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Change of granular and molecular structures of waxy maize and potato starches after treated in alcohols with or without hydrochloric acid

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Abstract

Starches from waxy maize and potato were treated in methanol and 2-propanol either with or without 0.36% hydrochloric acid at 65 °C for 1 h. The granule morphology, molecular structure and pasting properties of the starches were determined and the effects of treatments on the granule and molecular structures of starch were investigated. Starch treated in alcohols without acid showed loss of native order through the hilum of granules, and no obvious molecular degradation was found. However, acid–alcohol treated starch showed many cracks inside granules, and both waxy maize and potato starches showed obvious molecular degradation after treated. Furthermore, the amylose chains and long chains of amylopectin of starch were more easily degraded with acid–alcohol treatment. The pasting viscosity of acid–alcohol treated starches were also obviously less than that of their counterpart native starch and starch after alcohol treatment. The extent of degradation of molecules and the decrease of pasting viscosity on potato starch after acid–alcohol treated were more obvious than that of waxy maize starch. The result indicates that the degradation preferentially occur in the amorphous region when starch treated by acid–alcohol, and the degradation of starch molecules enhances the amorphous excretion and the occurrence of cracks inside the granules.

Keywords: Granular structure; Molecular structure; Acid–alcohol treatment; Starch

1. Introduction

Acid hydrolysis has been used to modify starch granule structure and produce 'soluble starch' for many years. In the industry, acid-modified starch is prepared with dilute hydrochloric acid (HCl) or sulfuric acid (H_2SO_4) at 25–55 °C for various time periods. The product, thin-boiling starch, is used extensively in food, textile and paper industries (Wurzburg, 1986). Although the viscosity or fluidity property of acid-hydrolyzed starch varies with the conditions used during modification, the yield of the modified starch consistently decreases with the increases of acid concentration and hydrolysis time. For obtaining the maximum conversion of raw starch into soluble starch with minimal production of low-molecular-weight dextrins, Small (1919) proposed a preparation procedure by refluxing starch granules in 95% ethanol containing 0.2–1.6% (w/v) HCl for 6–15 min. 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), with the highest value being obtained in methanol and the lowest value in 1-butanol. The yields of the modified starches were high (ranging from 100 to 88%), and the size distribution of the starch chains was narrower and more homogeneous than that of native starch. It was proposed that the different alcohols produced different concentrations of acid inside the granules, and hydrolysis of the glycosidic linkage was taking place exclusively inside the granule with the granule-bound water (Robyt, Choe, Hahn, & Fuchs, 1996).

Most studies on acid–alcohol modification of starch concerned the effects of acid concentrations, starch types and concentrations, and alcohol types and concentrations on

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the DP values (Fox & Robyt, 1992; Robyt, Choe, Fox, Hahn, & Fuchs, 1996; Robyt, Choe, Hahn, et al., 1996), the particle size and morphology of the modified starches (Jane, Shen, Wang, & Maningat, 1992), and the viscosity and stability of the emulsion made from the modified starches (Chun, Lim, Takeda, & Shoki, 1997). Few reports concerned the effect of acid–alcohol modification on the granules structure and molecular size distribution of the modified starch (Lin, Lee, & Chang, 2003; Ma & Robyt, 1987). For comprehending the change of granular and molecular structures of starch treated by acid–alcohol, study on the effect of alcohol on granular and molecular structures of starch is necessary. Jane, Craig, Seib, and Hoseney (1986) have postulated that the double helices in the starch granules 'melt' or dissolve when heated in aqueous 1-propanol and then rapidly form single helices with 1-propanol complexed in the center cavity of the helix. Polaczek, Starzyk, and Tomasik (1999) refluxed the potato starch in 1-pentanol for 24 h, and indicated that 1-pentanol could penetrate starch granule and expel the amorphous content of starch through the hilum. This phenomenon was named as 'amorphous excretion'. These results revealed the starch treated in alcohol caused change of the native order of granule. However, the effects of alcohol on the molecular structure and physicochemical properties of starch were not clear.

For further elucidating the effect of acid–alcohol treatment on granular and molecular structures of starch, in this study commercial waxy maize and potato starches were treated in alcohol (methanol or 2-propanol) with or without 0.36% HCl at 65 °C for 1 h. The effect of treatment on the granule structure of starch was investigated, and the pasting properties, molecular size distribution and chain length distribution of starches before and after treatments were also determined. Then, the differences on the granular and molecular structures between the starches treated in alcohol and acid–alcohol were discussed.

2. Materials and methods

2.1. Materials

2.1.1. Starches

Waxy maize starch was a product of National Starch and Chemical Company (Bridgewater, MA) and potato starch was obtained from Parachem Company (Brande, Denmark). The moisture content of waxy maize starch was 12.2% (w/w), and potato starch was 13.7%.

