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ACID-ALCOHOL DEGRADATION OF STARCH

Summary

For revealing the degradation effect of acid-alcohol modification on starch, commercial corn and potato starches were hydrolyzed by 0.36% HCl in methanol at 25°C for 1 to 15 days. Results showed the yields of the modified starches ranged from 91 to 100%, and the average granule size of the modified starch was slightly smaller as compared to their counterpart native starches. After 15 days of modification, no significant configuration change was found whereas the solubility of starches obviously increased with the increase in the hydrolysis time. Corn starch showed a slower increasing tendency than that of potato starch. Gelatinization onset temperatures (T_o) of starches after modification showed a decrease tendency in corn starch, and an increase tendency in potato starch. For both corn and potato starches, the peak (T_p) and conclusion (T_c) temperatures of gelatinization increased with the increase in the treatment time.

Consequently, the range of gelatinization increased from 12.2 to 23.9°C for corn starch, and from 12.8 to 19.8°C for potato starch. However, the gelatinization enthalpies (• H) of modified starches showed 1 to 2 J/g lower than their counterpart native starches. For the acid-alcohol treated starches, the area of amylopectin fractions determined by high-performance size exclusion chromatography (HPSEC) decreased with the treatment time. Compared to the gradual degradation pattern of corn starch, potato starch showed a stepwise pattern. The weight average degree of polymerization (DP_w) of starches rapidly decreased within the first 5 days of treatment, and potato starch had a higher rate of decrease than corn starch. The number average degree of polymerization (DP_n) of corn and potato starches after acid-alcohol treated for 15 days were 162 and 183, respectively. It was concluded that the degradation of amylopectin by the acid-alcohol modification might alter the molecular structure of starch, which resulted in the fast disruption of granule and the decrease of viscosity of starch paste during heating.

Introduction

For many years, acid hydrolysis has been used to modify starch granule structure and produce "soluble starch". In industry, acid-modified starch is prepared on treatment of starches with dilute HCl or 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

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[1]. Although the viscosity or fluidity properties 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 maximum conversion of raw starch into soluble starch with minimal production of low-molecular-weight dextrins, Small [2] 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 [3] showed that treatments of potato and waxy maize starches 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 more narrow and homogeneous than that of native starch. It was proposed that the different alcohols produced different concentrations of acid inside the granules. Hydrolysis of the glycosidic linkage took place exclusively inside granules with the granule-bound water. However, the hydrolysis of potato starch granules in the presence of the above alcohols with 0.36% HCl did not proceed indefinitely, the DP values of the modified products rapidly decreased and became constant after 72 h of reaction [4].

Most studies on acid-alcohol modification of starch concerned the effects of acid concentrations, starch types and concentrations, and alcohol types and concentrations on the DP values [4, 5, 6], the particle size and morphology [7] of the modified starches, and the viscosity and stability of the emulsion made from the modified starches [8]. Few reports [3] considered the effect of acid-alcohol modification on the molecular size distribution, and its final results on the physicochemical properties of the modified starch. In this report, commercial corn and potato starches were hydrolyzed by 0.36% HCl in methanol at 25°C for 1 to 15 days. The treatment effects on the granule size, morphology, solubility, and gelatinization properties of starch were investigated. The changes in molecular size distribution of modified starches were also determined by high-performance size exclusion chromatography (HPSEC) and discussed with the change in the physicochemical properties for further elucidating the effects of acid-alcohol modification on starch.

Materials and methods

Starches

Corn starch and potato starch were obtained from National Starch and Chemical Company (Bridgewater, NJ). The moisture content of corn starch was 12.6% (w/w), and potato starch 13.7%.

Acid-alcohol modification

Starch (25g) was suspended in 100 mL methanol (< 0.3% water) in a 250 mL flask. The suspension was stirred at 25°C. Reaction was started by adding of 1 mL of concentrated (36% by weight) hydrochloric acid, and allowed to proceed for 1, 3, 5, 7, 9, 11, 13, and 15 days, respectively. The reaction was stopped by adding of 14 mL of 1M $NaHCO_3$. The starch was centrifuged at 2,500 × g for 5 min and washed four times with 50% ethanol. The precipitate was air-oven dried at 40°C. The yield was calculated by weight of the recovery starch to the initial weight of dry starch.

Size distribution and morphology of starch granule

The size distribution of starch granule was determined by using a laser light scattering based particle size analyzer (Mastersizer Micro, Malvern Instruments, UK.). Granule morphology of starch was studied with a scanning electron microscope (SEM ABT150S, Topcon, Japan). Starch samples were mounted on circular aluminum stubs with double sticky tape, coated with gold, and then examined and photographed at an accelerating potential of 10 kV.

