



Type and concentration of acid on solubility and molecular size of acid–methanol-treated rice starches differing in amylose content

Yung-Ho Chang^{a,*}, Jheng-Hua Lin^b, Ciao-Ling Pan^a

^a Department of Food and Nutrition, Providence University, 200 Chung-Chi Road, Shalu 43301, Taiwan

^b Department of Hospitality Management, MingDao University, Peetow 52345, Taiwan

ARTICLE INFO

Article history:

Received 7 July 2009

Received in revised form 30 September 2009

Accepted 5 October 2009

Available online 13 October 2009

Keywords:

Starch

Acid–methanol treatment

Hydrogen-ion concentration

Solubility

Molecular size

ABSTRACT

Rice starches differing in amylose content were treated in methanol with acid at 45 °C for 1 h, effects of acid type and concentration on the solubility and molecular size of starch were investigated. Significant differences were found in solubility and weight-average degree of polymerization (DP_w) among starches treated with different acids at the equal normality. Starch treated with HCl had lower DP_w than treated with H_2SO_4 or HNO_3 . The hydrogen-ion concentration in methanol was negatively correlated ($p < 0.001$) with the DP_w of starch despite of type and concentration of acid used. The increment in degradation extent of waxy rice starch with increasing hydrogen-ion concentration in methanol was lower than that of non-waxy rice starch. Results indicate that the distinct deionization extent of acids in methanol influences the degradation of starch, and that the hydrogen-ion concentration can be used to monitor the degradation of rice starch during acid-treatment in methanol.

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

Acid–alcohol treatment was proposed by Small (1919) for obtaining the maximum conversion of raw starch into soluble starch with minimal production of low molecular weight dextrin. Acid hydrolysis of starch in alcohols has high recovery of starch granules, uses less amount of acid and retains the granular structure of starch (Lin, Lee, & Chang, 2003; Ma & Robyt, 1987). The average degree of polymerization (DP) of acid–alcohol-treated starch is affected by the botanical source and concentration of starch, type and concentration of alcohols, acid concentration, treatment time and temperature (Chang, Lin, & Lii, 2004; Ma & Robyt, 1987; Robyt, Choe, Hahn, & Fuchs, 1996). Results of previous studies indicate that the solubility of starch granules after acid–alcohol treatment profoundly increases during heating in water, and the molecular weight and pasting viscosity of treated starch obviously decrease to lower than that of the counterpart native starch (Chang et al., 2004; Lin et al., 2003). Chang, Lin, and Chang (2006) demonstrated that the solubility of acid–alcohol-treated waxy starch linearly correlated ($r^2 = 0.983$) with the T/T_0 value, as T stands for the measuring temperature (°C) used for solubility determination and T_0 stands for the gelatinization onset temperature of starch. Ma and Robyt (1987) proposed that different alcohols introduced different concentrations of acid into starch granules, and hydrolysis of glycosidic linkage took place exclu-

sively inside the granule with the granule-bound water. Robyt et al. (1996) showed the DP of acid–alcohol-treated starch decreased with increasing concentration of acid.

Singh and Ali (2000) studied the hydrolysis of starch at 50 °C for 1.5 h in aqueous solutions containing 0.5 N of different types of acid. Their results showed that H_3PO_4 solution caused the least, and HCl and HNO_3 solutions the highest, reduction of molecular weight of starch. Sulfuric acid, though a strong acid, promoted less degradation at equal normality as compared with HCl and HNO_3 . This result indicates that even at equal normality, the extent of acid hydrolysis of starch granules treated with different types of acid is distinct.

Hydrochloric acid, nitric acid and sulfuric acid are strong acids, being completely ionized in dilute aqueous solution. Strong acids appear to have equal strengths in aqueous solution, for the same acid – hydronium ion – is common to all such solutions. This means that the apparent acidity in aqueous solutions of all strong acids is reduced to the same mediocre level, that of the hydronium ion. This phenomenon is known as “leveling effect” (VanderWefer, 1961). However, the pK_a of acid in methanol is larger than that of the same acid in the water, owing to the lower dielectric content of methanol than water (Rived, Roses, & Bosch, 1998). It means that hydrogen-ion dissolves easier in the water than in methanol, and that the difference on ionization of different strong acids in aqueous solution could be more profound than in methanol or other non-aqueous solutions. The difference in degradation of starch treated with various strong acids in aqueous solution system has been observed by Singh and Ali (2000). However, the

* Corresponding author. Tel.: +886 4 2632 8001x15302; fax: +886 4 2653 0027.
E-mail address: yhchang@pu.edu.tw (Y.-H. Chang).

influences of acid type on acid degradation of starch treated in non-aqueous solution is still absent, while the information is valuable for treating starch in non-aqueous system.