2.1.2. Enzyme

Isoamylase (EC 3.2.1.68) of *Pseudomonas amyloderamosa* (59,000 U/mg) was purchased from Hayashibara Biochemical Laboratories Inc. (Okayama, Japan). All reagents used were of analytical grade.

2.2. Methods

2.2.1. Sample preparation

Treated starch was prepared by stirring 25 g (dry basis) of native starch in 100 mL anhydrous (<0.03% water) methanol or 2-propanol with or without 0.36% hydrochloric acid (HCl) at 65 °C for 1 h. For acid–alcohol treatment, the reaction started by adding 1 mL of concentrated (36% by weight) HCl, and allowed to proceed for 1 h. The reaction was stopped by adding 14 mL of 1 M $NaHCO_3$. Starch suspension after alcohol or acid–alcohol treatment was cooled in ice-bath for 5 min, and centrifuged at $3500 \times g$ for 5 min. The precipitate was washed with 50% ethanol until the NaCl was absent (detected by adding 1% $AgNO_3$) in supernatant, and was dried in an air-oven at 40 °C. The yield was calculated by the weight of recovery starch to the initial weight of dry starch.

2.2.2. Granule size distribution

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

2.2.3. 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.

2.2.4. Polarized-light microscopy

Starch granules were dispersed onto a microscope slide and observed by a light microscope (BH-2, Olympus, Tokyo, Japan) equipped with a cross-polarizer at room temperature. Photomicrogram of a calibration slide at the same magnification was taken and was used as a reference for size determination.

2.2.5. Light microscopy

Starch granules were stained by iodine solution (containing 2 mg I_2 and 2 mg KI per mL). The suspensions was dispersed onto a microscope slide and observed by a light microscope (BH-2, Olympus, Tokyo, Japan) at room temperature. The reference of size determination was the same as that used in polarized-light microscopy.

2.2.6. Pasting properties

Pasting properties of starch was determined by using a rapid viscoanalyzer (model 3D+, Newport Scientific, Warriewood, Australia). Each starch suspension (28 g total weight; 7%, w/w, dry basis, for all waxy maize starch samples, native potato starch and alcohol treated potato starches; 14% for acid–alcohol treated potato 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.

2.2.7. Molecular weight distribution

The solution of native and alcohol treated starches 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–alcohol treated starches, 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 (Lin et al., 2003).

Each starch solution was filtered through a 5.0 μm syringe filter (Millipore, Billerica, MA), and then the filtrate (100 μL) was injected into an HPSEC system. The system consisted of an isocratic pump (G1310A, Hewlett Packard, Wilmington, DE), a refractive index (RI) detector (HP 1047A), and a multiangle laser light-scattering (MALLS) detector (Dawn DSP, Wyatt Tech., Santa Barbara, CA). 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 ranged from 5.6 to 769.5 kDa; Polymer Standards Service, Silver Spring, MD).

2.2.8. Chain length distribution

Starch solution (2.5 mg starch/2.45 mL H_2O) was prepared according to the procedures described above. After cooling to room temperature, acetate buffer (0.05 mL, 1.0 M, pH 3.5) and isoamylase solution (10 μL, 5.9 U/μL) were added to the starch solution, then the mixture incubated in a shaker bath at 45 °C for 24 h (Suzuki, Hizukuri, & Takeda, 1981). 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 boiling water bath for 10 min. Debranched starch solutions were then filtered using a 0.45 μm syringe filter (Millipore). The filtrate (100 μL) was injected 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 and the mobile phase was 100 mM phosphate buffer (pH 6.2) containing 0.02% NaN_3. 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 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 ranged from 1.0 to 46.0 kDa; Polymer Standards Service)(Lin et al., 2003).

3. Results and discussion

3.1. Yields and average granule size of starches

The yields of the starches treated in alcohols at 65 °C for 1 h either with or without 0.36% HCl are shown in Table 1. Starches treated in alcohol with 0.36% HCl showed lower yields than those treated without acid. The yields of the starches treated in alcohols with acid ranged from 80.5 to 94.4%, while the yields of starches treated in alcohols without acid were all above 99% (99.3–99.8%). Robyt, Choe, Fox, et al. (1996) and Robyt, Choe, Hahn, et al. (1996) treated the potato starch in methanol, ethanol, 2-propanol, and 1-butanol using different acid concentrations (0.36 to 5.0%, w/v) and temperatures (5–65 °C) for 72 h. The results indicated that the granule structure of potato starch was completely destroyed after treated in 2-propanol or 1-butanol at temperature above 35 °C and acid concentration above 0.71%. Results of Table 1 also

