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# Molecular Degradation Rate of Rice and Corn Starches during Acid–Methanol Treatment and Its Relation to the Molecular Structure of Starch

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The degradation rates of rice and corn starches with different contents of amylose treated in methanol containing 0.36% HCl at 25 °C for 1−15 days were evaluated by monitoring the weight average degree of polymerization of starch. A two-stage degradation pattern during acid−methanol treatment was found for the starches studied, which were the slow (first) and the rapid (second) degradation stages. Waxy starches showed a shorter time period of the first stage than that of nonwaxy starch. Rice starch showed a shorter time period of the first stage and a higher degradation rate of the second stage than the counterpart corn starch with similar amylose content. Despite the botanic source and amylose content of starch, the degradation rate of starch in the second stage significantly ($p < 0.05$) correlated to the S/L ratio ($r = -0.886$) and polydispersity ($r = 0.859$) of amylopectin branch chains of native starch.

**KEYWORDS: Molecular degradation rate; acid−methanol treatment; molecular structure; rice starch; corn starch**

## INTRODUCTION

"Acid−alcohol treatment" of starch means modifying starch with acid in alcohols. In an acid−alcohol treatment, starch can be treated at a temperature higher than its gelatinization onset temperature, and consequently, a shorter treatment time is needed for starch degradation. Generally speaking, the degradation of starch treated with acid−alcohol depends on acid concentration, starch type and concentration, alcohol type and concentration, and treatment temperature. Ma and Robyt (*1*) showed that potato and waxy maize starches treated with different alcohols (methanol, ethanol, 2-propanol, and 1-butanol) containing 0.36% HCl at 65 °C for 1 h produced starches with different values of an average degree of polymerization (DP); the molecular sizes of treated starch progressively decreased in the order of methanol > ethanol > 2-propanol > 1-butanol. Robyt et al. (*2*) indicated that the limiting DP of starch after acid−alcohol treated at different conditions was obtained in 72 h. The limiting DP of starch significantly varied with its botanic source and the alcohol solution used. Most studies on acid−alcohol modification of starch treated the starch granules with HCl in the solution of single alcohols (*1*−*3*) or alcohols combined in different ratios (*4*).

Examinations on the recovery yield or solubilization of starch during treatment have been widely used for studying the degradation kinetic of acid hydrolysis of starch (*5*−*10*). However, observations on the yield or solubilization of starch focus on the degradation of starch granules but not on the degradation of starch molecules. Ma and Robyt (*1*) indicated that the yields of starch granules after treatment at 65 °C for 1 h in different alcohols (methanol, ethanol, 2-propanol, and 1-butanol) containing 0.36% HCl were all higher than 88%; however, the number average DP ($DP_n$) of acid−alcohol-treated starches was similar to that of commercial lintner starch. The $DP_n$ of waxy corn starch after treatment at 65 °C for 1 h in 50−90% (v/v) ethanol solution containing 1.39% HCl ranged from 209 to 111 AGU (anhydrous glucose unit), and the yields of treated starch were higher than 96% (w/w) (*11*). This reveals that the degradation of starch molecules can be profound even though most of the acid−methanol-treated starch granule is recovered and the production of solutes is negligible.

A high-performance size-exclusion chromatograph (HPSEC) equipped with light scattering (LS) and refractive index (RI) detectors has been used for observing the molecular weight distribution and to determine the average molecular weight of biopolymers such as starch (*12*−*14*). The molecular weight distribution of most of starch molecules shows bimodal distribution. McPherson and Jane (*15*) indicated the reduced sensitivity of LS for small molecular weight species such as amylose or degraded amylopectin fragments of starch after degradation, which suggested that the molecular weight standards were also necessary for molecular weight calculation for starch.

Degradation of starch during acid−alcohol treatment is strongly dependent on its botanical source (*1*−*4*, *16*), which implies that the degradation of starch may depend on the amylose content and structure of amylose or amylopectin. Therefore, the purpose of this report was to study the relationship between the molecular structure of starch and its degradation

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rate during acid−alcohol treatment. For monitoring the molecular degradation of starch during acid−alcohol treatment and elucidating the effect of molecular structure of native starch on the degradation rate, rice (TCW70, TNG67, and TCS17) and corn (waxy corn, normal corn, and Hylon V) starches with different amylose contents were treated at 25 °C for 1−15 days in methanol containing 0.36% HCl. The amylose content, granule size, and chain length distribution of native starches were examined. The degradation rate of starch during acid−methanol treatment was calculated from the change of DP of starch, and its relation to the molecular structure of native starches, from different botanical sources and with different amylose contents, was studied.

## MATERIALS AND METHODS

**Materials and Chemicals.** Polished rice kernels of TCW70, a waxy rice, were obtained from Miaoli District Agricultural Research and Extension Station (Miaoli, Taiwan); TNG67, a japonica rice, was obtained from Taiwan Agricultural Research Institute (Wufong, Taiwan); and TCS17, an indica rice, was obtained from Taichung District Agricultural Research and Extension Station (Changhua, Taiwan). The crops for rice samples were harvested in 2003. Normal corn starch was obtained from Roquette Co. (Lestrem, France). Waxy corn starch and Hylon V were products of the National Starch and Chemical Co. (Bridgewater, NJ). The moisture contents of waxy corn, normal corn, and Hylon V starches were 9.5, 8.4, and 10.4%, respectively. Isoamylase (EC 3.2.1.68) of *Pseudomonas amyloderamosa* (59000 IU/mg) was purchased from Hayashibara Biochemical Laboratories, Inc. (Tokyo, Japan). All reagents used were of analytical grade.

