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Effect of ethanol concentration on the physicochemical properties of waxy corn starch treated by hydrochloric acid

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Abstract

Waxy corn starch was treated in 50, 70 or 90% of ethanol solutions containing 0.36 or 1.39% HCl at 65 °C for 1 h. The recovery yields of treated starch were higher than 96%. The average granule size, maximum wavelength of the spectrum of iodine coloration (λ_{max}), pasting viscosity, gelatinization onset temperature (T_o), average molecular weight and average chain length of starch decreased after treated, while no obvious change on X-ray diffraction pattern and increase in solubility of treated starch was also found. The changes on pasting viscosity, T_o, average molecular weight and solubility of treated starch increased with increasing ethanol and acid concentrations. It was found that the solubility of acid–ethanol treated waxy starch was a function of T/T_o (T was the temperature used for solubility measurement). At T/T_o below 1.2, the solubility of starch linearly correlated with T/T_o value ($r^2 = 0.984$).

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1. Introduction

Acid–alcohol treatment has been proposed by Small (1919) for obtaining the maximum conversion of raw starch into soluble starch with minimal production of low-molecular-weight dextrins. Ma and Robyt (1987) showed that potato and waxy maize starches treated with different anhydrous alcohols (methanol, ethanol, 2-propanol, and 1-butanol) containing 0.36% HCl at 65 °C for 60 min produced starches with different values of average degree of polymerization (DP), the molecular sizes progressively decreased in the order of methanol > ethanol > 2-propanol > 1-butanol. The yields of the modified starches were high (ranging from 100 to 88%), and the starch granule morphology was retained. The size distribution of the starch chains was narrower and more homogeneous than that of native starch. It was proposed that different alcohols produced different concentrations of acid inside the granules (Ma & Robyt, 1987), and the hydrolysis of glycosidic linkage took place exclusively inside the granule with the granule-bound water (Robyt, Choe, Hahn, & Fuchs, 1996).

Most studies on acid–alcohol modification of starch treated the granules with HCl in the solution of single anhydrous alcohols (Ma & Robyt, 1987; Robyt, Choe, Hahn et al., 1996; Lin, Lee, & Chang, 2003) or anhydrous alcohols combined in different ratios (Robyt, Choe, Fox, Hahn, & Fuchs, 1996). Fewer reports deliberated the starch treated in solution with different concentrations of alcohols. For obtaining the high-crystalline amylodextrins as fat replacers, Chun, Lim, Takeda, and Shoki (1997) refluxing the rice starch granules in different concentrations (70–99%) of ethanol solutions containing 2–4% HCl at 78–80 °C for 1–5 h. The results showed that the degree of hydrolysis and average DP of modified rice starch decreased as the ethanol concentration increased at the same acid concentration. It implied that the susceptibility of starch degradation by HCl in different concentrations of alcohols were different. Whereas the differences of molecular and physicochemical properties among the starches treated in different concentrations of alcohol solution were not clear.

For elucidating the effect of alcohol concentration on the degradation and physicochemical properties of starch, in this study waxy corn starch was treated in different

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concentrations (50, 70 and 90%) of ethanol solution containing 0.36 or 1.39% HCl at 65 °C for 1 h. The granule size, X-ray diffraction pattern, scanning electron micrography (SEM), maximum wavelength of the spectrum of iodine coloration (λ_{max}), pasting properties, solubility, gelatinization thermal properties of starch were examined. The molecular weight and chain length distribution of starch before and after treated were also determined by high performance size exclusion chromatography (HPSEC). Then the differences on the granular structures, physicochemical properties and molecular structures among the waxy corn starch treated in ethanol solutions with different concentrations will be discussed.

2. Materials and methods

2.1. Materials

Waxy corn starch was a product of National Starch and Chemical Company (Bridgewater, USA), moisture content of the starch was 12.2% (w/w). Isoamylase (EC 3.2.1.68) of *Pseudomonas amyloderamosa* (59,000 IU/mg) was purchased from Hayashibara Biochemical Laboratories, Inc. (Tokyo, Japan). All reagents used were of analytical grade.

2.2. Methods

2.2.1. Preparation of treated starch

Acid–alcohol treatment on starch was performed according to the procedure described previously (Lin et al., 2003). Starch (25 g, dry basis) was suspended in 100 ml 50, 70 or 90% ethanol solutions, then the reaction was started by adding 1 or 4 ml of concentrated (36% by weight) HCl and allowed to proceed at 65 °C for 1 h. The reaction was stopped by adding 14 or 56 ml of 1 M $NaHCO_3$. The solution was cooled in ice-bath 2 for 5 min, and centrifuged at 3500 × *g* for 5 min. The precipitate was washed four times with 50% ethanol and dried at 40 °C air-oven. The yield was calculated by weight of the recovery starch to the initial weight of dry starch.