Table 1
Yields and average granule sizes of starches

Starch	Yield[a] (%)	Average granule size (μm)
Waxy maize		
Native	–	15.8±0.1
Methanol[b]	99.3	15.3±0.0
Methanol+acid[c]	94.4	15.4±0.0
2-Propanol[b]	99.8	14.9±0.1
2-Propanol+acid[c]	91.6	15.0±0.1
Potato		
Native	–	47.4±0.1
Methanol	99.8	47.9±0.2
Methanol+acid	80.5	46.6±0.1
2-Propanol	99.6	47.6±0.0
2-Propanol+acid	82.0	45.2±0.1

[a] (Weight of starch after treated)/(weight of starch before treated)× 100%.
[b] Starch treated in methanol or 2-propanol without acid at 65 °C for 1 h.
[c] Starch treated in methanol or 2-propanol with 0.36% HCl at 65 °C for 1 h.

suggested that a part of starch granules was destroyed and lost during acid–alcohol treatment. However, more than 80% of starch granules were recovered after treated in methanol or 2-propanol containing 0.36% HCl at 65 °C for 1 h. Compared to the native starch, the average granule sizes of the treated waxy maize starches either with or without acid were slightly smaller. While the potato starch treated in alcohols without acid showed similar average granule size to the native one, and the granule size decreased after treated in alcohols with acid.

3.2. Granule structure of starches

Examined by SEM, the native waxy maize starch (Fig. 1A) showed polygonal, irregular shape, while potato starch (Fig. 1F) had oval or spherical-like shape. The granule surface of the native starches are either smooth for potato or with naturally-occurred, randomly-distributed surface openings (Fannon, Hauber, & BeMiller, 1992) on some waxy maize granule. After treatment, morphological changes on starch granules were found. The granule surface of waxy maize starch (Fig. 1B–E) changed to rough, and caves were also found. Potato starch treated in methanol (Fig. 1G) showed no obvious changes on granule surface, whereas the granules surface changed rough after treated in 2-propanol without acid and in both alcohols with acid (Fig. 1H–J). Furthermore, fragmentation of starch granule was also found after treated in 2-propanol with 0.36% HCl (Fig. 1J).

Native starch granules showed a clearly recognizable 'Maltese cross' pattern under polarizing light (Fig. 2A and F).

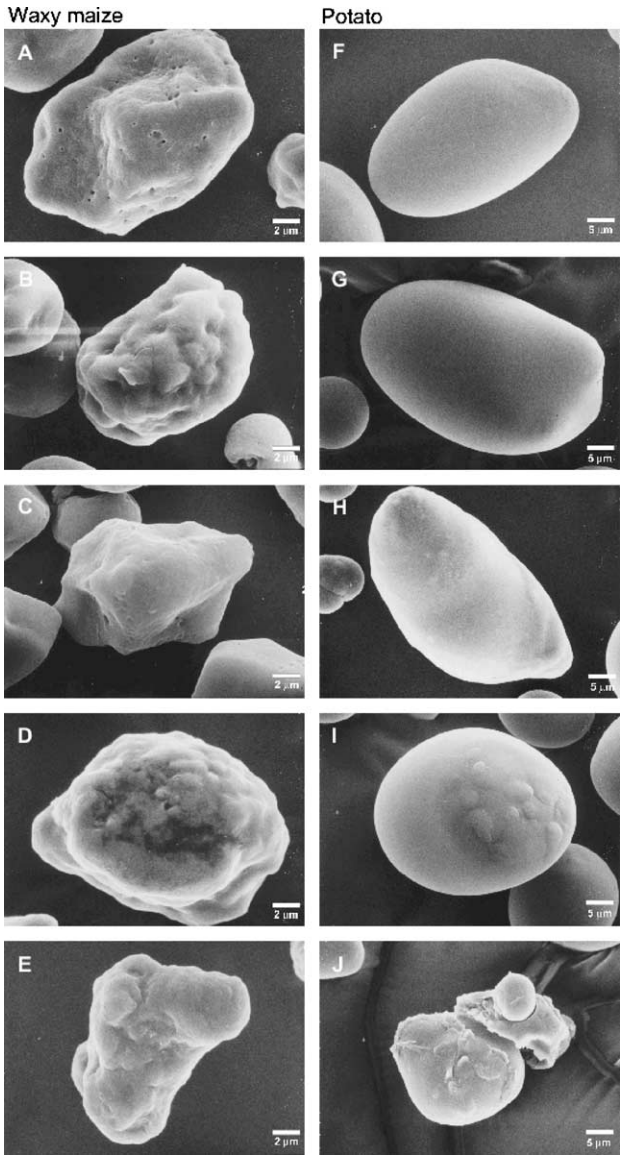

Fig. 1. Scanning electron micrograms of native starches (A, F) and starches treated at 65 °C for 1 h in methanol (B, G), methanol with 0.36% HCl (C, H), 2-propanol (D, I), and 2-propanol with 0.36% HCl (E, J), respectively.

