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Mild hydrolysis of resistant starch from maize

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Abstract

To investigate structural changes of resistant starch (RS) caused by mild-acid treatment, native maize starch, retrograded (RS3), and cross-linked (RS4) resistant starches, prepared from maize starch, were hydrolyzed with 0.1 M HCl at 35 °C for 30 days. The hydrolysis rate of RS3 was shown to be the highest, at 44.1% after 30 days of the hydrolysis. The hydrolysis rapidly progressed upto 10 days but gradually changed after that. Native starch and RS4 showed less than 5% of hydrolysis during the period of hydrolysis. As for the RS level of the residue after the hydrolysis, RS4 did not show any significant change, but RS3 exhibited an increase of up to 25.9% after 30 days, which led to 88% increase in comparison with 13.8% at the initial stage. As a result of examining the molecular weight (MW) of RS3 by the SEC-MALLS-RI system, the non-hydrolyzed RS3 exhibited three peaks, having MW 53.4×10^6, 7.4×10^6, and 0.8×10^6, respectively, but the MW of the molecules decreased to 4.9×10^6 and 0.6×10^6 after 7 days of hydrolysis. It was difficult to verify the MW of RS4 because this was not dispersed in 1 M NaOH. The crystallinity of native starches, RS3 and RS4, by X-ray diffractometry of the residue hydrolyzed with 0.1 M HCl was equal to that of the non-hydrolyzed starch. The peak intensity at $2\theta = 17°$ of RS3 increased sharply after hydrolysis.

Keywords: Retrograded resistant starch; Cross-linked resistant starch; Mild acid; Structural changes

1. Introduction

Resistant starch (RS) is the sum of starch and the products of starch degradation not absorbed in the small intestine of healthy individuals (Asp, 1992; Sievert, Czuchajowska, & Pomeranz, 1991). The RS is reported to have physiological effects similar to those of dietary fibre, and the physiological effects associated with RS include, mainly, reduced levels of plasma glucose and insulin, increased faecal bulk, and short-chain fatty acid (SCFA) production through fermentation in the large intestine (Bingham, 1988; Birkett, Muir, Phillips, Jane, & O'Dea, 1996; Carins, Sun, Morris, & Ring, 1995; Lin & Visek, 1991; Ranhotra, Gelroth, & Glaser, 1996). The RS is classified into four different types (Eerlingen, Deceuninck, & Delcour, 1993; Englyst, Kingman, & Cummings, 1992). Because RS1 and RS2 are native starches, they will lose the potential of RS if gelatinized during the processing of food. RS3, formed through a heating and cooling cycle, is stable when heating above 100 °C because retrograded starch (amylose) melts at ~155 °C (Keren, Hazazelet, & Eyal, 2003). On the other hand, RS4 formed by cross-linking, is also known to be stable when treated by enzyme or acid.

Until now, the cross-linking of starch is used industrially to stabilize granule structure and to restrict swelling. However, the degree of cross-linking of starch used as thickeners in food is too low to elicit resistance to α-amylase. Increasing the degree of cross-linking of starch may be expected to inhibit the entrance of α-amylase molecules into the starch granule. Moreover, the cross-linking between starch molecules inhibits the migration of the molecules into a combining site with α-amylase

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(Björck, Gunnarsson, & Östergard, 1989; Casset, Imberty, Haser, Payan, & Perez, 1995; Han & Hamaker, 2002; Lim, Mun, & Shin, 2004; Valetudie, Colonna, Bonchet, & Gallant, 1993).

Accordingly, the addition of RS3 and RS4, manufactured by a heating-cooling process and chemical modification, respectively, can increase RS levels in food. The RS can be added to a variety of foods due to diverse physicochemical properties by type, and can be applied to the development of low-fat and low-calorie foodstuffs by adding the RS to dressing or sauce as a fat replacer. Nevertheless, this foodstuff generally has low pH and, subsequently, it is necessary to confirm the RS stability of acidic food for maintaining food quality by adding RS to food.

