C for 240 h (Fig. 7b). Since the crystalline regions

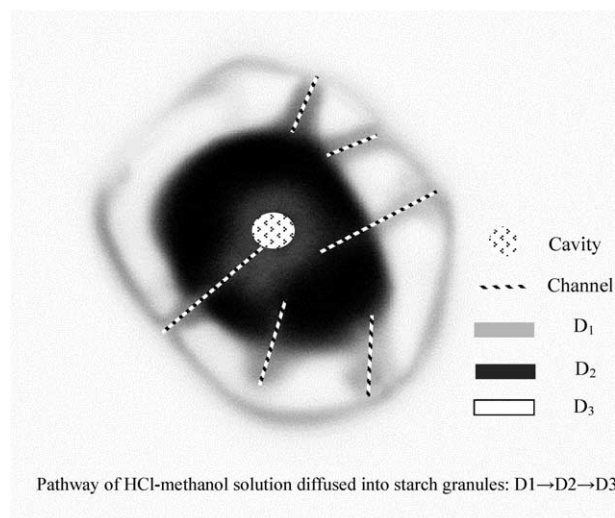


Fig. 8. Diagram to illustrate the possible diffusion pathway of HCl-methanol solution into starch granule. D_1 : the granular matrix surrounding cavity and channels, D_2 : the intermediate organized area, and D_3 : dense packed layer beneath the surface of starch granule.

of the starch formed by clusters of A and B₁ chains of amylopectin, the amorphous regions of the B₂, B₃, B₄ chains of amylopectin linking several crystalline regions were preferentially attacked and degraded into short chains by acid (Jane et al., 1997; Wang & Wang, 2001).

3.5. A proposed model of HCl-methanol hydrolysis on starch influenced by starch granular architecture

The heterogeneity and complexity of starch structure within granules influence the acid modification of starch molecules. HCl-methanol solution primarily diffused from the surface of starch granules, passed through the channels to the cavity and lateral diffused from cavity and channels throughout the granule matrix. From the diffusion process, the matrix of native corn starch granules was supposed to be divided into three regions: the granule matrix surrounding cavity and channels (D_1), dense packed layer beneath the surface (D_3), and the intermediate organized area (D_2) (Fig. 8). The mild hydrolysis condition of 0.36% HCl-methanol solution diffused into starch matrix with different rates due to the different structural organizations. The glycosidic linkage within the amorphous region and around the less-organized branching point was preferentially attacked by acid.

4. Conclusion

Starch architecture features possibly dictated the acid hydrolysis model within starch granules. The CLSM images of native corn starch stained with APTS for different time periods revealed the influence of diffusion process on chemical modification. The elevated temperature not only increased the reactivity of the hydrolysis of the glycosidic linkage, but also facilitated the diffusion of acid solution throughout starch matrix. The glycosidic bond surrounding the branching point in

the amorphous region was considered preferentially attacked and degraded by acid since the organization of the molecules in this area is more disordered. The HCl-methanol treatment highlights some possible molecular and granular structure of native corn starch and the characteristics of 0.36% HCl-methanol hydrolyzed at 25 and 45 °C.

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