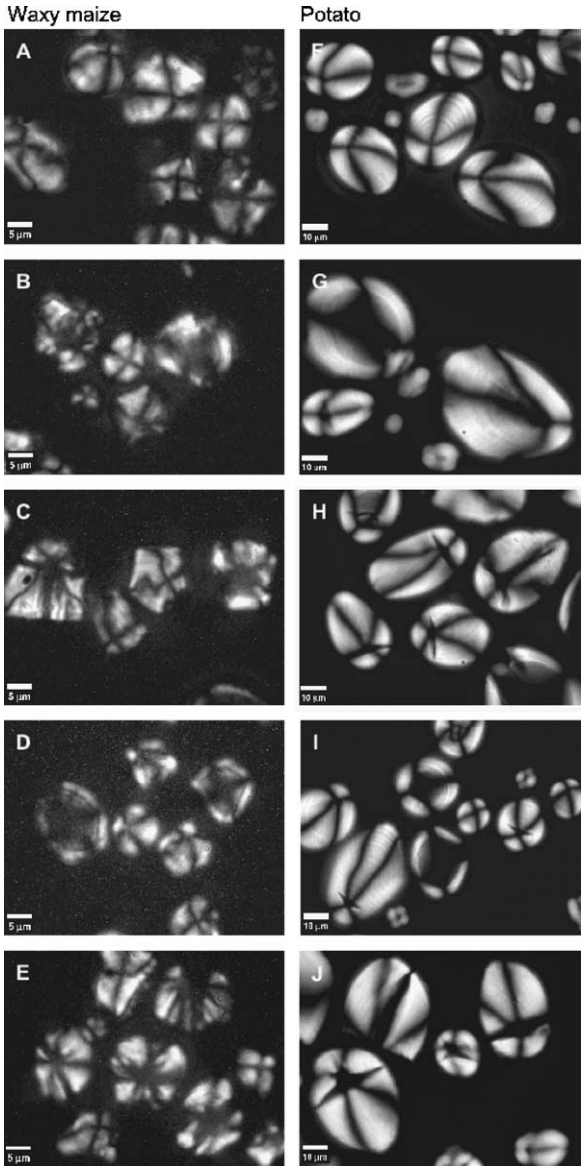

Fig. 2. Polarized-light micrograms of native starches (A, F) and starches treated at 65 °C for 1 h in methanol (B, G), methanol with 0.36% HCl (C, H), 2-propanol (D, I), and 2-propanol with 0.36% HCl (E, J), respectively.

However, after treated in alcohols either with or without acid the 'Maltese cross' pattern in the center of some starch granules disappeared (Fig. 2B–E and G–J). The loss of polarized light in the center of starch granule after treated reflects the loss of native order. A similar result was found in potato starch granule after refluxed in 1-pentanol, and the loss of 'Maltese cross' of starch granule was attributed to the alcohol penetrated the starch and expelled the amorphous content of starch granule through the hilum (Polaczek et al., 1999). After staining by iodine solution, waxy maize starch showed brown color under light microscope (Fig. 3A–E) (web version only), which was the typical color of amylopectin from iodine staining (Obanni & BeMiller, 1995). Native and alcohol treated waxy maize starch granules (Fig. 3A, B and D) showed intact granule morphology, and no obvious cracks were found inside the granules. However, waxy maize starch after acid–alcohol treatment (Fig. 3C and E) had many cracks inside the granule. After staining by iodine solution, potato starch granules showed blue color (web version only) (Fig. 3F–I) except for the starch treated in 2-propanol with 0.36% HCl (Fig. 3J), which showed purple color after staining (web version only). The blue color of potato starch was due to the complex formed mainly by amylose with iodine solution. The purple color of potato starch treated in 2-propanol with 0.36% HCl (Fig. 3J) (web version only) after iodine staining, was different from that of counterpart native starch and that of starch treated under other conditions. This implies that the amylose of potato starch was degraded after treated in 2-propanol with 0.36% HCl. Native potato starch showed intact granule morphology (Fig. 3F), and no cracks could be found inside the granules. Nevertheless, after treated in alcohol without acid (Fig. 3G, I), some cracks were observed inside potato starch granules. Moreover, after acid–alcohol treatment (Fig. 3H, J) more obvious cracks inside the potato starch granules were found.