2.2.2. Granule size distribution

The granule size distribution of starches was determined by using a laser light scattering based particle size analyzer (Mastersizer Micro, Malvern Instruments, Malvern, UK).

2.2.3. Maximum wavelength of the spectrum of iodine coloration (λ_{max})

The λ_{max} of starch was measured according to the method reported by Biliaders, Grant, and Vose (1979). Starch (10 mg, dry basis) was dissolved by 1 ml 1N NaOH, and heating in boiling water bath for 3 min. The solution was cooled to room temperature, and neutralized by 1N HCl. The $C_4H_5KO_6$ (potassium hydrogen tartrate, 0.14 g) and iodine solution (1 ml, containing 0.2% I_2 and 2% KI) were added, and then the mixed solution was made up to 50 ml by deionized water. The λ_{max} was determined by measuring the wavelength of maximum absorbance at 1 nm intervals from 500 to 700 nm in a spectra photometer (U2001, Hitachi, Tokyo, Japan).

2.2.4. Scanning electron microscopy

Starch granules were mounted on circular aluminum stubs with double sticky tape, coated with gold, and then examined by a scanning electron microscope (ABT-150S, Topcon, Tokyo, Japan) and photographed at an accelerating potential of 10 kV.

2.2.5. X-ray diffraction pattern

X-ray diffraction patterns were carried out by using a X-ray diffractometer (XRD-6000, Shimadzu, Hadano, Japan). The diffractometer was operated at 27 mA and 50 kV. The scanning region of the diffraction angle (2θ) was from 4 to 40° at 0.02° step size with a count time of 1.2 s.

2.2.6. Solubility

Starch (0.1 g, dry basis) was heated in 40 ml of water to the desired temperature (60, 70, 80 and 90 °C) for 30 min. The formation of lump was prevented by continuous stirring. The mixture was centrifuged at 4000 × *g* for 15 min, and then the supernatant was collected. An aliquot of supernatant was evaporated at 130 °C and weighed. The solubility was the ratio in weight of the dried supernatant to the initial weight of the dry starch.

2.2.7. Pasting properties

Pasting properties of starch was determined by using a Rapid ViscoAnalyzer (RVA, model 3D + , Newport Scientific, Warriewood, Australia). Starch suspension (28 g total weight; 7%, w/w, dry basis for native waxy corn starch and 14% for acid–ethanol treated starches) was equilibrated at 50 °C for 1 min, heated to 95 °C at a rate of 12 °C/min, maintained at 95 °C for 2.5 min, and then cooled to 50 °C at a rate of 12 °C/min. Paddle speed was set at 960 rpm for the first 10 s and then 160 rpm for the rest of the analysis.

2.2.8. Gelatinization thermal properties

Thermal properties of starch during heating were determined by using a differential scanning calorimeter (DSC, model 2910, TA Instruments, Surrey, England). Starch sample (about 2.5 mg, dry basis) was weighed in the sample pan, mixed with distilled water (about 7.5 mg), and sealed. The samples were heated from 25 to 120 °C at a heating rate of 10 °C/min. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures together with gelatinization enthalpy (ΔH) were quantified.

2.2.9. Molecular weight distribution

The molecular weight distribution of starch was determined as described by Lin et al. (2003). In general, the solution of native starch was prepared by solubilizing 75 mg (dry basis) of starch with 15 ml, 90% dimethyl sulfoxide (DMSO) solution in a boiling water bath for 1 h with constant stirring, then continuously stirred for 24 h at room temperature. 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 resolubilized in deionized water (15 ml, 95 °C) and stirred with a magnetic stirrer in a boiling water bath for 30 min. To the acid–ethanol treated starch, the starch solution was prepared by solubilizing 10 mg (dry basis) of starch with 15 ml deionized water and stirred with a magnetic stirrer in a boiling water bath for 1 h.

Each starch solution was filtered through a 5.0 μm syringe filter (Millipore, Billerica, USA), and then the filtrate (100 μl) was injected into an HPSEC system. The system consisted of an HP G1310A isocratic pump (G1310A, Hewlett Packard, Wilmington, USA), a refractive index (RI) detector (HP 1047A), and a multiangle laser light-scattering (MALLS) detector (Dawn DSP, Wyatt Tech., Santa Barbara, USA). The columns used were PWH (guard column), G5000PW and G4000PW (TSK-Gel, Tosoh, Tokyo, Japan) columns connected in series and kept at 70 °C. The mobile phase was 100 mM $NaNO_3$ 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 (ver. 4.50, Wyatt Tech.). Peaks were assigned according to the RI chromatograms. The MALLS and RI signals were used to determine the molecular weight of amylopectin (first peak). Because of the reduced sensitivity of MALLS for small molecular weight species, 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 (molecular weight 5.8, 12.2, 22.8, 48.0, 100.0, 212.0, 380.0, and 863.0 kDa; Polymer Standards Service, Silver Spring, USA).