Solubility

Starch (0.1g, dry basis) was heated in 40 ml of water to the desired temperature for 30 min. The formation of lump was prevented by continuously stirring. The mixture was centrifuged at 4 000 × g for 15 min, then the supernatant was decanted and the swollen starch sediment weighed. An aliquot of supernatant was evaporated overnight at 130°C and weighed. The solubility was the ratio in weight of the dried supernatant to the initial weight of the dry starch.

Pasting properties

Pasting properties of starch were determined with a Rapid Visco-Analyzer (RVA 3D°C, Newport Scientific, Australia). Each starch suspension (7%, w/w, dry basis for corn and 6% for potato; 28g total weight) 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 sec and then 160 rpm for the rest of the analysis.

Gelatinization properties

Gelatinization properties of starch were determined by using a differential scanning calorimeter (Micro DSC VII, Setaram, France). Starch sample (about 150 mg, dry basis) was weighed in the sample pan, mixed with distilled water (about 450 mg), and sealed. The samples were heated from 25 to 120°C at a heating rate of 1.2°C /min. Onset (T_o), peak (T_p) and conclusion (T_c) temperatures together with gelatinization enthalpy (ΔH) were quantified.

Molecular weight distribution

The molecular weight distribution of starch was determined by HPSEC. 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, and 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 4,000 × 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 modified starch, the starch solution was prepared by solubilizing 10 mg (dry basis) of starch with 15 mL deionized water and stirred in a boiling water bath for 1 h.

Each starch solution was filtered through a 5.0 μm syringe filter, and then the filtrate (100 μL) was injected into an HPSEC system. This system consisted of an HP G1310A isocratic pump (Hewlett Packard, USA), refractive index (RI) detector (HP 1047A), and a multiangle laser light-scattering (MALLS) detector (Dawn DSP, Wyatt Tech., USA) with a helium-neon laser light source ($\lambda = 632$ nm) and a K-5 flow cell. The columns used were PWH (guard column), G5000PW and G4000PW (TSK-Gel, Tosoh, Japan) HPSEC columns connected in series and kept at 70°C. The mobile phase was 0.1M $NaNO_3$ solution containing 0.02% NaN_3 at a flow rate of 0.5 mL/min.

Results and discussion

The yields of the starches modified by 0.36% HCl in methanol at 25°C for 1 to 15 days were high, ranging from 91 to 100% (Table 1). Compared to their counterpart native starches, the average granule size of the modified starch was slightly smaller. For the potato starch after 15 days of treatment, the average granule size decreased from 47.2 μm of native starch to 44.8 μm (Table 1). The changes of granule size of corn starch after modification were less obvious.

Examined by SEM, the native corn starch (Fig. 1, A and B) showed polygonal, irregular shape, while potato starch (Fig. 1, E and F) had oval or spherical-like shape. The granule surface of the native starches was smooth without obvious fissures or cavities. After 15 days of modification, no significant configuration change such as fragmentation or swelling was found. However, the granule surface of corn starch changed rough, and the naturally-occurred, randomly-distributed surface openings [9] of some granules became more obvious (Fig. 1, C, and D). The granule surface of potato starch also changed rough with partial protuberances (Fig. 1, G and H).

Table 1

Yields and average granule sizes of native starches and starches treated by 0.36% HCl in methanol at 25°C for 1 to 15 days.

Time (days)	Yield*(%)		Average granule size (μm)	
	Corn	Potato	Corn	Potato
Native	–	–	13.7±0.1	47.2±0.2
1	98.5	98.0	13.4±0.0	47.6±0.1
3	98.4	98.4	13.6±0.0	46.1±0.9
5	98.4	99.8	13.6±0.0	47.5±0.4
7	100.0	100.0	13.6±0.0	47.9±0.1
9	97.1	94.6	12.4±0.2	47.6±0.0
11	97.2	96.4	12.5±0.3	45.4±0.1
13	91.7	92.7	12.8±0.0	43.3±0.2
15	97.7	95.2	13.2±0.0	44.8±0.7

*(Weight of starch after acid-alcohol treatment)/(weight of starch before treatment) x 100%.

Fig. 2 shows the solubility of corn and potato starches measured at different temperatures (65–95°C). In spite of the measurement temperature, the solubility of native corn and potato starch was below 10% and 22%, respectively. After acid-alcohol modification, the solubility of starches obviously increased with the increase in the hydrolysis time. Corn starch showed a slower increasing tendency, and the solubility value stabilized after 11 days of treatment. In contrast, the solubility of potato starch increased rapidly, and reached its maximum value after 3 days of treatment. As the measurement temperature was above 75°C, the solubility of modified potato starches were higher than 90%. This indicated the starch granules were nearly fully dissolved. Results of pasting properties (Fig. 3) of starches measured by RVA confirmed the high solubility of modified starches. Native potato starch had higher peak viscosity and lower pasting temperature than the native cornstarch. After the acid-alcohol treatment the pasting viscosity of the modified starches decreased obviously for both potato and corn starches. Among the modified starches prepared, only the cornstarch after acid-alcohol treated for 1 day showed the entire profile of the RVA amylograph similar to that of the native starch. Other modified starches showed either very lower (less than 100 cps) or undetectable peak viscosity.