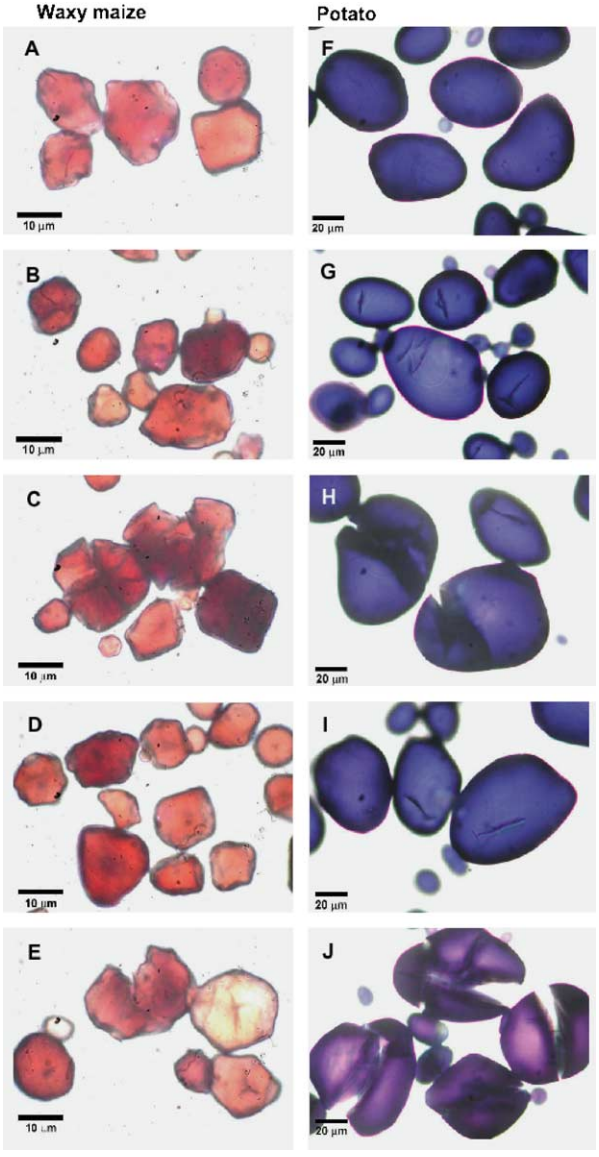

Fig. 3. Light micrograms of iodine stained starches: native (A, F) and starches treated at 65 °C for 1 h in methanol (B, G), methanol with 0.36% HCl (C, H), 2-propanol (D, I), and 2-propanol with 0.36% HCl (E, J), respectively.

As shown in Figs. 2 and 3, acid–alcohol treatment causes not only the disappearance of 'Maltese cross' pattern in the center of granule but also the occurrence of cracks inside the granule. Furthermore, the changes on potato starch after treated are more profound than that of waxy maize starch. Consequently, lower yield of potato starch after acid–alcohol treatment is obtained.

3.3. Pasting viscosity of starches

The pasting properties of native starches and starches after treatment are shown in Table 2. Compared to the acid–alcohol treated starches the pasting viscosity of alcohol treated starches were higher, and the pasting profiles of

Table 2
Pasting properties of starches

Starch	Viscosity (cP)				
	Peak	Hot paste	Final	Break-down	Set-back
Waxy maize					
Native[a]	2166	1123	1220	1043	97
Methanol[a]	1957	1034	1084	923	50
Methanol + acid[a]	775	394	325	381	−69
2-Propanol[a]	1972	1122	1143	850	21
2-Propanol + acid[a]	239	188	225	120	37
Potato					
Native[a]	7645	1815	2486	5830	671
Methanol[a]	8008	2182	2522	5826	341
Methanol + acid[b]	186	–[c]	62	–	–
2-Propanol[a]	8652	2346	2626	6306	280
2-Propanol + acid[b]	–	–	–	–	–

[a] Measured at 7% (d.b.) starch suspension.
[b] Measured at 14% (d.b.) starch suspension.
[c] Undetectable.