The hydrolysis of starch by acid takes place in two stages (Jayakody & Hoover, 2002; Robin, Mercier, Duprat, & Guilbot, 1975; Singh & Ali, 2000; Wang, Truong, & Wang, 2003): first, the hydrolysis of an amorphous region that rapidly progresses and second, the hydrolysis of a crystalline region that progresses slowly. Kim, Kang, and Kim (1996) reported that rice starch and kidney bean starch showed a three-step pattern during hydrolysis with 2.2 N HCl; the first and the second steps occurred in amorphous regions with gradual hydrolysis in the first step but rapid hydrolysis in the second step while almost no change in the third step. Robin et al. (1975) explained that, when potato starch was hydrolyzed with 2.2 N HCl, rapid hydrolysis occurred in the amorphous regions of the starch granules at an early step and gradual hydrolysis occurred in the crystalline region at the second step. Cereal and legume starches are known to show similar tendencies (Hoover & Vasanthan, 1994; Inouchi, Glover, & Fuwa, 1987; Jane, Wong, & McPherson, 1997; Vasanthan & Bhatty, 1996).

While lintnerized starch is generally manufactured by reacting starch with 2.2 N HCl at 30–40 °C, this study utilized mild acidic conditions for estimating the food processability. The objectives of this study were to examine the RS applicability to acidic food by comparing the acid hydrolytic patterns of native maize starch, RS3 and RS4, prepared from maize starch using 0.1 M HCl, and to measure the RS level and physicochemical properties of residue after the hydrolysis.

2. Materials and methods

2.1. Materials

Normal maize starch was obtained from Samyang Genex Co., Korea. Sodium trimetaphosphate (STMP), sodium tripolyphosphate (STPP), and pancreatin (from *Porcine pancreas*, Cat No. P 7545) were purchased from Sigma Chemical Co. (St. Louis, MO). Pullulanase (Promozyme ID #115193) was obtained from Novo Nordisk, Denmark.

2.2. Preparation of retrograded RS3

Retrograded resistant starch (RS3) was prepared according to Sievert and Pomeranz's method (Sievert & Pomeranz, 1989). Maize starch (50 g, db) was mixed with 175 ml of distilled water (starch:water = 1:3.5), and the mixture was pressure-cooked in an autoclave at 121 °C for 1 h. The paste was then cooled at room temperature and stored at 4 °C overnight. After two repetitions of the autoclaving/cooling cycle, the sample was dried in an oven (105 °C) and ground into fine particles (<150 μm).

2.3. Preparation of cross-linked RS4

Cross-linked resistant starch (RS4) was prepared using Woo and Seib's (2002) and Shin, Song, and Seib's (2004) methods. Starch (50 g, db), distilled water (70 ml), and sodium sulfate (5.0 g, 10%, starch basis, sb) were placed in a beaker and stirred with magnetic bar for 10 min and then STMP (5.99 g, 11.98%, sb) and STPP (0.01 g, 0.02%, sb) were added with stirring. The mixture was adjusted to pH 11.5 by adding 1.0 M sodium hydroxide solution (25 ml, 2.0%, sb). The slurry was stirred continuously, warmed to 45 °C, and reacted at 45 °C over 3 h. After the reaction period, the slurry was adjusted to pH 6.5 by adding 1.0 M hydrochloric acid, and the starch was collected by centrifugation, washed with water (>250 ml, five times), dried at 40 °C (oven) and passed through a 100 mesh sieve (<150 μm).