In this study, rice starches (9–11% moisture) with different amylose contents (TKW1, TNG67 and TCS17) were stirred at 45 °C for 1 h in methanol/water (about 99/1, v/v) containing various types (HCl, HNO₃ and H₂SO₄) and concentrations (25, 50, 75 and 100 mN) of acid. The solubility and molecular size of starch after acid–methanol treatment were examined to elucidate the effects of type and concentration of acid on the degradation of starch. Furthermore, the effect of hydrogen-ion concentration in methanol containing various acids on the degradation extent of rice starch was observed.

2. Materials and methods

2.1. Materials

Polished rice kernels of TKW1, a waxy rice, and TNG67, a japonica rice, were obtained from Taiwan Agricultural Research Institute, Wufong, Taiwan. Polished rice kernels of TCS17, an indica rice, were obtained from Taichung Agricultural Research and Extension Station, Changhua, Taiwan. All reagents used were of analytical grade.

2.2. Methods

2.2.1. Isolation of starch

The isolation of starch was performed according to the method proposed by Yang, Lai, and Lii (1984) with some modifications. Polished rice kernels (2 kg) was steeped overnight in 5 l of 0.1% NaOH solution, then the supernatant was decanted and the kernels were washed with fresh 0.1% NaOH solution. After washed, the kernels were milled with 10 l of 0.1% NaOH solution by using of a Stone Wet-Mill (CL-010, Ladyship, Taoyuan, Taiwan). The slurry was diluted to 35 l and poured into a glass container (30 cm in diameter and 60 cm in height). After standing for 10 min, the slurry separated into three layers. The top and bottom layers were yellow in color, which were impurity layers. The middle layer was starch layer, which was siphoned out. The impurity layers were collected to repeat the diluting with 0.1% NaOH solution, standing and siphoning procedures until the starch layer was clear. The collected starch layers were centrifuged (10,000 g) by using of a continuous phase centrifuge (T1A, Sharples, Warminster, PA, USA). The precipitate was suspended in distilled water, neutralized with 0.1% HCl, then the slurry was repeatedly washed and centrifuged until the absence of salt, detecting by 1% AgNO₃, in the supernatant. The precipitate was washed with 95% ethanol, air-oven dried at 40 °C, and ground and passed through a 100-mesh sieve. The moisture content of the isolated TKW1, TNG67 and TCS17 rice starch was 8.9%, 10.7% and 9.1%, respectively. Amylose content of the isolated native starch, determining from its iodine affinity value (BeMiller, 1964), was 0.1%, 18.3% and 29.2% for TKW1, TNG67 and TCS17 rice starch, respectively.

2.2.2. Acid–methanol treatment

Acid solutions with different concentrations (2.5, 5.0, 7.5 and 10.0 N) were prepared by diluting concentrated HCl, HNO₃ and H₂SO₄ with distilled water, respectively. Fifty grams (db) of the isolated native starch was suspended in 200 ml methanol (<0.03% water) at 45 °C. The reaction was started by adding 2 ml of the prepared acid solutions and allowed to proceed at 45 °C for 1 h. The acid concentrations in methanol after adding different concentrations of acids were 25, 50, 75 and 100 mN, respectively. The ratio of methanol-to-water for the acid–methanol solutions used was

about 99–1 (v/v). The reaction was stopped by neutralizing with 1 M NaHCO₃, cooled in ice-bath for 5 min and centrifuged at 3500g for 5 min. The precipitate was repeatedly washed with 50% ethanol until salt was absent from supernatant, and dried in an air-oven at 40 °C. The recovery of starch granules was determined by the weight of recovery starch to the initial weight of native starch used (Lin et al., 2003).

2.2.3. Solubility

Starch (0.1 g, db) was heated in 40 ml of distilled water to the desired temperature (60, 70, 80 and 90 °C) for 30 min. The formation of lump was prevented by continuously stirring. The mixture was centrifuged at 4000g 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.

2.2.4. Average molecular weight

Starch solution was prepared by dissolving 75 mg (db) 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 stirred at room temperature for 24 h. Starch was precipitated from an aliquot of DMSO solution (2.1 ml) with excess amount of absolute ethanol and centrifuged at 4000g for 10 min. The precipitated amorphous starch pellet was re-dissolved in de-ionized water (15 ml, 95 °C) and stirred with a magnetic stirrer in a boiling water bath for 30 min.

Each starch solution was then filtered through a 5.0 μm syringe filter (Millipore, Billerica, MA, USA), and the filtrate (100 μl) was injected into a high-performance size-exclusion chromatograph (HPSEC) system. The system consisted of an isocratic pump (G1310A, Hewlett Packard, Wilmington, DE, USA), a refractive index (RI) detector (HP 1047A), and a multi-angle laser light-scattering (MALLS) detector (Dawn DSP, Wyatt Tech., Santa Barbara, CA, 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 NaNO₃ containing 0.02% NaN₃ at a flow rate of 0.5 ml/min. The electronic outputs of the RI and MALLS detectors were collected by ASTRA software (version 4.50, Wyatt Tech.) and were used to determine the molecular weight of starch (Chang et al., 2006).