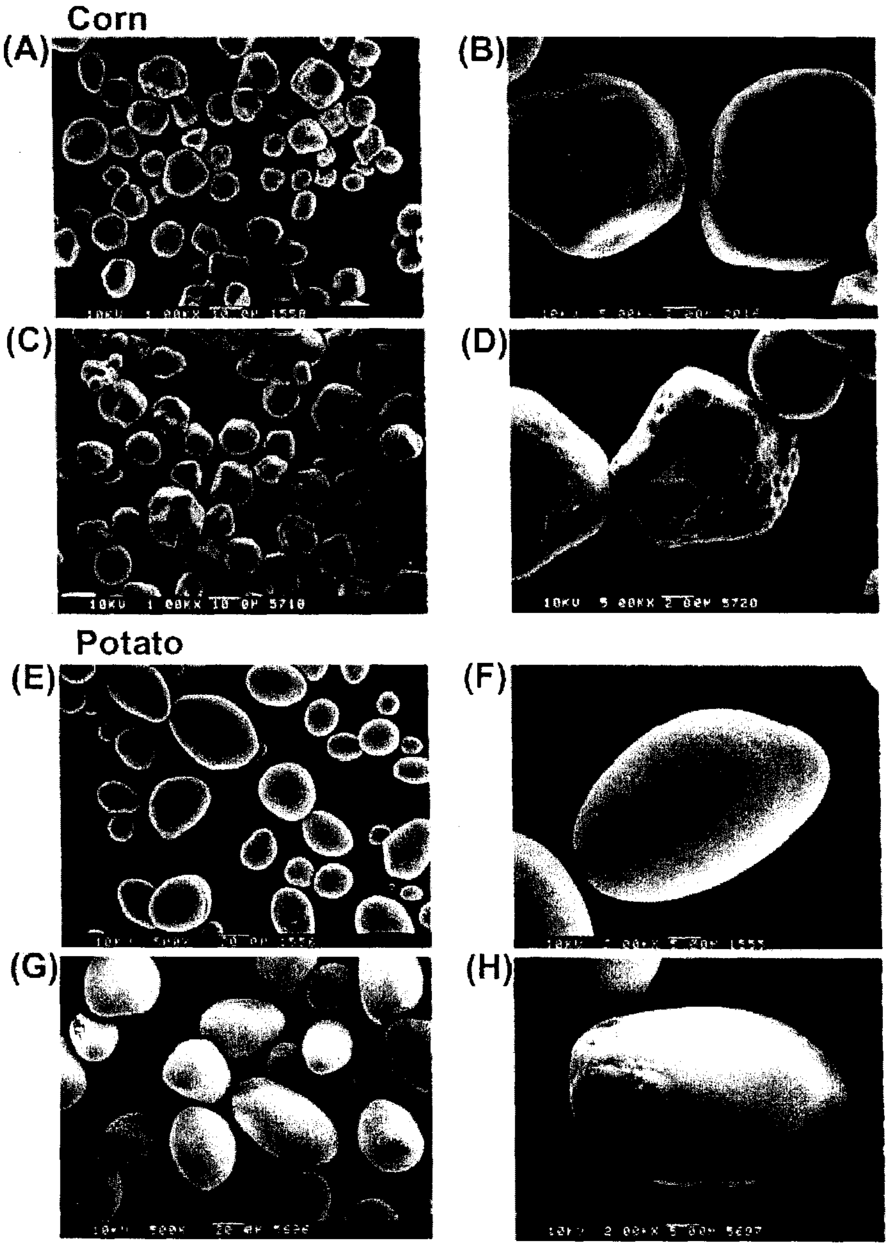

Fig. 1. Scanning electron micrograms of corn and potato starches: native (A, B, E, F), and treated by 0.36% HCl in methanol at 25°C for 15 days (C, D, G, H).