2.4. Determination of RS level using the pancreatin-gravimetric method

RS levels of samples were determined by using the pancreatin-gravimetric method (Shin et al., 2004). Sample (1.0 g, db) was placed in 50 ml screw cap centrifuge tube (Nalgene Cat. No. 3139-0050) with magnetic stirring bar ($\phi 3.2 \times 13$ mm) and suspended in 20 ml sodium acetate buffer (pH 5.2). The sample tubes were heated in a boiling water bath for 1 h with continuous stirring and immediately cooled to 40 °C. Enzyme solution (2 ml) was added to each sample tube and the mixture was incubated for 16 h at 37 °C in a water bath with stirring. After incubation, the reaction mixture was transferred to a 250 ml beaker with 117 ml of 95% ethanol to make an 80% concentration of alcohol, and allowed to stand for over 1 h at room temperature to precipitate the residue. The precipitate was collected in a tared sintered glass crucible (porosity No. 2) over a dried bed of acid-washed Celite as filter aid. After washing the residue with 78% and 95% ethanol and acetone in a sequence, the insoluble residue was dried at 105 °C in an

oven to determine the RS level. Because all resistant starch samples had low levels of protein and ash, they were not analyzed in the digested starch. Enzyme solution was prepared by the following method: 1.0 g of pancreatin and 12 ml of deionized water were placed in a centrifuge tube with magnetic stirring bar and then mixed well. After centrifugation (3000 rpm, 10 min), 10 ml of supernatant were mixed with 0.2 ml of Promozyme and 1.8 ml of deionized water.

$$\text{RS level}(\%) = \frac{\text{Weight of insoluble residue(g, db)}}{\text{Weight of sample(g, db)}} \times 100$$

2.5. Mild acid hydrolysis of resistant starches

Native starches, RS3 and RS4, were dispersed individually in 0.1 M HCl at 35 °C (10.0 g starch/40 ml acid solution) for the hydrolysis period, ranging from 6 h to 30 days. The starch slurries were shaken daily by hand to resuspend the deposited granules. After digestion, each sample was centrifuged at 7000 rpm for 10 min. The sugar content in the supernatant was measured to measure hydrolytic rate, using the phenol–sulfuric acid method (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956) and the residue was neutralized by adding 1 M NaOH solution to pH 6.5, washed with distilled water, dried at 40 °C and ground to pass through a 100 mesh sieve. The hydrolytic rate was calculated by the Robin et al. (1975) method.

$$\text{Hydrolytic rate}(\%) = \frac{\text{Total sugar content (g) of supernatant} \times 0.9}{\text{Amount of starch(g)}} \times 100$$

2.6. Starch solution preparation for SEC-MALLS

Starch sample was moistened with 50 μl ethanol and then dissolved in a capped glass vial containing 1 M NaOH (1 ml) by vortexing. The starch solution was diluted with 50 mM $NaNO_3$ solution (16 ml) and then neutralized with 1 M HCl. The starch solution was autoclaved (121 °C) for 20 min. Autoclaved solution was further treated by boiling–stirring in a hot plate/stirrer for 96 h. The dial settings for boiling and stirring were No. 3.5 (~100 °C) and No. 4 (155 rpm), respectively.

2.7. SEC-MALLS-RI system

The system consisted of a pump (P2000, Spectra System, San Jose, CA), an injector valve with a 1 ml loop (model 7072, Rheodyne, Cotati, CA), the SEC column, a MALLS detector (632.8 nm, DAWN DSP-F, Wyatt Technology, Santa Barbara, CA), and a RI detector (Optilab DSP, Wyatt Technology, Santa Barbara, CA). The mobile phase used for the SEC was 50 mM $NaNO_3$ solution that had been filtered through a 0.1 μm cellulose acetate membrane (Whatman, Kent, UK) and degassed. Two SEC columns (TSK G5,000 and 3,000 PWXL, $\phi 7.8 \times 600$ mm) were used and column temperature and flow rate were 60 °C and 0.4 mM/min, respectively. The starch solution was filtered through a 5.0 μm membrane filter before injection into the column. A specific refractive index increment value (dn/dc) of 0.146 ml/g was used (Han & Lim, 2004a; Han & Lim, 2004b).