2.2.10. Chain length distribution

Starch solution (2.5 mg starch/2.45 ml H_2O) was prepared according to the procedures described above. The solution was cooled, then acetate buffer (0.05 ml, 1.0 M, pH 3.5) and isoamylase solution (10 μl, 5.9 U/μl) was added, and the mixture incubated in a shaker bath at 45 °C for 24 h (Lin et al., 2003). The solution was neutralized with 0.1 M NaOH, and deionized with Amberlite IR-120P and IR-93 (Sigma, St Louis, USA) ion exchangers. The solution was diluted to 5 ml, and heated in boiling water bath for 10 min. Debranched starch solutions were then filtered using a 0.45 μm syringe filter (Millipore). The filtrate was injected (100 μl) into the HPSEC system used for the determination of molecular weight distribution, except the columns used were one G3000PW$_{XL}$ and two G2500PW$_{XL}$ (TSK-Gel, Tosoh) connected in series. 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. A typical HPSEC profile of debranched starch showed trimodel distribution. The molecular weight of first peak (amylose) was determined by using MALLS and RI signals, and the molecular weight 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 weight 1.0, 5.8, 12.2, 22.8, and 48.0 kDa; Polymer Standards Service).

2.2.11. Percentage acid inside the starch granule

Starch (25 g, dry basis) was stirred in 100 ml of different concentrations of ethanol solution containing 0.36 or 1.39% HCl at 65 °C for 3 min. The starch granules was filtered by using of Whatman No. 4 filter papers (Whatman, Maidstone, England), and washed by 95% ethanol. Then starch granules was stirred in 100 ml of deionized water for 1 h at 25 °C, the supernatant was collected after filtered out the granules. The amount of acid in the supernatant was determined and the percentage acid inside the starch granule was calculated as HCl weight basis.

3. Results and discussion

3.1. Yield, granule size and λ_{max} of starch

As shown in Table 1 at least 95% of starch granules were recovered after treated in 50–90% ethanol solutions containing 0.36 or 1.39% HCl at 65 °C for 1 h. Compared to the native starch, the average granule size of the waxy corn starches treated in 50% ethanol solution containing 0.36% HCl showed no obvious changes, and slight decrease in granule size was found for the starches treated in other conditions. The λ_{max} of the acid–ethanol treated starch was lower than that of native starch. The λ_{max} of starch treated in

Table 1
Yield, average granule size and λ_{max} of waxy corn starch after treated in ethanol solutions at 65 °C for 1 h

Treatment		Yield[a] (%)	Average granule size (μm)	λ_{max} (nm)
HCl (%)	Ethanol (%)			
Native[b]	–	–	13.8 ± 0.1	530 ± 0.6
0.36	50	99	13.8 ± 0.0	530 ± 0.6
	70	98	13.6 ± 0.0	529 ± 0.4
	90	99	13.6 ± 0.1	528 ± 0.8
1.39	50	96	13.4 ± 0.1	524 ± 0.6
	70	97	13.4 ± 0.1	523 ± 1.0
	90	97	13.4 ± 0.0	523 ± 0.8

[a] (Weight of starch after acid–ethanol treated)/(weight of starch before treated) × 100%.

[b] Waxy corn starch without treated.