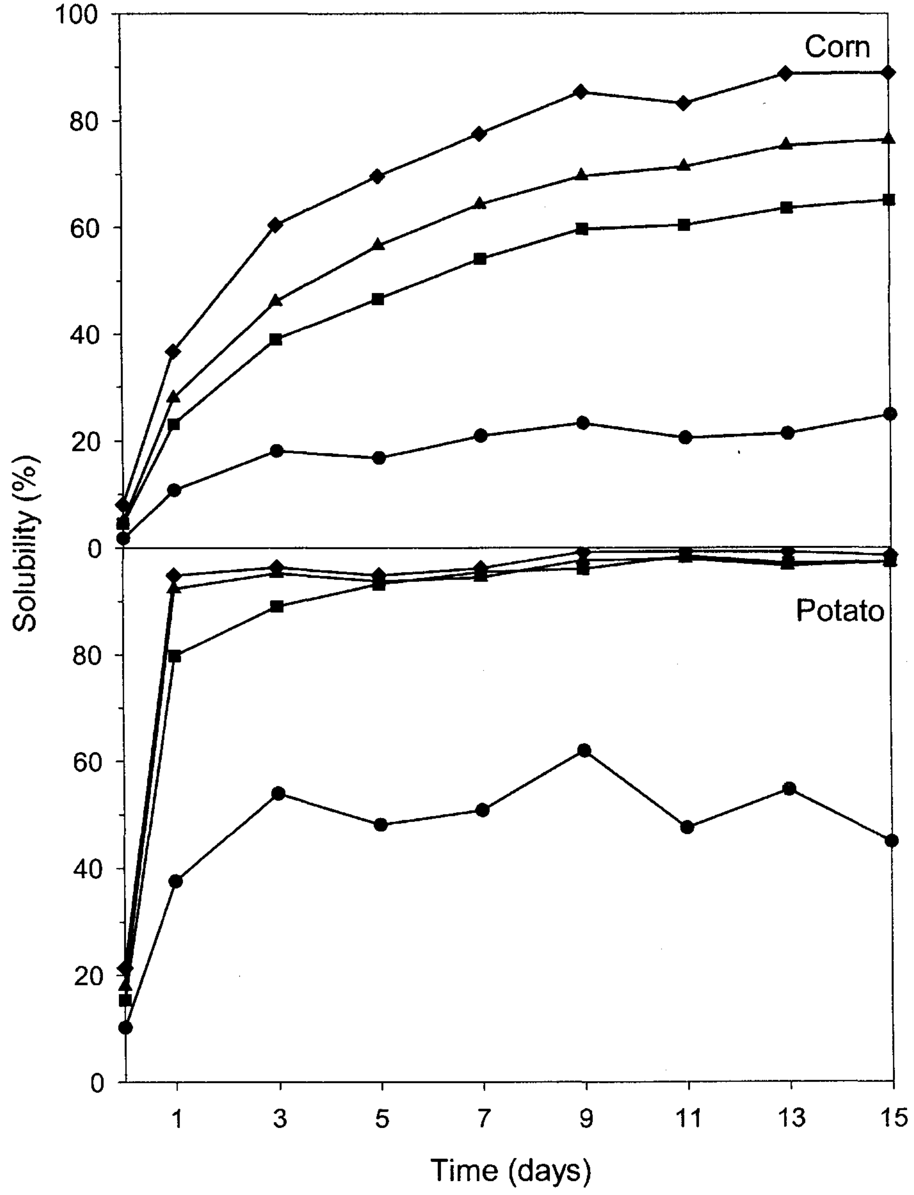

Fig. 2. Solubility of starches measured at 65 (●), 75 (■), 85 (▲), and 95°C (□), respectively. Starches were hydrolyzed by 0.36% HCl in methanol at 25°C for 1 to 15 days.