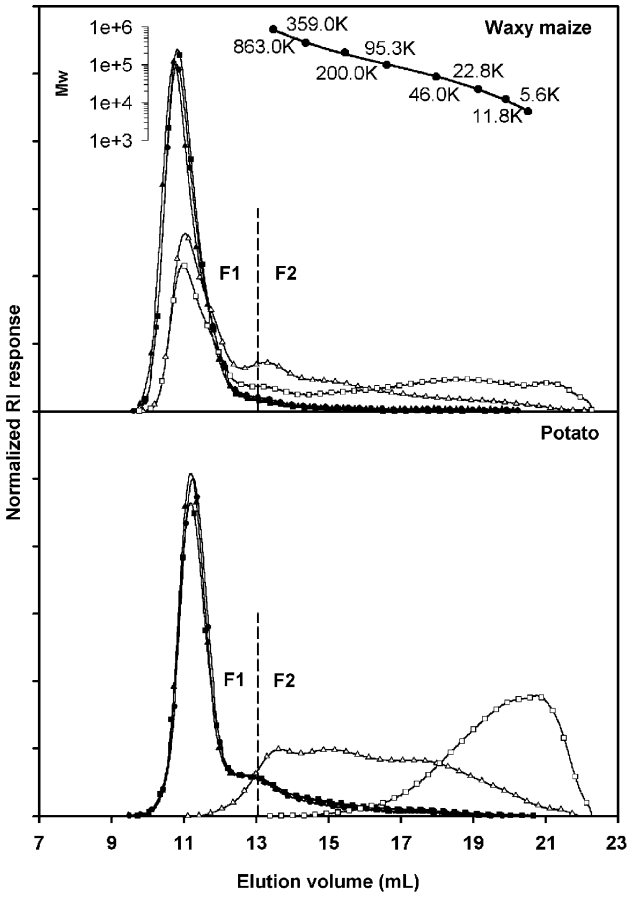

Fig. 4. HPSEC profiles of native starches (●) and starches treated at 65 °C for 1 h in methanol (▲), methanol with 0.36% HCl (△), 2-propanol (■), and 2-propanol with 0.36% HCl (□), respectively.

alcohol treated starch were quite similar to their counterpart native starch. Waxy maize starch treated in methanol or 2-propanol without acid had slightly lower value in pasting viscosity than that of the native starch. While potato starch had slightly higher value in pasting viscosity after alcohol treated. After acid–alcohol treatment, the pasting viscosity of starches obviously decreased for both waxy maize and potato starches. In spite of the sample concentration used during the RVA measurement, the viscosity of acid–alcohol treated starch was obviously less than that of the counterpart native starch and alcohol treated starch. After acid–alcohol treatment, waxy maize starch showed a lower decreasing extent on pasting viscosity than potato starch. Furthermore, starches treated in 2-propanol showed a higher decreasing extent on pasting viscosity than those treated in methanol. Even at 14% of dry solid concentration, the pasting viscosity of potato starch treated in 2-propanol was too low to be detected.

3.4. Molecular weight distributions of starches

Molecular weight distributions of starches are shown in Fig. 4. The first (F1) fraction of the HPSEC profile corresponded to the amylopectin of starch, and the second (F2) fraction to the amylose or low molecular weight molecules. Both waxy maize and potato starches treated in methanol or 2-propanol without acid showed similar HPSEC profiles to their counterpart native starches. After acid–alcohol treatment, the peak of F1 fraction on HPSEC profile of both waxy maize and potato starches shifted toward lower molecular weight. Table 3 summarizes the relative content (weight percentage) and average degree of polymerization (DP) of each fraction of starches before and after treatment. No obvious differences were found on the relative contents and DP values of F1 and F2 fractions of starches treated in alcohols without acid from their counterpart native starches. For the acid–alcohol treated starch, the content of F1 fraction decreased and that of F2 fraction increased. Furthermore the average degree of polymerization (DP), either in weight (DP_w) or in number (DP_n) of the F1 fraction also decreased. Approximately 40% (from 93.0 to 56.2%) content of F1 fraction of waxy maize

Table 3
Weight percentages and average degrees of polymerization of HPSEC fractions of starches

Starch	F1			F2		
	%	DP_w[a] ($\times 10^3$)	DP_n[b] ($\times 10^3$)	%	DP_w	DP_n
Waxy maize						
Native	93.0±0.7	460.2±7.9	382.1±17.1	7.0±0.7	2588±201	589±90
Methanol	94.3±0.3	440.6±0.5	388.6±1.0	5.7±0.3	2664±107	591±46
Methanol+acid	56.2±0.8	449.2±12.4	252.6±8.5	43.8±0.8	2125±31	158±33
2-Propanol	95.6±1.0	466.3±1.9	397.6±3.4	4.0±1.0	2631±141	548±65
2-Propanol+acid	44.8±2.4	465.0±12.4	312.1±17.0	55.2±2.4	999±28	53±3
Potato						
Native	83.8±1.6	459.2±2.5	314.6±10.4	16.2±1.6	2993±192	1080±32
Methanol	82.4±3.1	482.2±12.7	351.9±17.5	17.6±3.1	3008±175	1154±75
Methanol+acid	6.0±0.2	433.1±9.3	112.8±12.4	94.0±0.2	1526±7	251±12
2-Propanol	81.8±1.1	424.8±16.8	269.6±23.6	18.2±1.1	2936±119	966±52
2-Propanol+acid	–[c]	–	–	100.0±0.0	127±22	31±2

[a] Weight average degree of polymerization.
[b] Number average degree of polymerization.
[c] Undetectable.