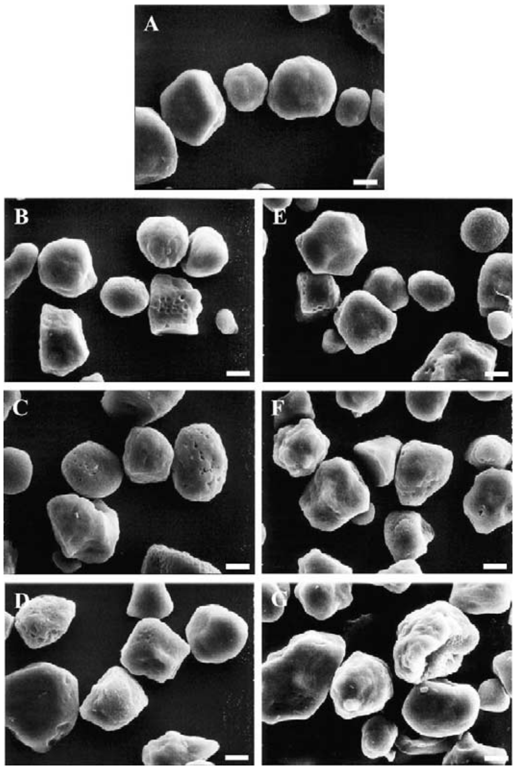

Fig. 1. Scanning electron micrograms of waxy corn starch after treated at 65 °C for 1 h: native (A); treated with 0.36% HCl in 50% (B); 70% (C); and 90% ethanol (D); treated with 1.39% HCl in 50% (E); 70% (F); and 90% ethanol (G), respectively. (Bar, 4 μm).

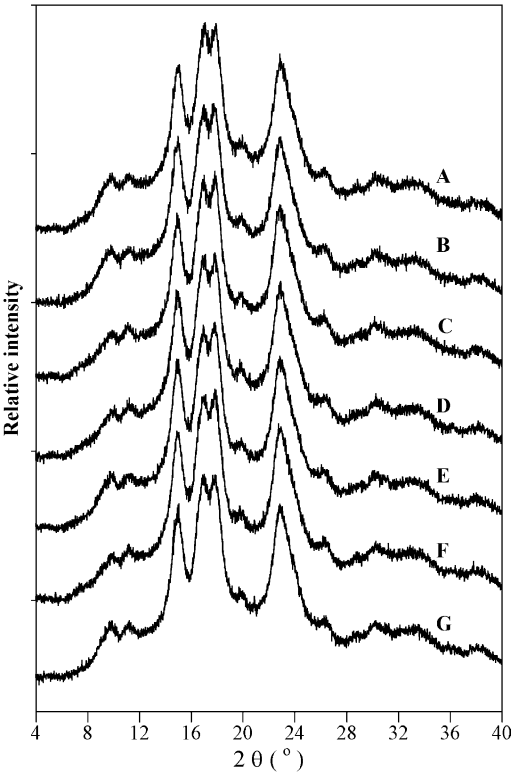

Fig. 2. X-ray diffraction patterns of waxy corn starch after treated at 65 °C for 1 h: native (A); treated with 0.36% HCl in 50% (B); 70% (C); and 90% ethanol (D); treated with 1.39% HCl in 50% (E); 70% (F); and 90% ethanol (G), respectively.

Table 2
Solubility of waxy corn starch after treated in ethanol solutions at 65 °C for 1 h

Treatment		60 °C[a]	70 °C	80 °C	90 °C
HCl (%)	Ethanol (%)				
Native[b]	–	1.1	2.6	8.2	17.5
0.36	50	6.4	54.3	87.2	88.4
	70	7.0	59.2	87.4	89.8
	90	8.7	64.7	87.8	90.8
1.39	50	16.2	77.1	88.3	92.1
	70	22.9	81.4	90.7	93.8
	90	29.9	86.0	92.5	94.1

Solubility (%) = weight of soluble starch/weight of dry starch × 100%, and standard deviations were less than 3%.

[a] Temperature used during measurement.

[b] Waxy corn starch without treated.

ethanol solutions containing 1.39% HCl was slightly lower than that of starch treated in ethanol solutions containing 0.36% HCl. However, no obvious differences were found on λ_{max} among the starches treated in different concentrations of ethanol solution. In general, the value of λ_{max} is affected by the chain length of starch molecule, that is the λ_{max} increases with the increasing chain length of starch. Therefore, the lower λ_{max} of starch after treated with 1.39% HCl could be attributed to the lower average chain length of starch resulting from the higher extent of degradation caused by the higher acid concentration.

3.2. SEM and X-ray patterns of starch granule

Waxy corn starch granules examined by SEM showed polygonal or irregular shape for native starch (Fig. 1A). After acid–ethanol treated, the granule surface of starch (Fig. 1B–G) became rough, and some caves were also found, especially for starch treated in 90% ethanol containing 1.39% HCl (Fig. 1G). The native waxy corn starch showed typical A-type X-ray diffraction pattern (Fig. 2).

Table 3
Pasting properties of waxy corn starch after treated in ethanol solutions at 65 °C for 1 h

Treatment		Viscosity (cP)[a]				
HCl (%)	Ethanol (%)	Peak	Hot paste	Final	Breakdown	Setback
Native[b]	–	2396	906	1193	1490	287
0.36[c]	50	325	85	138	240	53
	70	299	97	141	202	44
	90	122	79	106	43	27
1.39[c]	50	51	31	48	20	17
	70	50	35	45	15	10
	90	18	–[d]	21	18	21

[a] Waxy corn starch without treated, and measured at 7% (d.b.) starch suspension.