2.2.5. pH value of solution

The pH values of the treatment solutions were determined according to the method proposed by Boraie (2001) with some modifications. One hundred milliliter of methanol solutions containing 1 ml of acid with different concentrations were equilibrated at 45 °C in water bath for 5 min, the pH values of the solutions were then determined by using of a standardized pH meter (SP-2200, Suntext Instruments, Taipei, Taiwan).

2.2.6. Statistical analysis

The General Linear Model (GLM) procedure of Statistical Analysis System (SAS Institute Inc., Cary, NC, USA) was used for performing statistical analysis.

3. Results and discussion

Although different acid solutions was used for acid–methanol treatment in this study, the discrepancy in water content of different treatment systems was within 5% (w/w) of the total amount of water presented in treatment system, which includes water from starch granules, water from methanol, and water from acid solution. Moreover, Robyt et al. (1996) indicated that the hydrolysis of the glycosidic linkage of starch treated in alcohol system took

place exclusively inside the granules with the granule-bound water. Therefore, the effect of discrepancy in water content for different acid solutions on degradation of starch can be ignored in this study.

3.1. Recovery of starch granules

The recovery of rice starch granules after acid–methanol treatment ranged from 95.4% to 98.7%. No obvious difference was found for the recovery of starches treated with different types or concentrations of acid. Results showed that the most of starch granules were recovered after acid–methanol treatment. The high recovery of the modified starch granules is in line with the studies of Lin et al. (2003) on acid–methanol-treated rice and corn starches (above 93%) and You and Lzydorczyk (2007) on acid–alcohol-treated barley starches (above 91%). The high value of recovery could be attributed to the less extent of solubilization of starch during acid–alcohol hydrolysis. For acid-treatment in aqueous medium near room temperature, hydrolysis takes place in swollen amorphous region and the extent of solubilization of starch during treatment is high (Hoover, 2000).

3.2. Solubility of starch

Although the solubility of native and treated rice starches (0.25% in water) increased with increasing temperature (Fig. 1), native rice starches had obviously lower solubility at all measurement temperatures as compared to acid–methanol-treated starches. The solubility of native rice starches were below 10% (TKW1, 0.6 ~ 8.5%; TNG67, 1.4 ~ 7.5%; and TCS17, 1.7 ~ 7.4%) at all measurement temperatures studied. Results in Fig. 1 confirm the limited swelling properties and solubility of cereal starch granules during heating (Bowler, Williams, & Angold, 1980). However, after acid–methanol treatment, the solubility of treated starch was higher than that of the counterpart native starch. Moreover, the solubility of treated starch increased with increasing concentration of acid. With increasing acid concentration, the increment of solubility for starch treated with HCl was higher than that of the counterpart

starch treated with HNO_3 or H_2SO_4 . For starch treated with the same type and concentration of acid, solubility of TNG67 and TCS17 starches was lower than that of the TKW1 starch. Tester and Morrison (1990) indicated that the swelling behavior of cereal starch was primarily a property of its amylopectin, and amylose acted as both a diluent and an inhibitor of swelling. In other words, amylose restricts the swelling of starch and maintains the integrity of swollen granules. Therefore the low solubility of TNG67 and TCS17 could be attributed to the presence of sufficient amount of amylose which restricts the swelling of starch and contributes to the maintenance of the granular structure of starch during heating in water.

In this study, the increase in solubility of starch after acid–methanol treatment could be attributed to the degradation of starch molecules. The higher solubility of HCl–methanol-treated starch indicates that HCl has the highest degradation efficiency on starch in methanol among the acids studied.

3.3. Molecular size and degradation extent of starch

Fig. 2 shows the molecular weight distribution profiles of rice starches before and after acid–methanol treatment. The first fraction (F1) of the profiles corresponds to amylopectin, and the second (F2) fraction to the amylose or low molecular weight molecules (Lin et al., 2003). For the three acids studied, the F1 fraction of starches after acid–methanol-treated gradually shifted toward F2 fraction with the increasing concentration of acid. Table 1 summarizes the weight-average degree of polymerization (DP_w) and degradation extents of acid–methanol-treated starches. Results showed that the DP_w of treated starches were lower than that of the counterpart native starch, and that the DP_w decreased with increasing concentration of acid. Starch treated with HCl had the lowest DP_w among starches treated with different acids at the equal normality. The degradation extents of treated starches, ranging from 1.6% to 82%, depended on the concentration of acid used in acid–methanol treatment.