**Isolation of Rice Starches.** The isolation of starch from polished rice kernels was performed according to the method proposed by Yang et al. (*17*) with some modifications. Rice kernels (2 kg, dry basis) were steeping overnight in 5 L of 0.1% NaOH solution. The supernatant was decanted, and the kernels were washed with fresh 0.1% NaOH solution. After washing, the kernels were milled with 10 L of 0.1% NaOH solution by a stone Wet-Mill (CL-010, Ladyship, Taipei, Taiwan). The slurry was diluted to 35 L and poured into a glass container (diameter, 30 cm; length, 60 cm). After it stood for 30 min, the slurry separated into three layers. The top and bottom layers (impurities) were identified by their yellow color. The middle layer (starch) was recovered by siphoning. The impurity layers were collected, and the process of diluting, standing, and siphoning was repeated until the starch layer was clear. The starch layers were collected and centrifuged at 10000*g* in a continuous phase centrifuger (T1A, Sharples, Warminster, PA). The precipitate was suspended in distilled water and neutralized with 0.1% HCl. Then, the slurry was repeatedly washed and centrifuged by distilled water until the absence of NaCl from supernatant (detecting by 1% $AgNO_3$). The precipitate was resuspended in 95% ethanol and air−oven dried at 40 °C, and the starch passed through a 100-mesh sieve was stored. The moisture contents of TCW70, TNG67, and TCS17 rice starches prepared were 7.9, 9.9, and 10.2%, respectively.

**Preparation of Acid−Methanol-Treated Starch.** Starch (25 g) was suspended in 100 mL of methanol (<0.3% water) in a 250 mL flask. The suspension was stirred at 25 °C, and the reaction was started by adding 1 mL of concentrated (36 wt %) HCl and was then allowed to proceed for 1, 3, 5, 7, 9, 11, 13, and 15 days, respectively. The reaction was stopped by adding 14 mL of 1 M $NaHCO_3$ and then cooled in an ice bath. The treated starch was centrifuged at 3500*g* for 5 min and washed four times with 50% ethanol. The precipitate was air−oven dried at 40 °C, and the recovery (%, w/w) of treated starch was calculated by weight of the recovered starch to the initial weight of dry starch.

**Determination of Amylose Content and Average Granule Size.** The amylose content of starch was calculated from its iodine affinity value (*18*), and the iodine affinity of pure amylose was assumed as 20.0%. Starch was defatted in a Soxhlet extractor for 48 h with 85% methanol. Defatted starch (0.1 g) was suspended with 1 mL of water. Ten milliliters of 1 N KOH was added, and the sample was dispersed by stirring. An additional 10 mL of 1 N KOH was added, and the mixture was placed in a refrigerator for 30 min. This solution was then diluted with 20 mL of water to give 40 mL of starch solution in 0.5 N KOH. The iodine affinity of starch solution was determined in triplicate according to an amperometric titration method by using a titrator (702 SM Titrino, Metrohm, Herisau, Switzerland) equipped with a platinum electrode. The average granule size of a starch granule suspended in water at room temperature was determined by using a laser LS-based particle size analyzer (Mastersizer Micro, Malvern Instruments, Worcestershire, United Kingdom).

**Chain Length Distribution Analysis.** The chain length distributions of native rice and corn starches were determined by HPSEC according to the procedures described by Charles et al. (*19*). Starch solution was prepared by dissolving 75 mg (dry weight) of starch with 15 mL of 90% dimethyl sulfoxide (DMSO) solution in a boiling water bath for 1 h with constant stirring and then continuously stirring for 24 h at room temperature. Starch was precipitated from an aliquot of DMSO solution (5 mL) with excess absolute ethyl alcohol and centrifuged at 4000*g* for 10 min. The precipitated amorphous starch pellet was redissolved in deionized water (2.45 mL, 95 °C) and stirred with a magnetic stirrer in a boiling water bath for 30 min. After the mixture was cooled to room temperature, acetate buffer (0.05 mL, 1.0 M, pH 3.5) and isoamylase solution (10 $\mu$L, 5.9 U/$\mu$L) were added to the starch solution, and then, the mixture was incubated in a shaker bath at 45 °C for 24 h. The solution was neutralized with 0.1 M NaOH and deionized with Amberlite IR-120P and IR-93 (Sigma, St. Louis, MO) ion exchangers. The solution was diluted to 5 mL and heated in a boiling water bath for 10 min. Debranched starch solutions were then filtered using a 0.45 $\mu$m syringe filter (Millipore, Billerica, MA) and injected into an HPSEC system. The HPSEC system consisted of an HP G1310A isocratic pump (Hewlett-Packard, Wilmington, DE), a RI detector (HP 1047A), and a multiangle laser LS (MALLS) detector (Dawn DSP, Wyatt Tech., Santa Barbara, CA) with a helium−neon laser light source. The columns used were one PWXL (guard column), one G3000$PW_{XL}$, and two G2500$PW_{XL}$ (TSK-Gel, Tosoh, Japan) connected in series and kept at 70 °C. The mobile phase was 100 mM phosphate buffer (pH 6.2) containing 0.02% $NaN_3$ at a flow rate of 0.5 mL/min. The electronic outputs of the RI and MALLS detectors were collected by ASTRA software (version 4.70, Wyatt Tech.). Peaks were assigned using the RI chromatograms. A typical HPSEC profile of debranched starch showed trimodal distribution. The molecular weight of first peak (amylose) was determined by using MALLS and RI signals, and the molecular weights of the second and third peaks (long chain and short chain of amylopectin) were calculated from the RI signal using a calibration curve constructed from a series of pullulan standards (molecular masses ranged from 1.0 to 46.0 kDa; Polymer Standards Service, Silver Spring, MD).