[b] Measured at 14% (d.b.) starch suspension.

[c] Undetectable.

[d] Standard deviations were less than 50 cP.

Table 4
Gelatinization thermal properties of waxy corn starch after treated in ethanol solutions at 65 °C for 1 h

Treatment		Gelatinization temperature (°C)			$T_c - T_o$ (°C)	ΔH (J/g)
HCl (%)	Ethanol (%)	T_o	T_p	T_c		
Native[a]	–	63.6 ± 0.3	69.9 ± 0.2	80.4 ± 0.3	16.8 ± 0.5	15.2 ± 0.4
0.36	50	63.0 ± 0.2	70.3 ± 0.2	85.3 ± 0.2	22.3 ± 0.1	14.5 ± 0.8
	70	63.5 ± 0.8	70.6 ± 0.4	84.2 ± 0.2	20.7 ± 0.7	14.2 ± 0.8
	90	61.9 ± 0.1	70.6 ± 0.3	86.7 ± 0.4	24.8 ± 0.4	15.7 ± 0.3
1.39	50	60.6 ± 0.3	70.6 ± 0.1	88.2 ± 0.5	27.7 ± 0.6	14.8 ± 0.2
	70	59.9 ± 0.6	70.5 ± 0.1	88.8 ± 0.6	29.0 ± 0.9	14.1 ± 0.3
	90	57.3 ± 0.1	69.5 ± 0.5	89.1 ± 0.2	33.9 ± 0.3	15.1 ± 0.2

[a] Waxy corn starch without treated.

The diffraction intensity and diffraction patterns of acid–ethanol treated starches were similar to that of the native starch, which revealed the waxy corn starch remained in its crystalline structure after acid–ethanol treatment.

3.3. Solubility and pasting properties of starch

The solubility of acid–ethanol treated starches was obviously higher than that of native starch under any measuring temperature used (Table 2). The solubility of native starch was below 20% at 90 °C, however, acid–ethanol treated starches showed higher than 54% solubility at 70 °C. At the same ethanol concentration, the solubility of starch treated with 0.36% HCl were lower than starch treated with 1.39% HCl. The solubility of acid–ethanol treated starch increased with increasing ethanol or acid concentration. After acid–ethanol treated, the pasting viscosity of starch decreased obviously (Table 3). At 7% measuring concentration, the pasting viscosity of acid–ethanol treated starches was too low to be detected by the Rapid ViscoAnalyzer (data not shown). Even at 14% concentration, which is two times of that used (7%) for native starch, the peak viscosity of acid–ethanol treated starches was still obviously lower than that of native starch. The peak viscosity of acid–ethanol treated starches also decreased with the increasing ethanol or acid concentrations. The result confirms the high solubility of acid–ethanol treated starches, and implies that the acid–ethanol treated waxy corn starch was easily disrupted when heated in water.

3.4. Gelatinization thermal properties of starch

Table 4 summarizes the gelatinization thermal properties of waxy corn starch determined by DSC. The T_o of acid–ethanol treated starch was lower than that of native starch, while the T_c and gelatinization temperature range increased after acid–ethanol treatment. The ΔH of starches treated in 50 or 70% ethanol solutions containing either 0.36 or 1.39% HCl were lower than that of native starch. For starch treated in 90% ethanol solution, the ΔH of starch treated with 0.36% HCl increased slightly, and no obvious change was found for starch treated with 1.39% HCl. Under the same ethanol concentration, the changes in gelatinization temperature and gelatinization temperature range of starch

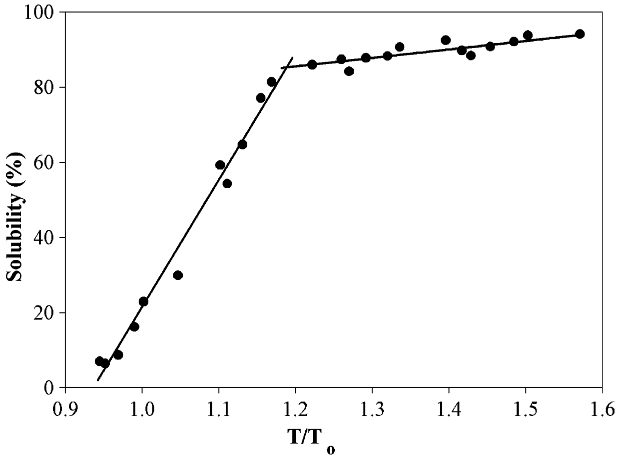

Fig. 3. Solubility as a function of T/T_o. T was the temperature used for solubility measurement, and T_o was gelatinization onset temperature determined by DSC.