Correlation between degradation extent of treated starch and concentration of acid used in this study was observed for elucidating

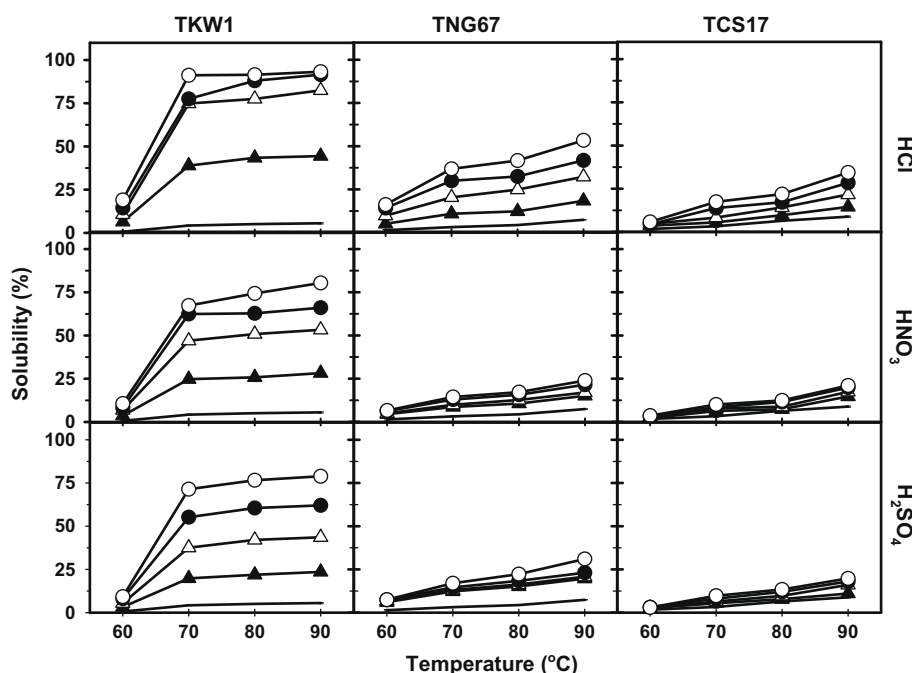


Fig. 1. Solubility index of native rice starches (—■) and rice starches after treated in methanol containing 25 mM (▲), 50 mM (△), 75 mM (●) and 100 mM (○) of acids.

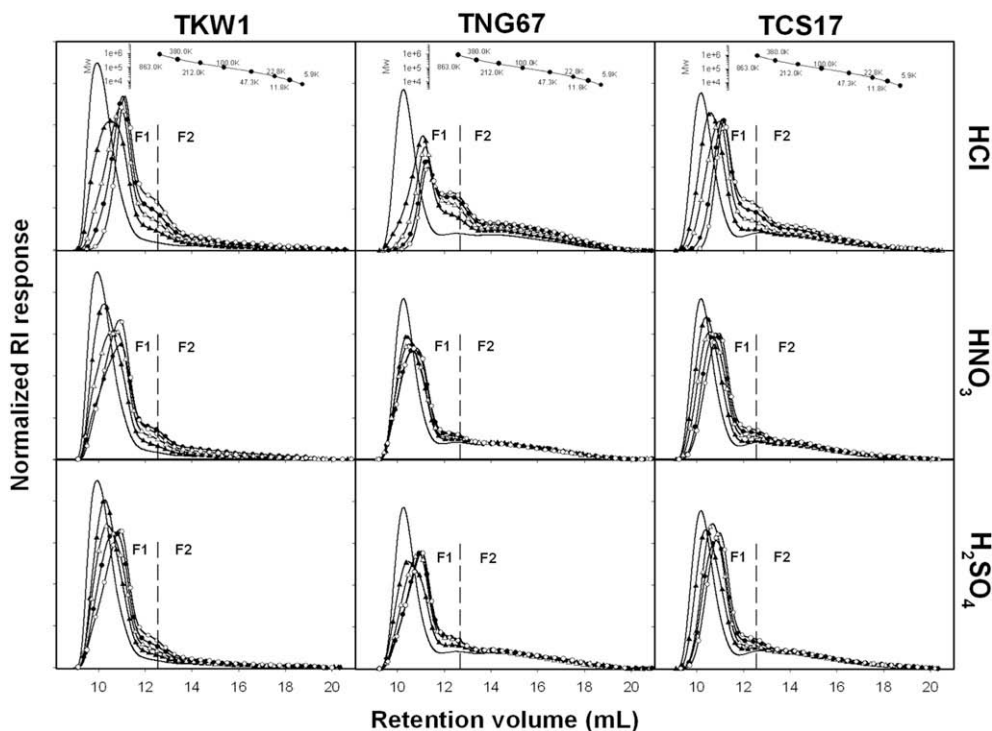


Fig. 2. Molecular weight distributions of native rice starches (—) and starches after acid-methanol-treated in methanol containing 25 mN (▲), 50 mN (△), 75 mN (●) and 100 mN (○) of acids.