**Molecular Weight Distribution Analysis.** The molecular weight distributions of starches before and after acid−methanol treatment were also determined by HPSEC following the method of McPherson and Jane (*15*). Starch−DMSO solution was prepared according to the procedures described above. Starch was precipitated from an aliquot of DMSO solution (2.1 mL) with excess absolute ethyl alcohol and centrifuged at 4000*g* for 10 min. The precipitated amorphous starch pellet was redissolved in deionized water (15 mL, 95 °C) and stirred with a magnetic stirrer in a boiling water bath for 30 min. Each starch solution was filtered through a 5.0 $\mu$m syringe filter, and then, the filtrate (100 $\mu$L) was injected into the HPSEC system used for the determination of chain length distribution, except the columns used were PWH (guard column), G5000PW, and G4000PW (TSK-Gel, Tosoh) columns connected in series and the mobile phase was 100 mM sodium nitrate solution containing 0.02% $NaN_3$. The MALLS and RI signals were used to determine the molecular weight of amylopectin (first peak), while the molecular weight of the second peak (amylose and degraded amylopectin fragments) was calculated from the RI signal using a calibration curve constructed from a series of pullulan standards (molecule masses ranged from 5.6 to 863.0 kDa; Polymer Standards Service).

**Statistical Analysis.** Statistical analysis was conducted with Statistical Analysis System (SAS Institute Inc., Cary, NC). Pearson correlation analysis was also conducted on the degradation rate constant of starches and their molecular structure parameters.

**Table 1.** Amylose Content and Average Granule Size of Native Rice and Corn Starches

| starch | amylose[a] (%) | granule size ($\mu$m) |
|---|---|---|
| | Rice | |
| TCW70 | 0.1 ± 0.0[b] | 4.9 ± 0.1 |
| TNG67 | 18.3 ± 0.2 | 5.0 ± 0.0 |
| TCS17 | 29.2 ± 0.4 | 5.5 ± 0.1 |
| | Corn | |
| waxy corn | 0.1 ± 0.0 | 14.0 ± 0.0 |
| normal corn | 25.9 ± 0.1 | 13.7 ± 0.1 |
| Hylon V | 50.8 ± 0.2 | 12.6 ± 0.0 |

[a] Amylose content = iodine affinity × (100/20). [b] Mean ± SD ($n$ = 3).

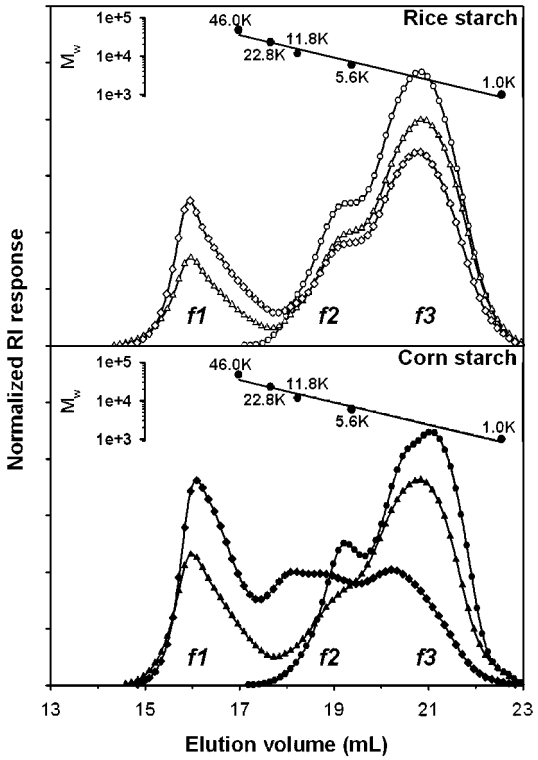


**Figure 1.** HPSEC profiles of isoamylase-debranched native starches: TCW70 rice (○), TNG67 rice (△), TCS17 rice (◇), waxy corn (●), normal corn (▲), and Hylon V (◆) starches, respectively.

## RESULTS AND DISCUSSION

**Amylose Content and Average Granule Size of Native Starch.** Amylose contents of native rice and corn starches examined by iodine affinity method are summarized in **Table 1**. Iodine affinities of the six native starches studied ranged from 0.01 to 10.16 (mg $I_2$/100 mg starch). Both waxy rice (TCW70) and waxy corn starches had extremely low (<0.02%) iodine affinity. Amylose contents of starches studied ranged from 0.1 to 29.2% for rice and from 0.1 to 50.8% for corn. The average granule sizes of rice starches (4.9−5.5 $\mu$m) were smaller than those of corn starches (12.6−14.0 $\mu$m). For starches from the same botanical source, the average granule sizes of starches were similar.