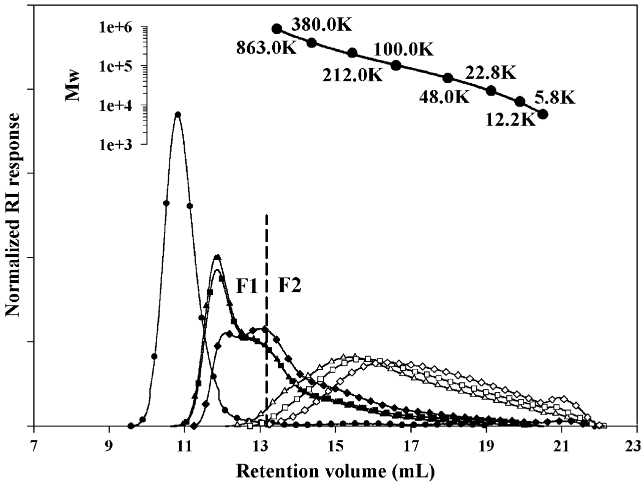

Fig. 4. HPSEC profiles of waxy corn starch after treated at 65 °C for 1 h: native (●), treated with 0.36% HCl in 50% (▲), 70% (■), and 90% ethanol (◆); treated with 1.39% HCl in 50% (△), 70% (□), and 90% ethanol (◇), respectively.

Table 5
Weight proportion and degree of polymerization (DP) of waxy corn starch after treated in ethanol solutions at 65 °C for 1 h

Treatment		$F1$			$F2$			$F1+F2$	
HCl (%)	Ethanol (%)	%	DP_n ($\times 10^3$)	DP_w ($\times 10^3$)	%	DP_n	DP_w	DP_n	DP_w
Native[a]	–	96.5 ± 0.4	415.8 ± 18.2	480.6 ± 17.8	3.5 ± 0.4	509 ± 69	1,797 ± 166	14339 ± 1461	463925 ± 18975
0.36	50	63.1 ± 1.0	137.1 ± 0.4	257.3 ± 0.8	36.9 ± 1.0	1,006 ± 69	2,866 ± 67	2695 ± 216	163324 ± 2598
	70	59.3 ± 0.3	122.0 ± 1.5	222.8 ± 2.8	40.7 ± 0.3	825 ± 12	2,658 ± 24	2009 ± 13	133251 ± 975
	90	42.4 ± 0.5	83.1 ± 0.8	122.6 ± 0.9	57.6 ± 0.5	509 ± 23	2,544 ± 53	881 ± 92	53450 ± 939
1.39	50	1.6 ± 0.1	43.3 ± 0.6	78.1 ± 0.6	98.4 ± 0.1	209 ± 12	1,200 ± 12	212 ± 21	2407 ± 101
	70	0.2 ± 0.0	62.8 ± 1.6	90.6 ± 1.8	99.8 ± 0.0	169 ± 3	926 ± 5	169 ± 3	1131 ± 55
	90	–[b]	–	–	100.0 ± 0.0	111 ± 1	659 ± 7	111 ± 1	659 ± 7

[a] Waxy corn starch without treated.
[b] Undetectable.

treated with 1.39% HCl were more obvious than those of starch treated with 0.36% HCl. The result reveals that the acid–ethanol treatment cause the crystalline of starch become more heterogeneous than native starch. The decrease of ΔH on starch treated in either 50 or 70% ethanol solutions may attribute to the partial gelatinization of starch after treated in higher water content at 65 °C.

Li and Yeh (2001) indicated that the swelling power of high amylose content corn starch showed well correlation with the T/T_p value (T was the temperature used for solubility measurement). It suggested the T_p was a good reference point for considering temperature effect (Li & Yeh, 2001). In this study, the solubility of starch was found as a function of T/T_o (T was the temperature used for solubility measurement) of starch after acid–ethanol treatment at different conditions. Fig. 3 illustrates that solubility is a second-order polynomial function of T/T_o for acid–ethanol treated waxy corn starch. At T/T_o below 1.2, the solubility of acid–ethanol treated starch linearly correlated with T/T_o value ($r^2 = 0.984$), in other words, the solubility of treated starch obviously increased with the increasing T/T_o. The solubility of acid–ethanol treated starch slowly increased as T/T_o was above 1.2. The result indicates that as heated in water at a temperature around T_o ($T/T_o = 0.9$–1.2), the granules of acid–ethanol treated waxy corn starch disrupt and consequently solubilize.