Table 1

Weight-average degree of polymerization (DP_w) and degradation extent of rice starch after acid-methanol treatment.

Acid	Concentration ^a (mN)	Rice starch					
		TKW1		TNG67		TCS17	
		$DP_w (\times 10^3)$	Degradation extent (%) ^b	$DP_w (\times 10^3)$	Degradation extent (%)	$DP_w (\times 10^3)$	Degradation extent (%)
HCl	25	644.8 ± 3.7	13.9	506.6 ± 24.2	15.7	634.2 ± 30.7	13.6
	50	620.5 ± 12.6	17.2	275.1 ± 15.1	54.2	578.3 ± 9.6	21.2
	75	575.5 ± 35.7	23.2	150.2 ± 2.3	75.0	386.0 ± 4.6	47.4
	100	396.7 ± 20.1	47.0	106.9 ± 1.4	82.2	240.0 ± 3.2	67.3
HNO ₃	25	705.2 ± 35.1	5.8	550.8 ± 43.6	8.3	715.4 ± 58.2	2.5
	50	690.4 ± 24.0	7.8	523.8 ± 9.6	12.8	669.7 ± 8.8	8.7
	75	648.9 ± 9.9	13.4	513.8 ± 17.4	14.5	622.2 ± 20.0	15.2
	100	575.1 ± 1.2	23.2	499.6 ± 10.8	16.8	528.8 ± 34.1	28.0
H ₂ SO ₄	25	737.2 ± 13.7	1.8	461.8 ± 9.6	23.1	707.9 ± 29.8	3.6
	50	719.3 ± 20.7	4.0	443.2 ± 6.1	26.2	649.5 ± 18.0	11.5
	75	710.3 ± 38.5	5.2	367.9 ± 12.1	38.7	626.6 ± 24.4	14.6
	100	672.5 ± 26.5	10.2	278.5 ± 1.1	53.6	532.4 ± 16.3	27.5
Native ^c	–	749.0 ± 66.9	–	600.6 ± 2.9	–	734.0 ± 25.4	–

^a Acid concentrations in methanol.

^b Degradation extent (%) = $(DP_w \text{ of native starch} - DP_w \text{ of treated starch}) / DP_w \text{ of native starch} \times 100\%$.

^c Starch before acid-methanol treatment.

the degradation efficiency of different acids (Table 2). Coefficients of determination (r^2) of the regressed correlation lines observed were equal to or above 0.809, while slopes of the regression lines showed discrepancy among types of acid used. In spite of rice variety, starch treated with HCl had higher slope than that of starch treated with the other two acids. The obviously higher slope of HCl-treated starches indicates that the degradation of starch molecules with increasing concentration of acid is more profound for starch treated by HCl. On the other hand, the trends in slope between starches treated with HNO₃ and H₂SO₄ depended on rice variety. The slope of TKW1 starch treated with HNO₃ was higher than treated with H₂SO₄, while reverse result was found for TNG67 starch. Moreover, comparable slopes were found for TCS17 starch treated with HNO₃ and H₂SO₄.

Table 2

Correlation between the degradation extent of rice starch and the concentration of acid used in the treatment.

Acid	Rice starch		
	TKW1	TNG67	TCS17
HCl	0.432 ^a (0.809) ^b	0.862 (0.870)	0.749 (0.962)
HNO ₃	0.230 (0.914)	0.102 (0.899)	0.330 (0.965)
H ₂ SO ₄	0.108 (0.924)	0.416 (0.935)	0.299 (0.944)

^a Slope of regression line.

^b Coefficient of determination (r^2) of regression line.

Results showed that acid–methanol treatment caused significant degradation of rice starches. Furthermore, starch treated in methanol with HCl had the highest degradation extent among the three acids studied, while the difference in the degradation extent of starch treated with HNO₃ or H₂SO₄ depended on the variety of rice. This reveals that the degradation of starch, being acid-treated in methanol, depends on the concentrations of acid, the variety of starch, as well as the type of acid.