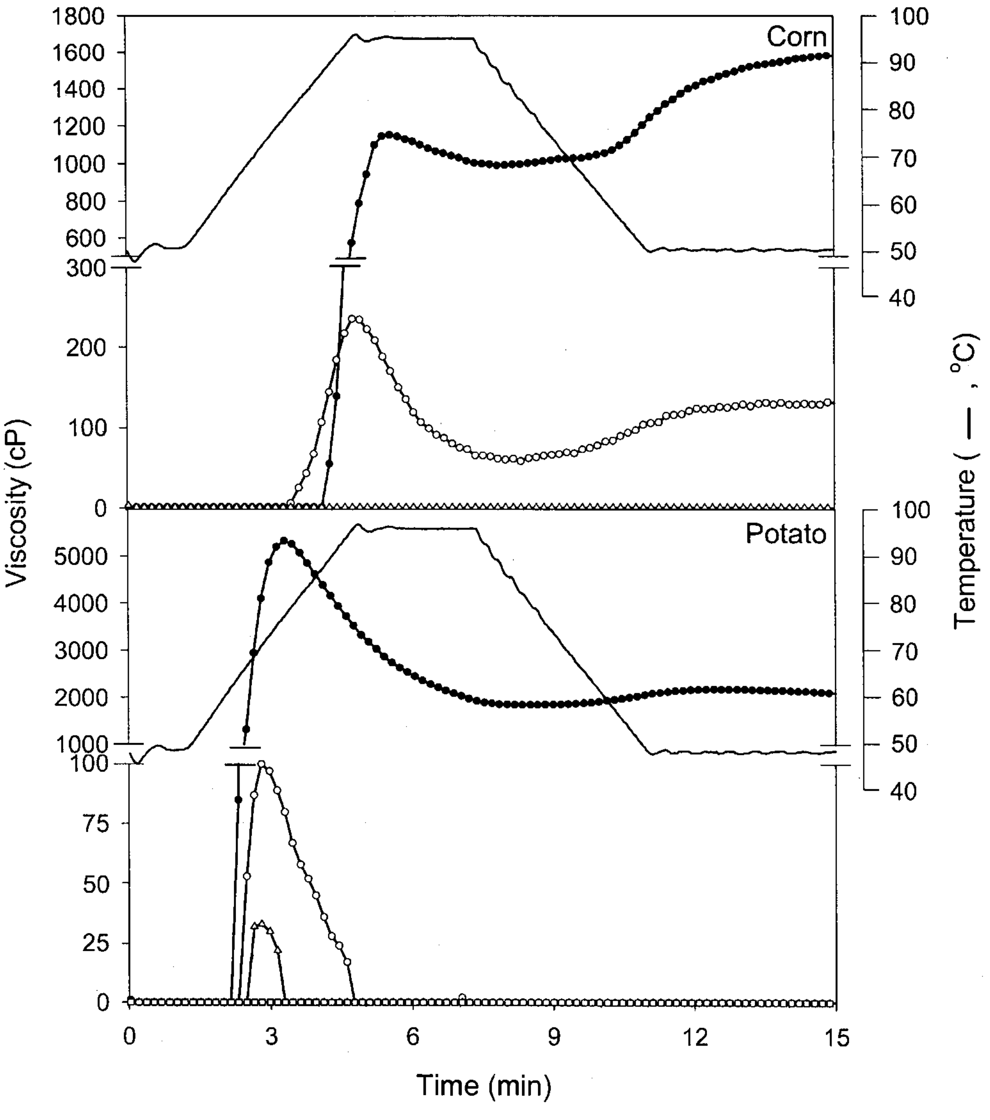

Fig. 3. RVA amylograms of native starches (●) and starches treated by 0.36% HCl in methanol at 25°C for 1 (○), 3 (Δ), and 5 days (□), respectively.

Gelatinization properties of starches determined by DSC are shown in Table 2. The gelatinization onset temperatures (T_0) of corn starches decreased from 61.1°C for native starch to 58.6°C for starch hydrolyzed for 15 days. The decrease tendency of T_0 of cornstarch during acid-hydrolysis treatment was the same as that of wheat starch hydrolyzed by 2.2N HCl at 35°C [10]. In contrast, the T_0 of potato starches increased from 56.1°C for native starch to 58.2°C for starch hydrolyzed for 15 days. This was the same tendency as potato starch hydrolyzed by 2.2N HCl at 35°C [10]. For both corn and potato starches, the peak (T_p) and conclusion (T_c) temperatures of gelatinization increased with the increase of treatment time. Accordingly, the range of gelatinization increased from 12.2 to 23.9°C for cornstarch, and from 12.8 to 19.8°C for potato starch. The time courses of T_p and T_c during acid-alcohol hydrolysis were the same as starches hydrolyzed by 2.2N HCl at 35°C [10].

Table 2

Gelatinization temperatures and enthalpies of native starches and starches treated by 0.36% HCl in methanol at 25°C for 1 to 15 days.

Time (days)	Gelatinization temperature[§](°C)			T_o-T_c (°C)	Enthalpy[#] (J/g)
	T_o	T_p	T_c		
Corn Native	61.1	66.6	73.3	12.2	11.9
1	59.4	65.0	72.9	13.5	10.9
3	58.6	66.2	77.6	19.0	9.5
5	58.8	66.8	77.2	18.4	10.6
7	58.4	67.3	79.0	20.6	9.8
9	58.4	68.5	80.9	22.5	9.9
11	59.0	68.6	81.1	22.1	9.6
13	58.8	69.6	82.7	23.9	10.1
15	58.6	69.9	82.5	23.9	10.3
Potato Native	56.1	61.5	68.9	12.8	16.9
1	56.8	63.0	73.1	16.3	15.8
3	55.6	61.3	71.6	16.0	14.7
5	56.2	62.5	73.2	17.0	14.6
7	57.0	63.5	74.6	17.6	15.2
9	56.5	62.8	74.6	18.1	15.1
11	57.4	63.5	74.9	17.5	15.8
13	57.5	63.8	76.8	19.3	15.8
15	58.2	64.5	78.0	19.8	15.4

[§] T_o, T_p, and T_c stands for the onset, peak, and conclusion temperature of gelatinization, respectively. Standard deviations ± 0.6°C.

[#] Standard deviations ± 0.5 J/g.

Although the gelatinization enthalpies (ΔH) of modified starches were 1 to 2 J/g lower than their counterpart native starches, there were no obvious differences among the enthalpies of starches treated with different times. Similar result was found on starches hydrolyzed by 2.2N HCl [10]. Although the crystallinity of starch granule was increased after acid hydrolysis [11–12] and the hydrolysis was preferential on the amorphous regions of the starch granule, the ΔH of acid-hydrolyzed starch did not show consistent correlation with the crystallinity of starch [10].