2.8. Amylose content and DSC measurement

Amylose content was determined by the Williams method (Williams, Kuzina, & Hlynka, 1970) and thermal characteristics of native starch were determined with a differential scanning calorimeter (DSC, Seiko Co., Japan). Sample (0.3 mg) was put in a DSC pan, into which distilled water was added, to make a ratio of starch to water of 1:2. The DSC pan was kept at room temperature for 4 h and heated from 20 to 180 °C at 5 °C/min.The DSC thermogram was recorded.

2.9. X-ray diffractometry

The crystallinities of acid hydrolyzed residues of native starches, RS3 and RS4, were examined by using an X-ray diffractometer (D/Max 1200, Rigaku Co., Japan) under the following conditions: diffraction angle (2θ) 40–5°; target, Cu Kα; filter, Ni; voltage, 40 kV; current, 20 mA; scanning speed, 8°/min.

3. Results and discussion

3.1. Hydrolysis rate and RS levels of residues after acid hydrolysis

Fig. 1 shows the hydrolysis patterns of native and resistant starches hydrolyzed with 0.1 M HCl. The native starch and RS4 hydrolyzed for 30 days showed gradual increases in the hydrolysis rates, up to 3.9% and 5.0%, respectively. In the hydrolysis with 2.2 N HCl, the native starch showed a two-step pattern in that the amorphous region was hydrolyzed and then the crystalline region was hydrolyzed at the beginning of the 8th day (Jayakody & Hoover, 2002). However, in the mild acid treatment of this study, the starch showed a low hydrolysis rate and a gradual increase up to 3.9%, which resulted in a different trend of hydrolysis according to the acid concentration.

RS3 exhibited a three-step hydrolysis pattern: gradual hydrolysis until 7 days, rapid hydrolysis until 10 days, and almost no change after that. Its hydrolysis rate on the 30th day was 44.1%. The early rapid hydrolysis of RS3 is usually shown by the hydrolysis of the

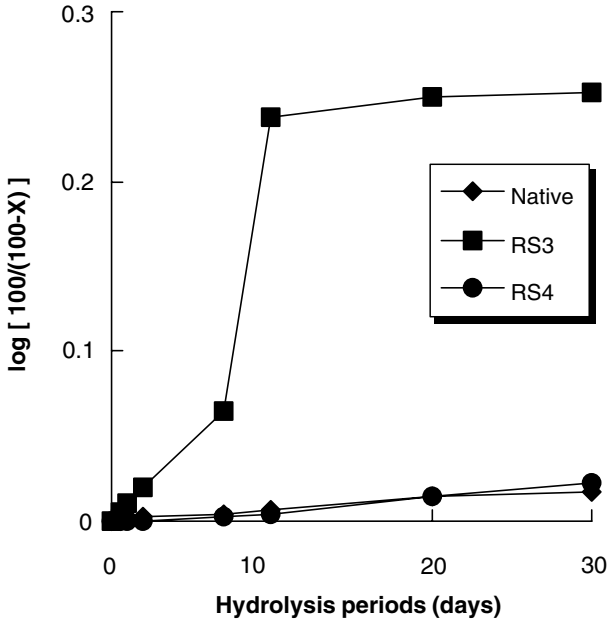

Fig. 1. Pattern of hydrolysis (%) of native starch, RS3 and RS4 prepared from maize starch treated with 0.1 M HCl at 35 °C for 30 days. *X*, extent of hydrolysis (%).

amorphous region and the next stage after that seems to correspond to the hydrolysis of the crystallized region formed by the cooling process.

It seems that RS3 is disintegrated through autoclaving with enough water and then recrystallized by association between linear amyloses or between amylose and amylopectin through cooling.