3.5. Molecular weight distribution of starch

HPSEC profile (Fig. 4) of native waxy corn starch showed a single peak at retention volume between 10 and 13 ml, which can be separated into two fractions according to the gyration radius (You, Fiedorowicz, & Lim, 1999). The first ($F1$) fraction corresponds to amylopectin, and the second ($F2$) fraction to the amylose or low molecular weight molecules. The profile of native starch showed that the waxy corn starch had a high amylopectin content, and the content of amylose or low molecular weight molecules ($F2$) was very low. After acid–ethanol treatment, $F1$ fraction shifted toward $F2$ fraction, especially for starch treated with 1.39% HCl. The weight proportion and average DP of starch before and after acid–ethanol treatment were summarized in Table 5. The content of $F1$ fraction and average DP ($F1+F2$) of starch decreased obviously after acid–ethanol treatment. Starch treated in 90% ethanol solution showed the lowest content of $F1$ fraction and average DP among starches treated in different ethanol solutions with the same amount of acid used. Starch treated with 1.39% HCl also showed a lower content of $F1$ fraction and average DP than treated with 0.36% HCl. This result indicates that the content of $F1$ fraction and average DP of the treated starch decrease with increasing ethanol and acid concentrations.

3.6. Chain length distribution of starch

Waxy corn starch debranched by isoamylase showed a bimodal chain length distribution (Fig. 5), which revealed that the amylose was absent in the waxy corn starch. Generally speaking, the $f1$ fraction consists of the longer chain (B2 or longer) fraction of amylopectin, and $f2$ the shorter chain (A and B1) fraction of amylopectin.

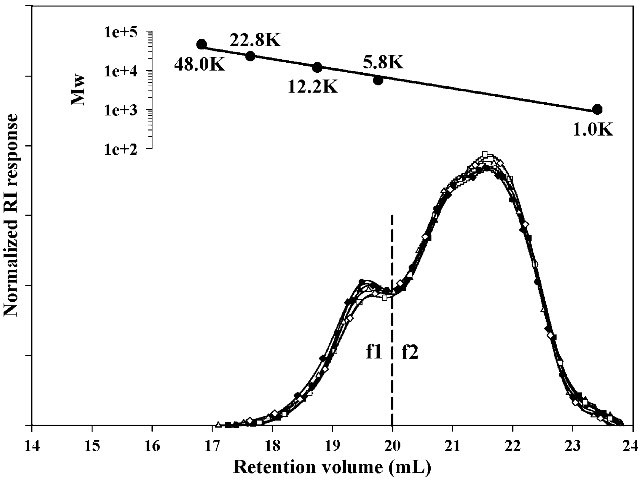

Fig. 5. Chain length distribution profiles of waxy corn starch after treated at 65 °C for 1 h: native (●), treated with 0.36% HCl in 50% (▲), 70% (■), and 90% ethanol (◆); treated with 1.39% HCl in 50% (△), 70% (□), and 90% ethanol (◇), respectively.

Table 6
Weight percentages and chain length distributions of isoamylase-debranched waxy corn starch after treated in ethanol solutions at 65 °C for 1 h

Treatment		$f1$			$f2$			$f1+f2$		
HCl (%)	Ethanol (%)	%	DP_n	DP_w	%	DP_n	DP_w	DP_n	DP_w	DP_w/DP_n
Native[a]	–	24.1 ± 1.0	54.5 ± 0.5	58.9 ± 0.7	75.9 ± 1.0	15.6 ± 0.1	18.6 ± 0.1	28.4 ± 0.6	18.8 ± 0.3	1.509
0.36	50	21.1 ± 0.2	53.0 ± 0.2	56.6 ± 0.4	78.9 ± 0.2	14.9 ± 0.0	18.1 ± 0.1	26.3 ± 0.2	17.6 ± 0.0	1.494
	70	21.3 ± 0.2	53.2 ± 0.2	57.0 ± 0.3	78.7 ± 0.2	15.0 ± 0.0	18.2 ± 0.0	26.4 ± 0.2	17.7 ± 0.1	1.492
	90	23.2 ± 0.1	54.5 ± 0.1	58.9 ± 0.2	76.8 ± 0.1	15.4 ± 0.0	18.5 ± 0.0	27.9 ± 0.1	18.5 ± 0.0	1.508
1.39	50	20.0 ± 0.1	52.6 ± 0.0	56.1 ± 0.1	80.0 ± 0.1	15.5 ± 0.0	18.5 ± 0.0	26.1 ± 0.1	18.0 ± 0.0	1.450
	70	19.9 ± 0.2	53.1 ± 0.1	56.9 ± 0.0	80.1 ± 0.2	15.1 ± 0.0	18.3 ± 0.1	26.0 ± 0.1	17.7 ± 0.1	1.469
	90	19.5 ± 0.5	53.2 ± 0.2	57.2 ± 0.3	80.5 ± 0.5	14.9 ± 0.0	18.2 ± 0.1	25.7 ± 0.3	17.6 ± 0.1	1.460

[a] Waxy corn starch without treated.