**Chain Length Distribution of Native Starch. Figure 1** shows the HPSEC profiles of native rice and corn starches after debranching by isoamylase. The profiles of nonwaxy starches show trimodal distributions, and the f1 fraction consists of amylose, f2 consists of the longer ($B_2$ or longer) chain fraction of amylopectin, and f3 consists of the shorter (A and $B_1$) chain fraction of amylopectin. However, waxy starches contain very low amylose; therefore, the chain length profiles are bimodal distributions. A similar profile of waxy corn starch was reported by Shi et al. (*20*).

The weight percentage (%), weight average chain length ($CL_w$), and number average chain length ($CL_n$) of each fraction of HPSEC profiles for the six starches are summarized in **Table 2**. Between the nonwaxy rice starches, TCS17 had a higher amylose content (weight percentage of f1 fraction) and lower $CL_w$ and $CL_n$ values of the f1 fraction than TNG67. A similar result was found between the nonwaxy corn starches studied, i.e., Hylon V, and the normal corn starches. **Table 3** shows that the $CL_n$ of amylopectin chains (f2 + f3) of rice starches ranged from 17.4 to 18.4 AGU, and the polydispersity of amylopectin chains increased with increasing amylose content of rice starch (TCS17 > TNG67 > TCW70). Furthermore, the $CL_n$ of amylopectin chains of corn starches ranged from 18.2 to 29.9 AGU, and the polydispersity of amylopectin chains of different corn starches also increased with increasing amylose content of starch (Hylon V > normal corn > waxy corn). The S/L ratio of amylopectin chain, i.e., ratio of the weight percentage of f3 fraction divided by the weight percentage of f2 fraction, of corn starch was slightly higher than that of rice starch except for Hylon V starch, which had the lowest S/L ratio of amylopectin chains (0.99) among the six starches. The average chain length (both $CL_w$ and $CL_n$) and polydispersity of amylopectin chains of Hylon V starch also had obviously higher values than those of other starches. Results of this study indicate that rice starch with high amylose content has a low average chain length of amylose and a low homogeneity of amylopectin chains. The same is also true for the corn starch studied.

**Recovery and Molecular Weight Distribution of Acid−Methanol-Treated Starch.** Recovery of starches after treatment in methanol containing 0.36% HCl at 25 °C for 1−15 days is summarized in **Table 4**. After acid−methanol treatment, the recovery of starch granules was high, ranging from 91.7 to 100.1%. This reveals that most of the starch granules were recovered even though the starches were treated for 15 days.

Molecular weight distributions of starches before and after acid−methanol treatment are shown in **Figures 2** and **3**. The first fractions (F1) of the profiles mainly correspond to amylopectin, and the second fractions (F2) correspond to the amylose or low molecular weight molecules. For the acid−methanol-treated starches, the areas of F1 fractions decreased with the increase of treatment time, while the areas of F2 fractions increased. This indicates the degradation of amylopectin due to the acid−methanol treatment. The weight average DP ($DP_w$) of starch showed that the $DP_w$ of the six starches progressively decreased with treatment in methanol containing 0.36% HCl at 25 °C for 1−15 days (**Table 5**). In other words, the $DP_w$ of starches decreased with increasing time of acid−methanol treatment. After treatment for 15 days, the $DP_w$ values of starches were lower than 1000 AGU except for that of normal corn starch. After acid−methanol treatment for 15 days, normal corn starch had a higher $DP_w$ (1388 AGU) than other starches (ranging from 482 to 805 AGU).

**Degradation Rate of Starches.** As the treatment time was plotted against the reciprocal of $DP_w$, a two-stage process was evident for the degradation of starch during acid−methanol treatment (**Figure 4**). In general, a relatively slower degradation rate during the first stage was observed and followed by a more rapid degradation at the second stage. A two-stage hydrolysis pattern was also proposed for the degradation of starch in acid aqueous solution (*5*−*7*, *21*). However, the degradation rate of starch at the first stage of acid hydrolysis was relatively more

**Table 2.** Weight Percentage and Average Chain Length of Native Rice and Corn Starches after Isoamylase Debranching

| | f1 | | | f2 | | | f3 | | |
|---|---|---|---|---|---|---|---|---|---|
| starch | %[a] | $CL_w$ | $CL_n$ | % | $CL_w$ | $CL_n$ | % | $CL_w$ | $CL_n$ |
| | | | | Rice | | | | | |
| TCW70 | *b* | | | 23.3 ± 0.0[c] | 63.6 ± 0.4 | 58.1 ± 0.2 | 76.7 ± 0.0 | 18.5 ± 0.1 | 15.0 ± 0.1 |
| TNG67 | 17.5 ± 0.2 | 3247 ± 186 | 1172 ± 28 | 19.4 ± 0.2 | 62.0 ± 0.2 | 55.9 ± 0.1 | 63.1 ± 0.1 | 17.7 ± 0.0 | 14.3 ± 0.0 |
| TCS17 | 29.2 ± 0.2 | 2416 ± 82 | 894 ± 43 | 17.9 ± 0.3 | 64.3 ± 0.4 | 58.2 ± 0.3 | 53.0 ± 0.2 | 18.5 ± 0.0 | 15.0 ± 0.1 |
| | | | | Corn | | | | | |
| waxy corn | | | | 23.2 ± 0.8 | 59.2 ± 0.9 | 54.8 ± 0.3 | 76.9 ± 0.8 | 19.0 ± 0.1 | 15.9 ± 0.1 |
| normal corn | 26.8 ± 0.3 | 2381 ± 94 | 938 ± 52 | 15.8 ± 0.6 | 64.8 ± 0.4 | 59.0 ± 0.3 | 57.5 ± 0.7 | 18.9 ± 0.1 | 15.3 ± 0.1 |
| Hylon V | 38.9 ± 0.2 | 1708 ± 35 | 826 ± 13 | 30.7 ± 0.0 | 88.3 ± 0.2 | 76.4 ± 0.2 | 30.4 ± 0.2 | 22.6 ± 0.0 | 18.5 ± 0.0 |