3.4. Relation between solubility and DP_w of starch

Comparing to the counterpart native starch, the solubility of treated starch increased but the DP_w decreased. This implies the degradation extent of starch affects its solubility. It was found that the solubility of starch measured at 70 °C negatively correlated ($p < 0.001$) with the DP_w of starch in spite of type and concentration of acid used in treatment (Fig. 3). In other words, the solubility of starch measured at 70 °C increased with decreasing DP_w of starch. The slope of regression line for the plot of DP_w of rice starch against its solubility measured at 70 °C was in the order of TKW1 > TNG67 > TCS17 starch. The increment in solubility with decreasing DP_w, expressed by the slope of regression line, of

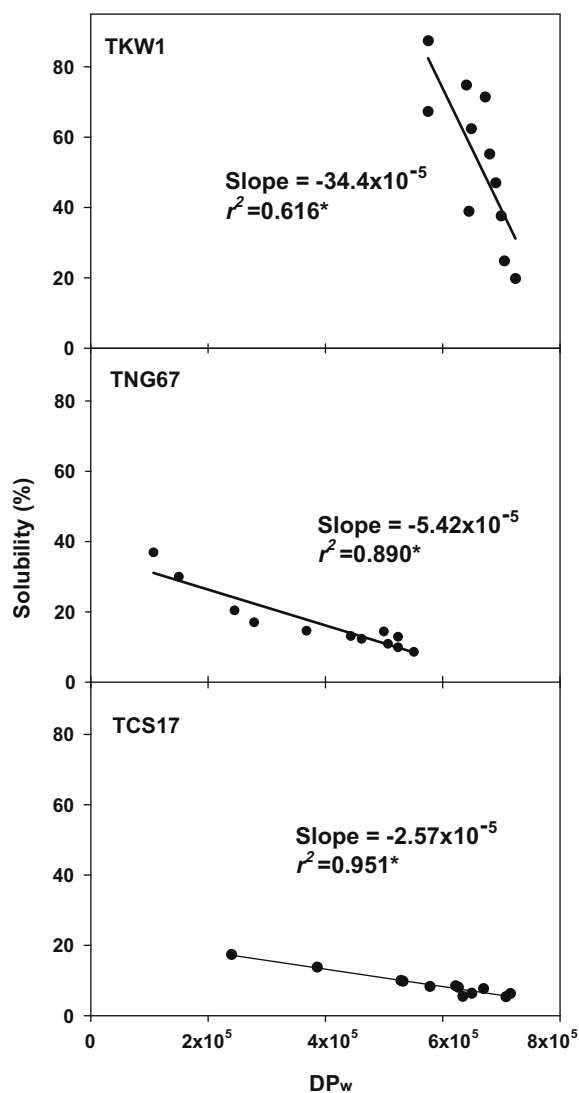


Fig. 3. Plots of the weight-average degree of polymerization (DP_w) of starch against its solubility measured at 70 °C. (* $p < 0.001$).

TKW1 was 6- and 13-fold higher than that of TNG67 and TCS17 starches, respectively. While the difference between TNG67 and TCS17 starches was less obvious than that of difference between TKW1 and TNG67 or TKW1 and TCS17 starches.

As mentioned, the increment in solubility with decreasing DP_w of starch was in the order of TKW1 > TNG67 > TCS17 starch. While the amylose content of rice starch used in this study was 0.1%, 18.3% and 29.2% for TKW1, TNG67 and TCS17 starches, respectively. The opposite order of amylose content to the increment in solubility of starch reveals that the increment in solubility with decreasing DP_w of starch negatively relates to the amylose content of starch. Amylose assists in maintaining the integrity of swollen starch granules (Tester & Morrison, 1990), therefore granules with low amount of amylose tends to rupture and dissolve during heating in water. The amylose content of TKW1 starch was too low to maintain the integrity of swollen granules. Consequently, the degradation of TKW1 starch after acid–methanol treatment resulted in the easily rupture of starch granules during gelatinization. Therefore, a dramatically increment in solubility with decreasing DP_w for TKW1 starch was observed. For starch granules with sufficient amount of amylose, the rupture of granules during heating in water was restricted and the increment in solubility reduced, as the results observed on TNG67 and TCS17 starches.

3.5. Relation between hydrogen-ion concentration in methanol and DP_w of starch

In this study, three strong acids were used for degrading starch in methanol. Generally, strong acids ionize completely in aqueous solutions; therefore aqueous solutions with the equal normality of strong acids have the same concentration of hydrogen-ion for acid hydrolysis. While results of this study show that the diversity among DP_w of starches treated with different acids in methanol are high, and that the degradation efficiency of different acids are also distinct. Neither molecular weight nor proton number of acid showed obvious effect on the DP_w of treated starches. This reveals that different acids in methanol have discrepancy ionizing extents. Table 3 shows that the pH value of HCl in methanol was lower than that of HNO₃ and H₂SO₄ in methanol at the equal normality, and an obviously higher hydrogen-ion concentration of HCl in methanol was observed. On the other hand, the pH values and hydrogen-ion concentrations of HNO₃ and H₂SO₄ in methanol at the equal normality were comparable. Similar result was reported by Rived et al. (1998). Results of pH and hydrogen-ion concentration in methanol showed agreements with the results of DP_w, degradation extent and solubility of starch. The highest hydrogen-ion concentration of HCl in methanol among the acids studied resulted in

Table 3

pH value of acid–methanol solutions prepared with anhydrous methanol containing different types and concentrations of acid.