The content and average DP of $f1$ fraction of starch decreased after acid–ethanol treatment (Table 6), and the content of $f2$ increased. Furthermore, the average chain length (CL), expressed as $(f1+f2)$ in Table 6, and polydispersity of starch decreased after acid–ethanol treatment with the exception of starch treated in 90% ethanol solution containing 0.36% HCl, which showed similar average CL and polydispersity to those of native starch. Although the starch treated in 90% ethanol solution containing 0.36% HCl showed no obvious difference on the chain length distribution (polydispersity) from the native starch, the average DP of the starch decreased significantly (Table 5). This result indicates that the starch molecules treated in 90% ethanol solution containing 0.36% HCl degrade significantly, and further implies that the degradation of waxy corn starch after treated in 90% ethanol solution containing 0.36% HCl mainly happens in the α-$(1 \rightarrow 6)$ linkage. However, this phenomenon was not observed in the starch treated with 1.39% HCl. The average CL of starches treated with 1.39% HCl at different ethanol concentrations were similar to each other. This should have resulted from the higher concentration of the HCl used.

3.7. Percentage of acid inside the starch granules

Table 7 shows the percentage of acid inside the starch granules treated in different concentrations of ethanol solution. The percentage of acid inside the granule increased as the ethanol concentration increasing from 50 to 70%. Nevertheless, when ethanol concentration was higher than 70%, the percentage of acid inside the starch granules decreased. The percentage of acid inside the starch granule treated in different concentrations of ethanol solution were in the order of 70 > 90 > 50%. The percentage of acid inside the granules during treatment did not show correlation with the molecular weight of acid–ethanol treated starch. This result disagrees with the result of study on starch granule treated with acid in different anhydrous alcohols (with less than 0.05% water content) (Ma & Robyt, 1987). This discrepancy implies that the degradation of starch treated with acid either in anhydrous alcohols or in alcohol solutions (with more than 10% water content) is different.

Table 7
Percentage of HCl in starch granule during acid–ethanol treatment

HCl (%)	Ethanol (%)	HCl added (mg)	mg HCl in granule	% HCl in granule
0.36	50	426.6	11.9 ± 0.1	2.8 ± 0.0[a]
	70	426.6	14.5 ± 0.2	3.4 ± 0.0
	90	426.6	9.0 ± 0.4	2.1 ± 0.1
1.39	50	1706.4	23.9 ± 3.4	1.4 ± 0.2[b]
	70	1706.4	35.8 ± 5.1	2.1 ± 0.3
	90	1706.4	27.3 ± 3.5	1.6 ± 0.2

[a] % HCl in granule = mg HCl in granule/426.6 × 100%.

[b] % HCl in granule = mg HCl in granule /1706.4 × 100%.

4. Conclusion

Waxy corn starch after acid–ethanol treatment had higher than 96% recovery yield of starch granules. Starch maintained its granular structure, and showed no obvious changes on its X-ray diffraction pattern after treated with acid–ethanol. The λ_{max} and pasting viscosity of starch decreased after acid–ethanol treated, and increasing on solubility of starch after treated was also found. The changes in λ_{max}, pasting viscosity and solubility of starch after treated increased with increasing ethanol and acid concentrations. The molecular weight of acid–ethanol treated starch decreased obviously with increasing ethanol concentration. This result confirms the properties of high solubility and low pasting viscosity of the treated starch.

In spite of the treated conditions, the solubility of acid–ethanol treated starch highly correlated with the T/T_o value, which suggests that T_o is a good index to estimate the solubility of acid–ethanol treated waxy corn starch.

The percentage of acid inside the starch granules during the acid–ethanol treatment did not show correlation with the molecular weight of treated starch. Nevertheless, the chain length distribution of waxy corn starch became narrower after treated with acid–ethanol, except for the starch treated with 0.36% HCl in 90% ethanol solution. Although a significant degradation on molecular weight of the starch treated with 0.36% HCl in 90% ethanol solution was found, the average values and distribution of chain length of the starch were similar to those of the native waxy corn starch. This indicates that ethanol concentration has an impact on the starch treated with acid–ethanol treatment, and that the starch treated with 0.36% HCl in 90% ethanol solution may have distinct degradation mechanism from starch treated under other conditions.

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