Molecular weight distributions of starches determined by HPSEC are shown in Fig. 4. The first fractions in the profiles corresponded to amylopectin, and the second fractions to the low molecular weight molecules. For the acid-alcohol treated starches, the areas of F1 fractions decreased with the increase of treatment time, while the areas of F2 fractions increased. This indicated the degradation of amylopectin to low molecular weight molecules due to the acid-alcohol hydrolysis. The degradation of amylopectin could cause the disruption of granular structure and the increase in leaching when starch was heated with water. Consequently, high extents of starch solubility (Fig. 2) and low pasting viscosity (Fig. 3) were observed.

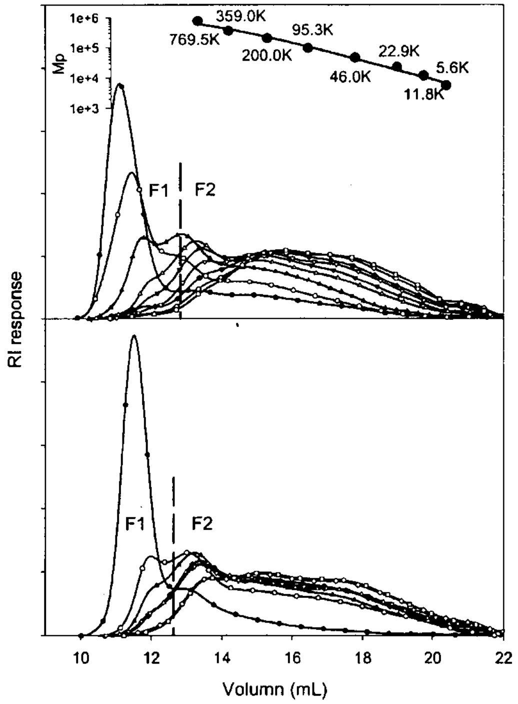

Fig. 4. HPSEC chromatograms of native starches (●) and starches treated by 0.36% HCl in methanol at 25°C for 1 (○), 3 (▲), 5 (△), 7 (▼), 9 (▽), 11 (◇), 13 (◆), and 15 days (□), respectively.

Compared to the gradual degradation pattern of cornstarch, potato starch showed a stepwise pattern (Fig. 4). The molecular weight distribution profiles of potato starches treated by 0.36% HCl in methanol at 25°C for 5 to 11 days were overlapped with each others. The same is true for the distribution profiles of potato starches treated for 13 and 15 days. Figs. 5 and 6 show the changes and the normalized percentage of changes on the weight average degree of polymerization (DP_w) of corn and potato starches as a function of hydrolysis time. The results indicated the decrease in DP_w occurred mostly within the first 5 days of treatment, and potato starch had a higher rate of decrease than cornstarch. While the changes of number average of polymerization (DP_n) of starches (Fig. 7) also showed that the potato starch had higher decreasing rate, the normalized percentage of changes of DP_n (Fig. 8) indicated a relatively higher decrease extent of corn starch.

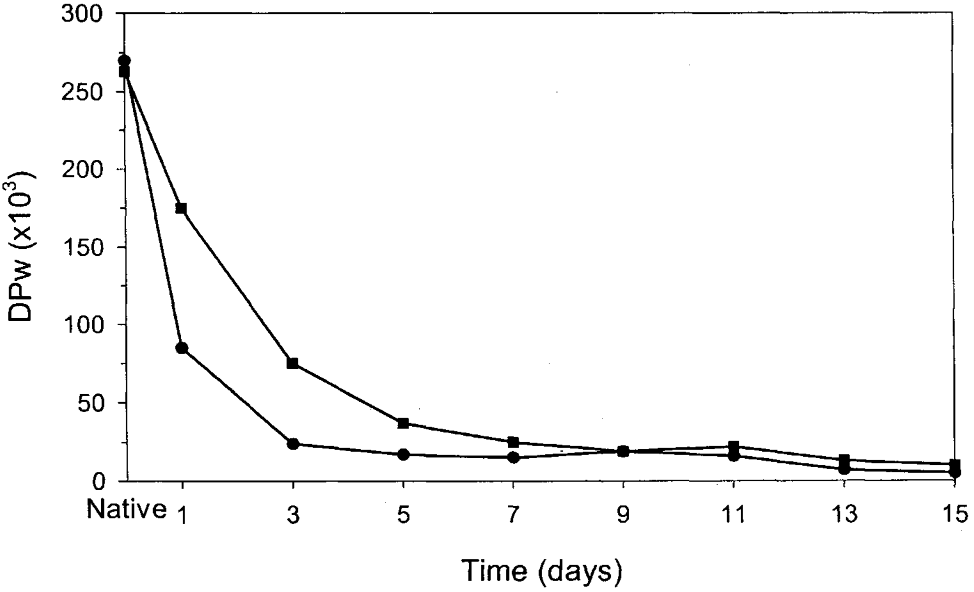

Fig. 5. Changes of weight average degree of polymerization (DP_w) of corn (■) and potato (●) starches as a function of hydrolysis time.