[a] %, $CL_w$, and $CL_n$ are the weight percentage, weight average chain length, and number average chain length of HPSEC fractions, respectively. [b] Not detectable. [c] Mean ± SD ($n = 3$).

**Table 3.** Average Chain Length, Polydispersity, and S/L Ratio of Amylopectin (f2 + f3) Fractions

| | average chain length[a] | | | |
|---|---|---|---|---|
| starch | $CL_w$ | $CL_n$ | $CL_w/CL_n$ [b] | S/L ratio[c] |
| | | Rice | | |
| TCW70 | 29.0 ± 0.1[d] | 18.1 ± 0.1 | 1.60 ± 0.01 | 3.29 ± 0.01 |
| TNG67 | 28.1 ± 0.1 | 17.4 ± 0.1 | 1.62 ± 0.00 | 3.25 ± 0.02 |
| TCS17 | 30.0 ± 0.3 | 18.3 ± 0.1 | 1.64 ± 0.01 | 2.97 ± 0.07 |
| | | Corn | | |
| waxy corn | 28.3 ± 0.6 | 19.1 ± 0.2 | 1.49 ± 0.01 | 3.32 ± 0.14 |
| normal corn | 28.7 ± 0.5 | 18.2 ± 0.3 | 1.58 ± 0.01 | 3.65 ± 0.17 |
| Hylon V | 55.7 ± 0.2 | 29.9 ± 0.1 | 1.86 ± 0.01 | 0.99 ± 0.01 |

[a] $CL_w$ and $CL_n$ are the weight average and number average chain lengths of amylopectin (f2 + f3) fractions, respectively. [b] $CL_w/CL_n$ is the polydispersity of amylopectin chains. [c] S/L ratio = (weight percentage of f3 fraction)/(weight percentage of f2 fraction). [d] Mean ± SD ($n = 3$).

**Table 4.** Recovery (%)[a] of Starch after Treatment for 1–15 Days

| time (days) | TCW70 | TNG67 | TCS17 | waxy corn | normal corn | Hylon V |
|---|---|---|---|---|---|---|
| 1 | 97.4 | 97.0 | 98.9 | 99.6 | 98.5 | 96.2 |
| 3 | 97.7 | 96.9 | 98.2 | 97.8 | 98.4 | 96.8 |
| 5 | 96.9 | 97.2 | 97.5 | 98.8 | 98.4 | 99.0 |
| 7 | 97.1 | 94.5 | 100.1 | 98.4 | 100.0 | 99.1 |
| 9 | 97.1 | 93.9 | 100.1 | 98.1 | 97.1 | 99.7 |
| 11 | 96.3 | 93.2 | 99.3 | 98.7 | 97.2 | 99.7 |
| 13 | 95.4 | 94.1 | 99.3 | 98.1 | 91.7 | 100.6 |
| 15 | 94.9 | 95.4 | 98.2 | 98.1 | 97.7 | 100.7 |

[a] Recovery (%, w/w) = (weight of starch after acid–alcohol treatment)/(weight of starch before treatment) × 100%.

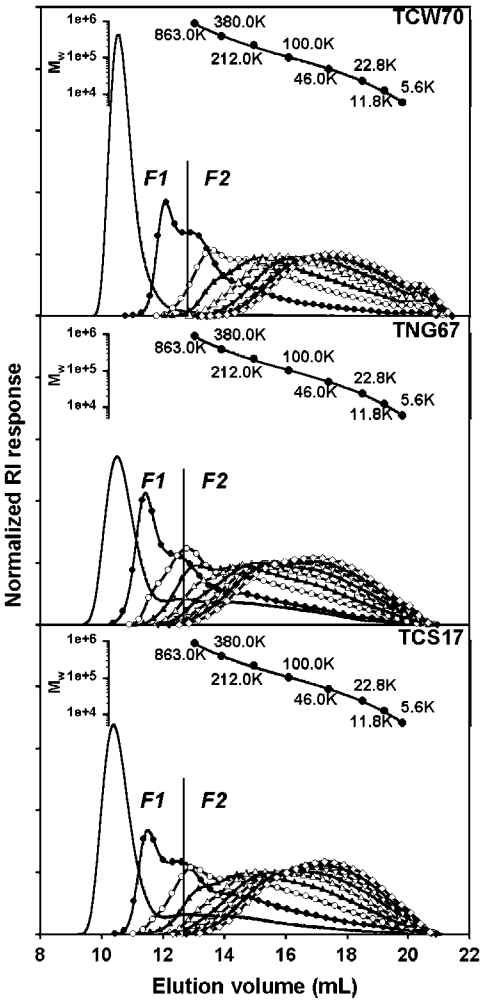


**Figure 2.** HPSEC profiles of native rice starches (—) and starches treated in methanol containing 0.36% HCl at 25 °C for 1 (●), 3 (○), 5 (▲), 7 (△), 9 (▼), 11 (▽), 13 (◆), and 15 (◇) days, respectively.