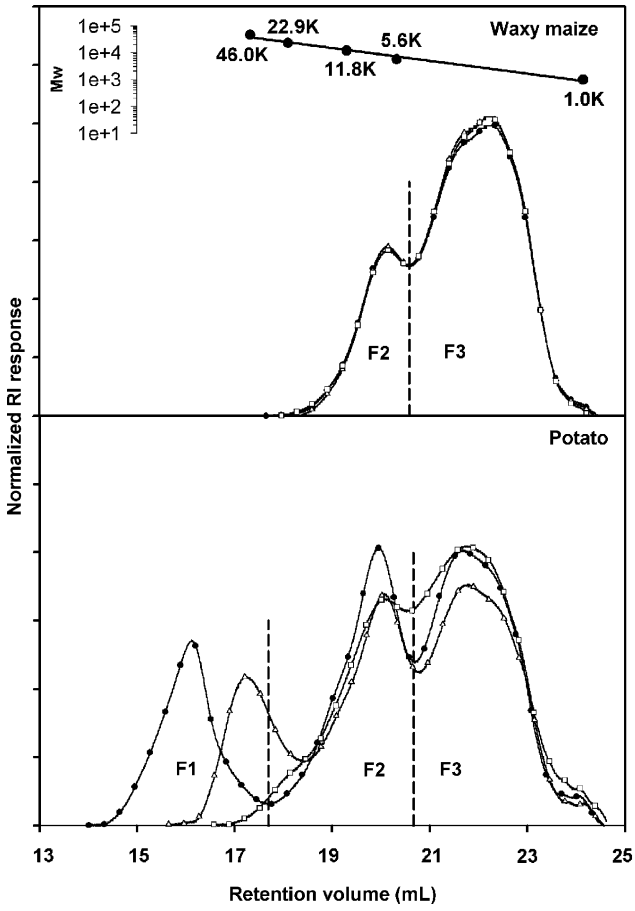

Fig. 5. HPSEC profiles of isoamylase-debranched starches after treated at 65 °C for 1 h with 0.36% HCl in different alcohols: native (●), methanol (△), and 2-propanol (□), respectively.

starch degraded to smaller molecules (F2 fraction) after treated in methanol with acid, and about 52% (from 93.0 to 44.8%) degradation was found on waxy maize starch treated in 2-propanol. However, about 93% (from 83.8 to 6.0%) and 100% (from 83.8 to 0%) of F1 fraction molecules of potato starch degraded after treated with acid in methanol and 2-propanol, respectively. The higher decreasing extent on relative content and DP value of F1 fraction of potato starch after acid–alcohol treatment, either in methanol or in 2-propanol, than the corresponding waxy maize starch indicates the higher sensitivity of potato starch to acid–alcohol treatment. This confirmed the higher extent of decreasing on the pasting viscosity of potato starch after acid–alcohol treated.

Native waxy maize contained about 7% F2 fraction (Table 3) and showed an A-type crystalline pattern (Jayakoda & Hoover, 2002), while potato starch contained about 16% F2 fraction and showed a typical B type crystalline pattern (Gunaratne & Hoover, 2002). As indicated above, potato starch showed a higher susceptibility to the acid–alcohol treatment than waxy maize starch, which should result from the differences on the amylose content and crystalline structure of these two starches. Lin et al. (2003) indicated that with a similar amylose content normal maize starch, which had an A-type crystalline pattern, showed less susceptibility to the acid–alcohol treatment than potato starch, which had a B-type pattern. Results indicated that in spite of the amylose content of starch, the susceptibility of waxy and normal maize (A-type starch) on acid–alcohol treatment was significantly less than that of potato starch (B-type starch). It implies that the crystalline structure of starch plays an important role on the degradation of starch when treated by acid–alcohol. More study is needed for further elucidating the effect of crystalline structure on the degradation of starch during acid–alcohol treatment.

Starch treated in 2-propanol showed a higher extent of degradation than that of starch treated in methanol. This agreed with the result of Ma and Robyt (1987). The different extent of degradation of starch treated by 0.36% HCl in methanol and 2-propanol could be attributed to the different amounts of acid inside the granule during treatment (Ma & Robyt, 1987).