Eerlingen et al. (1993) suggested two hypotheses about RS formation as follows: a micelle model and a lamellar model. They described that when the RS was formed by using amylose in a DPn range of 40–620, produced by hydrolysis with β-amylase, the DPn of the isolated RS was in a range of 19–26 (RS was formed in an aqueous solution of amylose of 0.83% at 4 °C for 13 h after a 10-min boiling step), which was not related to the chain length of the amylose used for forming RS. They also reported that RS was actually formed in an amylose solution by the aggregation of amylose helices over a specific part of the amylose chain.

Because RS3 has a higher portion of amorphous and gelatinized region (~80%) than do native starch or RS4, it was hydrolyzed easily by acid.

Woo and Seib (2002) reported that when wheat starch was cross-linked with STMP/STPP (99:1, w/w) mixture (12%) and sodium sulfate (10%) at 45 °C for 3 h, the level of phosphate remaining in the cross-linked starch was 0.32%, the swelling power was about 3.0, and the total dietary fibre (resistant starch) calculated by the AOAC method was about 76%. In this study, RS4 was manufactured with maize starch under the same conditions as above and the RS level measured by a pancreatin-gravimetric method was 14.6% showing 3.5 of the swelling power. RS level of RS4 obtained by the AOAC method was higher than that by the pancreatin-gravimetric method (Shin et al., 2004).

The reason that the RS level was lower when using the pancreatin instead of using heat stable α-amylase can be assumed to be as follows: first, the action of purified α-amylase (for AOAC method) is inhibited due to the formation of the strong cross-linked bond between the protein membrane on the starch surface and the starch molecules, or the protein blocks the channels of the granule surface to inhibit the entrance of enzyme. Second, pancreatin contains protease and lipase capable of hydrolyzing the protein membrane and, therefore, pancreatin is able to penetrate into the inside of the starch granules through the hydrolyzed cleavage.

Han and Hamaker (2002) reported that the protein observed in the gelatinized normal maize starch is granule-bound starch synthase (GBSS), and starch ghost is not destroyed owing to the combination with this type of protein and the amylopectin of starch ghost. The RS level can differ according to characteristics of the protein membrane surrounding the starch granules. Valetudie et al. (1993) reported that the hydrolysis rate increased after the protein in yam starch was treated with protease. RS4 also showed similar results to those of native starch and less than 5% of hydrolysis by 0.1 M HCl. It is considered that this result is probably due to the inhibition of the penetration of H_3O^+ into the starch granule as well as the enzyme activity.

Lim et al. (2004) explained that the hydrolysis of native maize starch, RS3 and RS4 with 1 N HCl exhibited hydrolysis rates of 47.4%, 82.7% and 50.3%, respectively, on the 20th day. Jayakody and Hoover (2002) reported that normal maize starch showed a hydrolysis rate of 73.4% when reacting with 2.2 N HCl at 35 °C for 15 days. The acidity of 0.1 M HCl, used in this experiment, did not significantly affect RS4 and, accordingly, the RS4 could be desirable as a resistant starch to be used in food having low pH.

Table 1 illustrates the results of the RS level on the residue after hydrolysis. The native starch showed a low increase, below 3%, and RS4 did not show any increase of the level by hydrolysis while RS3 showed an increase of the RS level from the 2nd day and the samples hydrolyzed for 30 days increased the RS level up to 25.9%. The hydrolysis of RS3 rapidly increased until the 10th day and then was slow. Moreover, the RS level also greatly increased up to 20.0% in the hydrolysis for 10 days and then showed a slow increase. As previously described, RS3 consists of a crystallized region and an amorphous region. The amorphous region, however, is easily decomposed by acid to leave only a stronger crystallization, which leads to the consideration that the relative RS level increases with a longer hydrolysis period. When estimating the RS level after acid hydrolysis with

Table 1
Extent of hydrolysis (%) and RS level of residue of native starch, RS3, and RS4 prepared from maize starch treated with 0.1 M HCl at 35 °C[a]