rapid than that of the second stage. The difference might be that an aqueous solution can easily penetrate the amorphous regions of granules, resulting in granules that are somewhat swollen and polymer chains within the swollen amorphous regions that are flexible (*22*). As hydrolysis takes place in these swollen amorphous regions, less and less readily hydrolyzable material is present so the reaction slows down to the rate of hydrolysis of crystallites. In methanol, starch granules do not swell as in water (*23*); in fact, the methanolic acid solution does not penetrate the granules readily. Slow polymer chain cleavage in the amorphous regions may allow some granule expansion (opening up) that becomes greater and greater with time.

For the acid hydrolysis of starch, the starch degradation pattern was observed by monitoring the solubilization of starch in acid aqueous solution (*5*−*7*). Because the solubilization of starch during acid−methanol treatment was less than 0.3% and the recovery of starch granule was above 91% in this study, the degradation pattern of starch during acid−methanol treatment could not be monitored either by the solubilization measurement or by the recovery determination. On the other hand, the change on $DP_w$ of starch well-described the degradation of starch during acid−methanol treatment. The reduction in $DP_w$ of starch during acid−methanol treatment could be expressed by the following equation:

$$1/M_t = 1/M_0 + kt/m = 1/M_0 + k't$$

where $k$ and $k'$ are the rate constants, $t$ is the reaction time, $M_0$ is the $DP_w$ of native starch, $M_t$ is the $DP_w$ of starch after

**Table 5.** $DP_w$ of Starch after Treatment for 1–15 Days

| time (days) | TCW70 | TNG67 | TCS17 | waxy corn | normal corn | Hylon V |
|---|---|---|---|---|---|---|
| native | 442154 ± 17008[a] | 581587 ± 45329 | 625911 ± 20319 | 460535 ± 3797 | 619775 ± 26966 | 275809 ± 18912 |
| 1 | 100767 ± 619 | 208902 ± 6336 | 101081 ± 3004 | 168419 ± 1958 | 177230 ± 1206 | 59675 ± 281 |
| 3 | 6908 ± 121 | 19464 ± 115 | 11425 ± 554 | 36340 ± 928 | 35104 ± 475 | 12011 ± 237 |
| 5 | 2134 ± 44 | 6244 ± 55 | 4137 ± 177 | 5151 ± 278 | 14432 ± 92 | 4827 ± 27 |
| 7 | 1135 ± 39 | 2720 ± 51 | 1597 ± 55 | 2638 ± 194 | 7044 ± 44 | 2797 ± 11 |
| 9 | 757 ± 15 | 1520 ± 16 | 835 ± 5 | 1537 ± 28 | 4154 ± 85 | 1797 ± 91 |
| 11 | 589 ± 5 | 1051 ± 25 | 672 ± 4 | 1188 ± 29 | 3109 ± 43 | 1097 ± 29 |
| 13 | 551 ± 3 | 761 ± 1 | 558 ± 9 | 992 ± 14 | 1783 ± 55 | 567 ± 5 |
| 15 | 482 ± 4 | 634 ± 7 | 486 ± 4 | 805 ± 23 | 1388 ± 42 | 493 ± 3 |

[a] Mean ± SD ($n = 3$).

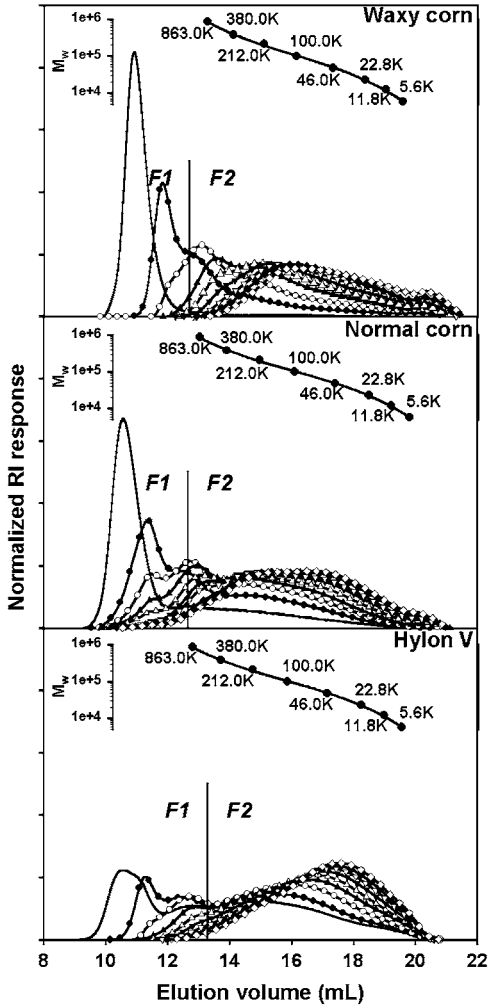


**Figure 3.** HPSEC profiles of native corn starches (—) and starches treated in methanol containing 0.36% HCl at 25 °C for 1 (●), 3 (○), 5 (▲), 7 (△), 9 (▼), 11 (▽), 13 (◆), and 15 (◇) days, respectively.