3.5. Chain length distribution of starches after acid–alcohol treatment

Since starches treated in alcohols without acid did not showed significant differences on molecular weight distribution from the counterpart native starches, Fig. 5 shows

Table 4
Weight percentages and chain length distributions of HPSEC fractions of isoamylase-debranched starches after treated with 0.36% HCl in alcohols at 65 °C for 1 h

Starch	F1			f2			f3		
	%	DP_w	DP_n	%	DP_w	DP_n	%	DP_w	DP_n
Waxy maize									
Native	–[a]	–	–	25.5±0.5	75±6	58±1	74.5±0.5	19±0	16±0
Methanol+acid	–	–	–	24.0±0.4	63±1	56±0	76.0±0.4	19±0	16±0
2-Propanol+acid	–	–	–	23.7±0.5	62±1	56±1	76.3±0.5	19±0	15±0
Potato									
Native	19.7±1.1	9014±860	3398±258	33.6±1.6	69±1	60±0	46.7±0.9	19±0	16±0
Methanol+acid	14.0±0.2	1244±434	576±59	37.3±0.5	77±0	64±0	48.7±0.8	19±0	16±0
2-Propanol+acid	1.0±0.1	1092±120	512±26	37.6±0.6	70±0	60±0	61.5±0.5	19±0	15±0

[a] Undetectable.

only the HPSEC profiles of native starches and acid–alcohol treated starches after isoamylase-debranched. Generally, the profiles showed trimodel distribution, and the f1 fraction consisted of amylose, f2 the longer (B2 or longer) chain fraction of amylopectin, and f3 the shorter (A and B1) chain fraction of amylopectin. However, waxy maize starch contains very low amylose, therefore the profile showed bimodal chain length distribution. Similar profile of waxy corn starch was proposed by Shi, Capitani, Trzasko, and Jeffcoat (1998). As shown in Fig. 5, no obvious differences were found on the elution time of each fraction of the waxy maize starches before and after acid–alcohol treatment. For potato starch, trimodal chain length distribution was found, after acid–alcohol treatment molecules in f1 fraction shifted to lower molecular weight, and the relative content (by weight) of f1 fraction decreased, especially for starch treated in 2-propanol.

Table 4 summarizes the chain length parameters of starch before and after treated with 0.36% HCl in alcohols. Waxy maize starch, with very low amylose content, showed undetectable content of f1 fraction. While potato starch had higher amylose content (19.7%) and showed significant decrease in both the content and chain length of f1 fraction after acid–alcohol treatment. For waxy maize starch, the content and DP value of f2 fraction decreased after acid–alcohol treatment. However, potato starch showed a reverse result. The increase of relative content and DP value of f2 fraction of potato starch could attribute to the degradation of amylose molecules (f1 fraction). The relative content of f3 fraction increased after acid–alcohol treatment for both waxy maize and potato starches, but no obvious differences on DP values were found. The results reveal that the amylose chain and the long chain of amylopectin are more easily degraded by acid–alcohol treatment. Hizukuri (1986) elaborated that A and B1 chains (short chain of amylopectin) made a single cluster, and B2, B3, and B4 chains (long chain of amylopectin) extend into a comprised 2, 3, and 4 or more clusters, respectively. Kasemsuwan and Jane (1994) proposed the amylose molecules were randomly interspersed in the starch granule instead of being in bundles. Furthermore, the amorphous region of starch was consisted of free amylose, lipid-complexed amylose, and mainly a frequently branched portion of amylopectin (Hizukuri, 1996). In this study, the average DP of f3 fraction (A and B1 chains) of starch showed no obvious changes after acid–alcohol treated, but the proportion of f3 fraction increased. This indicates that the single cluster of acid–alcohol treated starch remains unchanged. The higher extent of degradation on amylose and long chain of amylopectin of starch treated by acid–alcohol further implies that the degradation of starch granule treated by acid–alcohol is preferentially occurred in amorphous region. Similar results were observed in acid–alcohol treatment of starch (Lin et al., 2003), and in acid hydrolysis of starch in water (Robin, Mercier, Charbonnie're, & Guilbot, 1974). Gallant, Bouchet, and Baldwin (1997) proposed that the function of the amorphous fraction within the crystalline shells of the starch controlled the variation in granule volume. In this study, the chains in amorphous region (amylose or long chain of amylopectin) degraded during acid–alcohol treatment, which implies that the granule volume of treated starch should be more easily changed. It also infers that the phenomenon of amorphous excretion of starch granules during acid–alcohol treatment could be more profound, consequently the cracks inside the granules occur more obvious.

4. Conclusion

Starch treated in alcohol without acid results in the occurrence of amorphous excretion inside the starch granule without degrading the starch molecules. While treated by acid–alcohol both molecular degradation and amorphous excretion occurred in starch granules, which resulted in the cracks developing inside the granule and the lower pasting viscosity of treated starch during cooking. Furthermore, the changes on the molecular structure, granular structure and pasting viscosity of potato starch after acid–alcohol treatment are more profound than that of waxy maize starch.

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