Sample	Hydrolysis periods (days)						
	0.25	1	2	7	10	20	30
Extent of hydrolysis (%)							
Native	0.1 ± 0.0	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.1	1.6 ± 0.1	3.3 ± 0.3	3.9 ± 0.2
RS3	3.5 ± 0.3	6.0 ± 0.8	7.2 ± 0.8	13.9 ± 1.1	41.8 ± 3.8	43.7 ± 3.4	44.1 ± 2.7
RS4	0.0 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.6 ± 0.0	0.9 ± 0.1	3.3 ± 0.2	5.0 ± 0.4
RS level (%)							
Native (5.9 ± 0.45)[b]	6.1 ± 1.2	6.5 ± 1.3	7.3 ± 0.4	7.2 ± 0.4	7.3 ± 0.8	8.6 ± 0.7	9.1 ± 0.8
RS3 (13.8 ± 0.32)	14.9 ± 1.1	13.2 ± 1.6	14.8 ± 2.0	16.2 ± 2.3	20.0 ± 0.9	21.2 ± 1.3	25.9 ± 1.6
RS4 (14.6 ± 0.64)	14.2 ± 0.1	15.1 ± 0.2	15.0 ± 0.2	14.1 ± 1.4	13.9 ± 0.2	13.7 ± 1.3	13.1 ± 1.0

[a] Data expressed as means ± standard deviations. Data expressed on dry weight basis.
[b] RS levels of native starch, RS3 and RS4 without acid treatment.

1 N HCl, the hydrolysis rate and RS level of the samples were 38.3% and 27.6% at the 12 h hydrolysis, respectively, and were increased to 82.7% and 45.1% each on the 20th day of the hydrolysis (Lim et al., 2004). In this study, when the hydrolysis rate of RS3 was 38.3%, the RS level was about 22.5%, which was about 5% different from the 27.6% reported by Lim et al. (2004). Moreover, based on the fact that the level of the non-hydrolyzed RS3 was 17.4% by Lim et al. (2004) and 13.8% in this study. These two studies showed similar relationships between the hydrolysis rate and the RS level.

3.2. SEC-MALLS-RI system

Amylose content of native maize starch, used in preparation of RS3 and RS4, was 28.7%. Two melting endotherms of samples were shown by the DSC thermogram. The onset and end temperature of peak I were 63.3 and 85.0 °C, respectively, and those of peak II were 89.5 and 107.9 °C. The enthalpies of peak I and peak II were 10.1 and 1.5 J/g, respectively. Table 2 shows the MW of the native maize starch, RS3 and RS4 and their residues hydrolyzed for 6 h, 2 days, 7 days and 10 days measured by SEC-MALLS-RI system. According to the results, the native starch exhibited two peaks: peak I for amylopectin and peak II for amylose, with MWs of 104.5×10^6 and 21.3×10^6, respectively. Han and Lim (2004a, 2004b) reported that the SEC pattern of maize starch exhibited two peaks, and MWs of amylopectin and amylose showed 164×10^6 and 3.3×10^6, respectively, but MW was changed by heating, stirring, vortexing and solvent types used. When this sample was hydrolyzed for each different period, each MW of the amylopectin and amylose after the 2-day hydrolysis, was notably reduced, and the sample, after the 7-day hydrolysis, exhibited some intermediate fractions as shown by the

Table 2
Structural characteristics of acid treated native and resistant starches (with 0.1 M HCl at 35 °C)[a]