The DP_n of corn and potato starches after acid-alcohol treated for 15 days were 162 and 183, respectively, whereas the residue obtained after acid hydrolysis (2.2N HCl, 35°C, 14 days) of starch showed a bimodal distribution of chains on DP 14 and 28 [13]. The results of higher DP_n and higher yield of starch after acid-alcohol hydrolysis implied that the acid-alcohol treatment hydrolyzed the starch granule according to the mechanism different from that of acid hydrolysis in water. The mechanism of acid-alcohol hydrolysis in the starch granules can be further resolved by comparing the chain length distribution and X-ray patterns of native and acid-alcohol treated starches.

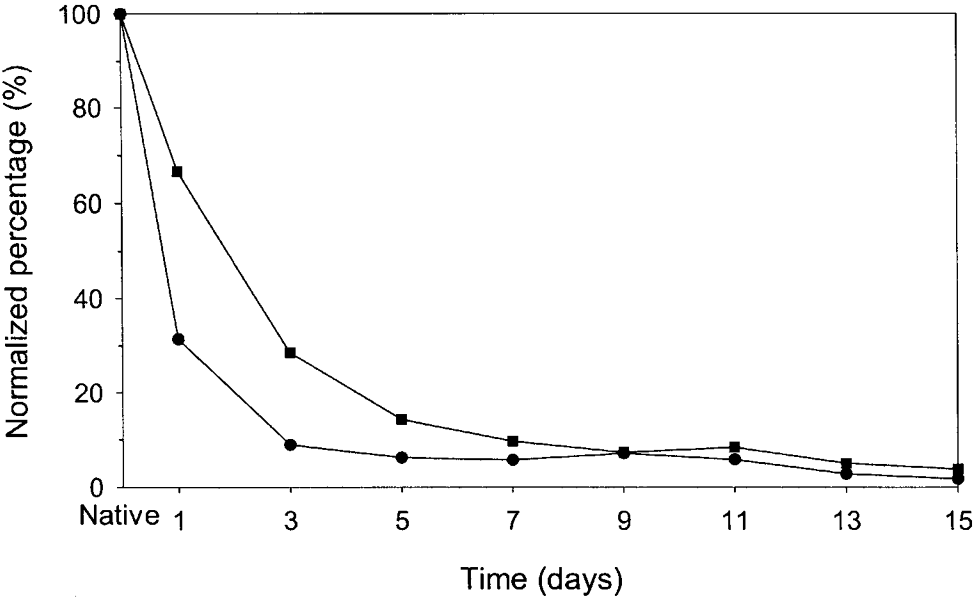

Fig. 6. Normalized changes of weight average degree of polymerization (DP_w) of corn (■) and potato (●) starches as a function of hydrolysis time.

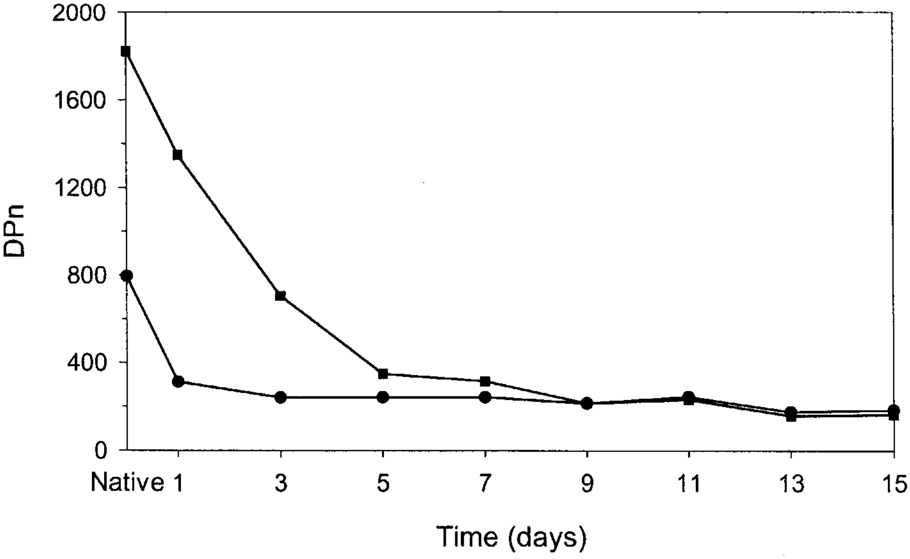

Fig. 7. Changes of number average degree of polymerization (DP_n) of corn (■) and potato (●) starches as a function of hydrolysis time.