Acid	Concentration ^a (mN)	pH	[H ⁺] ^b (N)
HCl	25	0.70	0.199
	50	0.56	0.275
	75	0.46	0.346
HNO ₃	100	0.32	0.478
	25	1.10	0.079
	50	0.95	0.112
H ₂ SO ₄	75	0.79	0.162
	100	0.75	0.177
	25	1.07	0.085
	50	0.93	0.117
	75	0.77	0.169
	100	0.71	0.195

^a Acid concentration in methanol.

^b $\text{pH} = -\log [\text{H}^+]$.

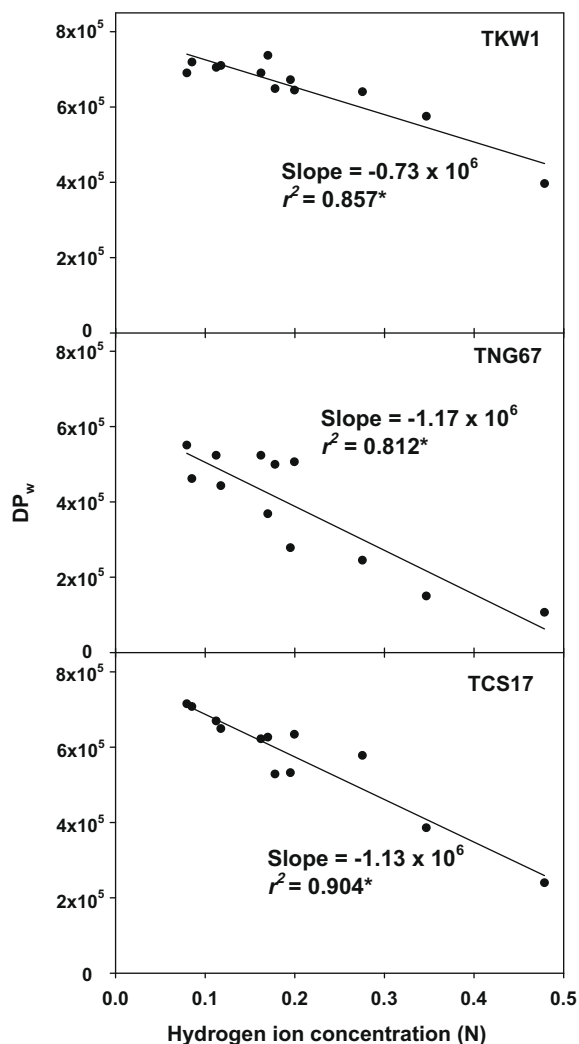


Fig. 4. Plots of the hydrogen-ion concentration in treatment solution against the weight-average degree of polymerization (DP_w) of starch. (* $p < 0.001$).

the lowest DP_w and the highest degradation extent and solubility of HCl-methanol-treated starch.

For further elucidating the relation between molecular degradation of starch and hydrogen-ion concentration in methanol, Fig. 4 illustrates plots of the hydrogen-ion concentration in treatment solution against the DP_w of starch in spite of acid type. Result showed that the DP_w of starch negatively correlated ($p < 0.001$) with hydrogen-ion concentration, indicating the higher hydrogen-ion concentration in treatment solution the greater degradation extent of starch after treated. In other words, the degradation extent of starch after acid-methanol treatment depends on the hydrogen-ion concentration in treatment solution.

The slope of regression line (Fig. 4) for TKW1 starch was obviously lower than that of TNG67 and TCS17 starches, while the slopes of regression lines for TNG67 and TCS17 starches were comparable. This indicates that the decrement in DP_w of TKW1 starch with increasing hydrogen-ion concentration was lower than that of TNG67 or TCS17 starch. Chang et al. (2006) concluded that acid-alcohol-treated waxy corn starch had higher gelatinization enthalpy than acid-alcohol-treated normal corn starch. Jenkins and Donald (1995) proposed that amylose disrupted the structural order within the amylopectin crystallites, hence more imperfect crystallites and amorphous regions were present in starch with higher amylose content. Furthermore, acid preferentially attacks

the amorphous region of starch as treated in methanol (Lin & Chang, 2006; Lin, Lii, & Chang, 2005). Therefore, the relatively lower slope of TKW1 starch than that of TNG67 and TCS17 starches could be attributed to a relatively less amount of amorphous region in TKW1 starch.