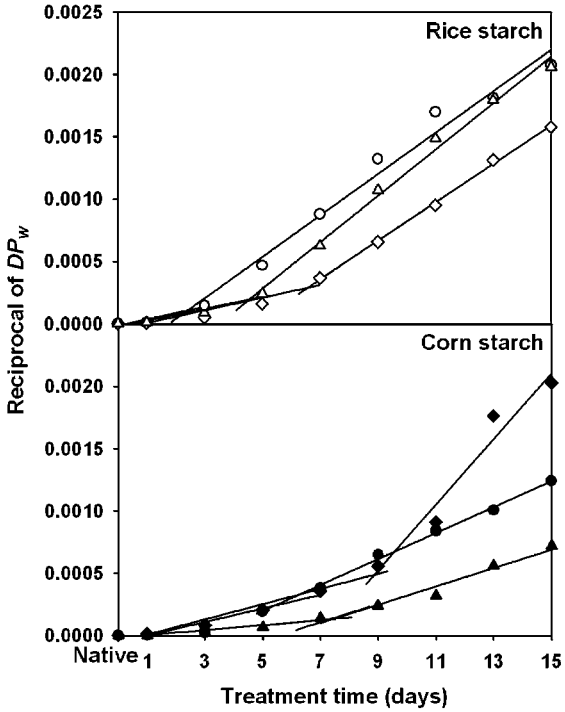


**Figure 4.** Plot of treatment time against the reciprocal of $DP_w$: TCW70 rice (○), TNG67 rice (△), TCS17 rice (◇), waxy corn (●), normal corn (▲), and Hylon V (◆) starches, respectively.

**Table 6.** Degradation Rate Constants and Time Period of the First Stage

| starch | degradation rate ($day^{-1}$)[a] $k_1$ | $k_2$ | $T_1$ (day)[b] |
|---|---|---|---|
| | rice | | |
| TCW70 | 0.081 (0.921[c]) | 0.269 (0.979) | 2.4 |
| TNG67 | 0.082 (0.884) | 0.249 (0.998) | 6.5 |
| TCS17 | 0.078 (0.931) | 0.301 (0.992) | 4.5 |
| | corn | | |
| waxy corn | 0.087 (0.878) | 0.169 (0.996) | 4.4 |
| normal corn | 0.032 (0.921) | 0.120 (0.963) | 7.4 |
| Hylon V | 0.099 (0.952) | 0.427 (0.958) | 8.9 |

[a] $k_1$ and $k_2$ are the degradation rate constants of the first and second stages, respectively. [b] $T_1$ is the time at the intersection of the first and second stages, representing the time period of the first stage. [c] Coefficient of determination ($r^2$).

treatment for time $t$, and $m$ is the monomer molecular weight (*24*). The degradation rate constants ($k$) of starches at different stages are summarized in **Table 7**.

The degradation rate constant of the first stage ($k_1$) was obviously lower than that of the second stage ($k_2$). The $k_1$ values of various rice starches were similar (from 0.078 to 0.082), while the $k_2$ values were in the order of TCS17 > TCW70 > TNG67. For corn starches, both $k_1$ and $k_2$ values were in the order of Hylon V > waxy corn > normal corn. Among the six starches studied, Hylon V starch had the highest $k_1$ value and normal corn starch had the lowest. The $k_2$ of corn starch was lower than that of rice starch except for Hylon V starch, which had the highest $k_2$ value among the six starches. Vasanthan and Bhatty (*25*) indicated that barley starch with a small granule size was hydrolyzed more rapidly and to a greater extent than barley starch with a larger granule size. Furthermore, the hydrolysis rate of starch treated with either α-amylase (*26*) or glucoamylase (*27*) was also found positively correlated with

**Table 7.** Correlation Coefficient ($r$) between the Degradation Rate Parameters and the Chain Length Distribution Parameters of Starch

| parameters[a] | $k_1$[b] | $k_2$ | $T_1$ |
|---|---|---|---|
| f1% | 0.477 | 0.753 | 0.636 |
| f2% | 0.711 | 0.654 | 0.251 |
| f2 $CL_w$ | 0.358 | 0.788 | 0.680 |
| f3% | –0.139 | –0.677 | –0.808 |
| f3 $CL_w$ | 0.370 | 0.657 | 0.618 |
| S/L ratio | –0.605 | –0.886[c] | –0.568 |
| $CL_w$ of amylopectin (f2+ f3) | 0.467 | 0.800 | 0.651 |
| polydispersity of amylopectin chains | 0.380 | 0.859[c] | 0.640 |

[a] f1%, f2%, and f3% are the weight percentages of f1, f2, and f3 fractions, respectively. $f2CL_w$ and $f3CL_w$ are the weight average chain lengths of f2 and f3 fractions, respectively. The S/L ratio is the content ratio of f3 to f2 fractions. [b] $k_1$ and $k_2$ are the degradation rate constants of the first and second stages, respectively. $T_1$ is the time period of the first stage. [c] $p < 0.05$.

the specific surface area of starch granules. Because methanol does not penetrate starch granules readily (*23*), the higher $k_2$ value of rice starch could be attributed to its smaller granule size (4.9−5.5 $\mu$m) as compared to that of corn starch (13.7−14.0 $\mu$m).