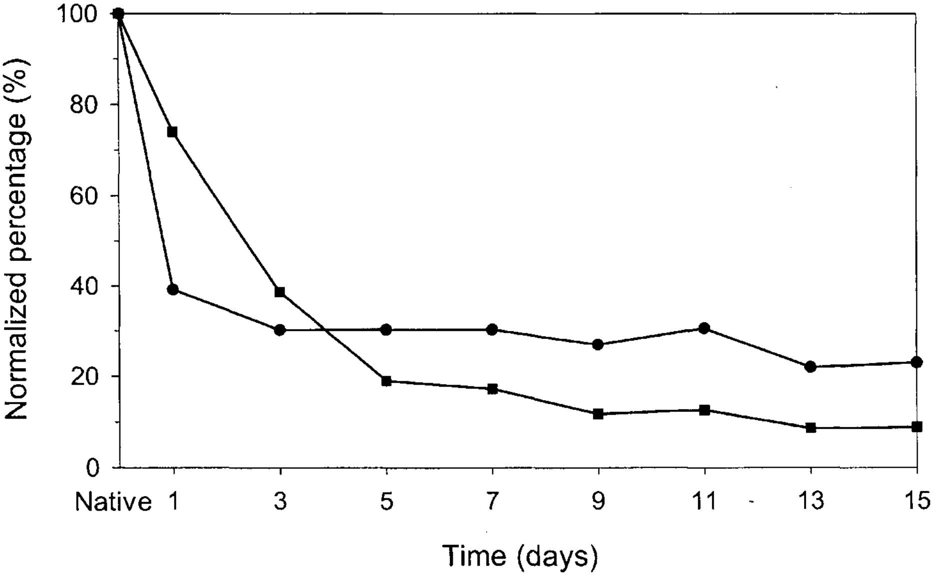

Fig. 8. Normalized changes of number average degree of polymerization (DP_n) of corn (■) and potato (●) starches as a function of hydrolysis time.

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DEGRADACJA SKROBI KWASAMI W ALKOHOLU

Streszczenie

W celu sprawdzenia degradacyjnego efektu modyfikacji skrobi za pomocą kwasów w alkoholu poddano hydrolizie, 0,36% roztworem chlorowodoru w metanolu, handlowe skrobię ziemniaczaną i kukurydzianą. Modyfikacje prowadzono w temperaturze 25°C w trakcie 1 do 15 dni. Wydajności modyfikowanych skrobi wynosiły od 91 do 100% a średni rozmiar tak modyfikowanych gałeczek skrobiowych był nieco mniejszy od rozmiaru gałeczek skrobi natywnych. Po piętnastodniowej modyfikacji nie stwierdzono zmian kształtu gałeczek, natomiast ich rozpuszczalność w wodzie wzrastała z czasem trwania modyfikacji. Zależność ta była wyraźniejsza w przypadku skrobi ziemniaczanej niż skrobi kukurydzianej. Temperatura początku żelowania (T_o) skrobi po modyfikacji malała w przypadku skrobi kukurydzianej i wzrastała w przypadku skrobi ziemniaczanej. W przypadku obu skrobi temperatura w maksimum (T_p) i końca żelowania (T_c) w wyniku tych modyfikacji wzrastała w miarę wydłużania czasu modyfikacji. W następstwie modyfikacji zakres temperaturowy, w którym zachodziło żelowanie rozszerzył się z 12.2 do 23.9°C w przypadku skrobi kukurydzianej i z 12.8 do 19.8°C w przypadku skrobi ziemniaczanej. Jednak entalpie żelowania (ΔH) skrobi modyfikowanych obniżały się zaledwie o 1 do 2 J/g. W tak modyfikowanych skrobiach obszar frakcji amylopektynowej wyznaczony za pomocą wysokosprawnej żelowej chromatografii podziałowej (HPSEC) malał wraz z czasem prowadzonej modyfikacji. Zmiany w przypadku skrobi kukurydzianej odbywały się w sposób ciągły, natomiast w przypadku skrobi etapowo. Średni stopień polimeryzacji (DP_w) gwałtownie obniżał się w ciągu pierwszych pięciu dni prowadzenia procesu, przy czym w przypadku skrobi ziemniaczanej był on gwałtowniejszy. Po 15 dniach obróbki stosowne wartości DP_w skrobi kukurydzianej i ziemniaczanej wynosiły odpowiednio 162 i 183.

Badania te pozwalają stwierdzić, że modyfikacje zmiany struktury molekularnej skrobi odbywają się za sprawą degradacji amylopektyny. Skutkiem modyfikacji jest zniszczenie gałeczek i obniżenie lepkości żeli.