4. Conclusion

Rice starches treated in methanol with various strong acids at 45 °C for 1 h showed distinct extent of degradation. The degradation of starch depends on not only the acid concentration but also the type of acid. Starch treated with HCl had higher degradation extent and more profound increase in the solubility than those of starches treated with HNO_3 or H_2SO_4 . The variation in degradation extent of starch treated with different acids at the equal normality could be attributed to the distinct deionization extent of acids in methanol. The degradation extent of rice starches with increasing hydrogen-ion concentration in methanol related to the presence of amylose in rice starch granules. The significant correlation between the hydrogen-ion concentration in methanol and the DP_w of the treated starch indicate the hydrogen-ion concentration in methanol can be used to monitor the degradation of starch despite of type and concentration of acid used in acid-methanol treatment. The solubility of starch was negatively correlated ($p < 0.001$) with the DP_w of starch and the relation was more profound for TKW1 starch, which could be attributed to the scarce amount of amylose in TKW1 starch for maintaining granule integrity during heating in water.

Acknowledgment

We thank the National Science Council, Taiwan, for financial support (NSC 95-2313-B-126-007-MY3).

References

- BeMiller, J. N. (1964). Iodimetric determination of amylose. In R. L. Whistler, R. J. Smith, J. N. BeMiller, & M. L. Wolfrom (Eds.), *Methods in carbohydrate chemistry* (Vol. IV, pp. 165–168). New York: Academic Press.
- Boraei, A. A. A. (2001). Acidity constants of some tetrazole compounds in various aqueous-organic solvent media. *Journal of Chemical and Engineering Data*, 46(4), 939–943.
- Bowler, P., Williams, M. R., & Angold, R. R. (1980). A hypothesis for the morphological changes which occur on heating lenticular wheat starch in water. *Starch*, 32(6), 186–189.
- Chang, Y. H., Lin, J. H., & Lii, C. Y. (2004). Effect of ethanol concentration on the physicochemical properties of waxy corn starch treated by hydrochloric acid. *Carbohydrate Polymers*, 57(1), 89–96.
- Chang, Y. H., Lin, J. H., & Chang, S. Y. (2006). Physicochemical properties of waxy and normal corn starches treated in different anhydrous alcohols with hydrochloric acid. *Food Hydrocolloids*, 20(2–3), 332–339.
- Hoover, R. (2000). Acid-treated starches. *Food Reviews International*, 16(3), 369–392.
- Jenkins, P. J., & Donald, A. M. (1995). The influence of amylose on starch granule structure. *International Journal of Biological Macromolecules*, 17(6), 315–321.
- Lin, J. H., & Chang, Y. H. (2006). Molecular degradation rate of rice and corn starches during acid-methanol treatment and its relation to the molecular structure of starch. *Journal of Agricultural and Food Chemistry*, 54(16), 5880–5886.
- Lin, J. H., Lee, S. Y., & Chang, Y. H. (2003). Effect of acid-alcohol treatment on the molecular structure and physicochemical properties of maize and potato starches. *Carbohydrate Polymers*, 53(4), 475–482.
- Lin, J. H., Lii, C. Y., & Chang, Y. H. (2005). Change of granular and molecular structures of waxy maize and potato starches after treated in alcohols with or without hydrochloric acid. *Carbohydrate Polymers*, 59(4), 507–515.
- Ma, P. O., & Robyt, J. F. (1987). Preparation and characterization of soluble starches having different molecular sizes and composition, by acid hydrolysis in different alcohols. *Carbohydrate Research*, 166(2), 283–297.
- Rived, F., Roses, M., & Bosch, E. (1998). Dissociation contents of neutral and charged acids methyl alcohol. The acid strength resolution. *Analytica Chimica Acta*, 374(2–3), 309–324.
- Robyt, J. F., Choe, J. Y., Hahn, R. S., & Fuchs, E. B. (1996). Acid modification of starch granules in alcohols: Effect of temperature, and acid concentration, and starch concentration. *Carbohydrate Research*, 281(2), 203–218.
- Singh, V., & Ali, S. Z. (2000). Acid degradation of starch. The effect of acid and starch type. *Carbohydrate Polymers*, 41(2), 191–195.

- Small, J. C. (1919). A method for preparation of soluble starch. *Journal of the American Chemical Society*, 41(1), 113–120.
- Tester, R. T., & Morrison, W. R. (1990). Swelling and gelatinization of cereal starches. I. Effect of amylopectin, amylose, and lipids. *Cereal Chemistry*, 67(6), 551–557.
- VanderWeft, C. A. (1961). The Bronsted–Lowry concept of acids and bases. In *Acids, bases, and the chemistry of the covalent bond* (pp. 23–26). New York: Reinhold.
- Yang, C. C., Lai, H. M., & Lii, C. Y. (1984). The modified alkaline steeping method for isolation of rice starch. *Food Science (Taiwan)*, 11(3–4), 158–162.
- You, S., & Izydorczyk, M. S. (2007). Comparison of the physicochemical properties of barley starches after partial α -amylolysis and acid/alcohol hydrolysis. *Carbohydrate Polymers*, 69(3), 489–502.