Hylon V starch had the highest $k_1$ and $k_2$ values among the six starches studied. Veregin et al. (*28*) indicated that amylopectin was the predominant crystalline component in starch; however, amylose of starch may be considered as a diluent to amylopectin especially for starch with high amylose content (*29*−*31*). Cheetham and Tao (*32*) proposed that amylose disrupted the crystallite formation of amylopectin. The $^{13}C$ cross-polarization/magic angle spinning-nuclear magnetic resonance ($^{13}C$ CP/MAS NMR) evidence (*15*, *33*) suggested that amylose could form double helices and potentially crystalline arrays in high amylose starch. Therefore, the highest degradation rate of Hylon V starch during acid−methanol treatment among the six starches studied could be attributed to its higher content of amylose or the specific molecular structure of amylopectin.

Waxy starch had similar $k_1$ values (0.081 for TCW70 and 0.087 for waxy corn), but the $k_2$ value of TCW70 (0.269) was higher than that of waxy corn starch (0.169) (**Table 6**). For nonwaxy starch, the $k_2$ value of starch with a higher amylose content (0.301 and 0.427 for TCS17 and Hylon V, respectively) was higher that that of the counterpart starch with a lower amylose content (0.249 and 0.120 for TNG67 and normal corn, respectively). This result is opposed to the study on the rate of acid hydrolysis of barley starch (*34*), which indicated that the rate of acid hydrolysis of barley starch increased with decreasing amylose content of starch. In this study, although the amylose contents of TNG67 (18.3%) and normal corn (25.9%) starches were lower than those of TCS17 (29.2%) and Hylon V (50.8%) starches (**Table 1**), the $CL_w$ of amylose chain (f1) fraction of TNG67 (3247 AGU) and normal corn (2381 AGU) starches were obviously higher than those of TCS17 (2416 AGU) and Hylon V (1708 AGU) starches, respectively (**Table 2**). This implies that the nonwaxy starch, containing amylose with a longer chain length, displayed a lower degradation rate on the second degradation stage during acid−methanol treatment.

The time period for the first stage ($T_1$) of starch degradation was quantified from the intersection of the regression lines of the two stages as shown in **Figure 4**. Results in **Table 7** show that waxy starch (2.4 and 4.5 days for TCW70 and waxy corn starches, respectively) has obviously shorter $T_1$ than that of nonwaxy starch (6.5, 4.5, 7.4, and 8.9 days for TNG67, TCS17, normal corn, and Hylon V starches, respectively). This implies that the presence of amylose in starch granules prolongs the first stage of degradation. For starch with similar amylose contents, such as TCW70 rice starch and waxy corn starch, the $T_1$ of rice starch was shorter than that of the counterpart corn starch. The same is true for TNG67 rice starch to normal corn starch.

**Correlation between the Molecular Structure of Native Starch and Its Degradation Rate during Acid−Methanol Treatment.** Despite the botanical source and amylose content of starch, the correlation between the molecular structure properties of native starch (fraction content and parameters of chain length distribution) and the degradation rate parameters ($k_1$, $k_2$, and $T_1$) of starch during acid−methanol treatment are summarized in **Table 7**. No significant correlations were found between the degradation rate parameters and the molecular structure properties of starch, except for the $k_2$ and the chain length parameters of amylopectin fraction. The $k_2$ was found significantly ($p < 0.05$) correlated with the S/L ratio ($r = -0.886$) and polydispersity ($r = 0.859$) of branch chains of amylopectin fraction. Results in **Table 7** reveal that the higher the heterogeneity of chain length distribution of an amylopectin fraction is, the higher the degradation rate of starch during the second degradation stage in acid−alcohol treatment is. In other words, with decreasing S/L ratio of amylopectin fraction, the starch degradation rate of the second stage in acid−methanol treatment increased. Long chains of amylopectin are B2 or longer branch chains, which extend through two or more clusters of starch molecules (*35*). The lower S/L ratio of amylopectin fraction indicates that the amylopectin has a relatively higher fraction content of long chains as compared to that of amylopectin with a higher S/L ratio, which further infers that more branch chains passing through the amorphous region for starch with low S/L ratio amylopectin. Because the acid hydrolysis preferentially occurred in the amorphous region (*16*), the branch chains passing through the amorphous region are more easily hydrolyzed; especially for the amorphous region around blocklet in the hard and soft shells, this amorphous fraction is responsible for the elasticity of the system (*22*). Therefore, higher degradation rate was found on starch with a lower S/L ratio in amylopectin.

In conclusion, rice and corn starches with different amylose contents exhibited recovery values higher than 91% after treatment in methanol containing 0.36% HCl at 25 °C for 1−15 days. The $DP_w$ of starch obviously decreased with increasing treatment time. The degradation of starch during acid−methanol treatment could be separated into the first and the second stages corresponding to the slow and rapid degradation rates, respectively. Waxy starch displayed a shorter time period of the first stage than nonwaxy starch. For nonwaxy starch, the degradation rate depended on the molecular size of amylose instead of the content of amylose. Rice starch showed a shorter time period at the first stage and a higher degradation rate at the second stage than those of the counterpart corn starch with similar amylose content. It was found that the degradation rates of both stages did not show significant correlation to the amylose content of native starch despite the botanical source of starch. The degradation rate of the second stage significantly ($p < 0.05$) correlated to the S/L ratio ($r = -0.886$) and polydispersity ($r = 0.859$) of branch chains of amylopectin fraction.

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