Period of hydrolysis (days)	Sample	Peak I		I.M		Peak II	
		Mw($\times 10^6$)g/mol	Rg (nm)	Mw($\times 10^6$)g/mol	Rg (nm)	Mw($\times 10^6$)g/mol	Rg (nm)
Non-acid treatment	Native	104.5 ± 1.3	160.4			21.3 ± 2.0	119.8
	RS3	53.4 ± 1.5	115.8	7.4 ± 1.0	71.0	0.8 ± 1.3	74.9
	RS4	–	–	–	–	–	–
0.25	Native	140.2 ± 2.4	172.1	–	–	14.7 ± 1.1	110.5
	RS3	67.4 ± 2.7	126.1	–	–	18.6 ± 2.0	129.4
	RS4	–		–	–	–	–
2	Native	36.9 ± 1.9	95.7	–	–	5.6 ± 0.5	64.0
	RS3	88.2 ± 1.7	127.3	–	–	15.7 ± 2.0	93.3
	RS4	–	–	–	–	–	–
7	Native	34.2 ± 1.5	93.9	3.2 ± 0.6	53.3	0.3 ± 2.3	61.5
	RS3	4.9 ± 1.5	86.0	–	–	0.6 ± 2.1	122.3
	RS4	–	–	–	–	0.7 ± 1.1	112.9
10	Native	–	–	–	–	0.5 ± 0.6	60.7
	RS3	–	–	–	–	0.2 ± 0.9	44.1
	RS4	–	–	–	–	0.9 ± 0.8	119.7

[a] Data expressed as means ± standard deviations.

hydrolysis of amylopectin. After the 10 day hydrolysis, only peak II remained. RS3 expressed three peaks in the non-hydrolysis. Because RS3 was formed by an autoclaving-cooling cycle, these peaks led to the assumption that RS3 consists of molecules having MWs of 53.4×10^6, 7.4×10^6 and 0.8×10^6, rather than consisting of amylopectin, intermediate fraction and amylose. When these were hydrolyzed for 2 days, the second peak disappeared and the MW and size of the first peak increased. These results are probably because of the entanglement of the fractions of low MW that were produced by the acid hydrolysis. After the 7 day hydrolysis, MWs of the two peaks decreased to of 4.9×10^6 and 0.6×10^6, respectively. Likewise, the decrease of the molecular weight was highly correlated with the increase of the hydrolysis rate after 7 days. In the samples after the 10 day hydrolysis, only the third peak was shown with low MW.

When RS4 was treated with 1 M NaOH to measure MW by using the SEC-MALLS-RI system, RS4 was not dispersed, on even with 2 M NaOH. The reason for this is probably that RS4 was not easily gelatinized by alkali, due to the strong cross-linking between the proteins in the membrane of the starch granule or between protein and starch molecule. In the cross-linked RS4, it was difficult to disperse the samples, and no peak was obtained because this starch did not penetrate through the filter. The samples after hydrolysis for 7 days and 10 days showed peaks of MW 0.7×10^6 and 0.9×10^6, which might be due to the elution of the hydrolyzed molecules by acid.

3.3. X-ray diffractometry

Native maize starch and RS4 exhibited peaks at $2\theta = 15°$, 17°, 20°, and 23° which were characteristic of A-type crystallinity (Fig. 2), while the diffraction patterns of RS3 were typical of B-type crystallinity, as indicated by the pronounced peaks at $2\theta = 17°$ and 23° (Keren et al., 2003). Besides the B-type profile, RS3 exhibited an additional peak at $2\theta = 20°$. A peak in this location is characteristic of V-type crystallinity. Similar combination of B- and V-type crystallinity in high amylose corn starch was previously reported by Sievert et al. (1991). When the native starch, and RS4 were hydrolyzed with 0.1 M HCl for 30 days (F), the crystallinity of the residue was not significantly changed, but RS3 showed high intensity of the peak at $2\theta = 17°$ compared to other peaks. As mentioned above, RS consists of crystallized and amorphous regions. However, the amorphous region was degraded easily by acid, which resulted in the residue having stronger crystallization. Therefore, a sharp X-ray pattern was shown in diffraction patterns of RS3. Thus, the crystalline region of RS3 was not affected by 0.1 M HCl, which allowed no change of the crystalline form on increase of the degree of crystallinity. The RS3 showed reduction of the peak at $2\theta = 20°$, probably due to the hydrolysis of the amylose–lipid complex formed during the heating process. The extent of hydrolysis was affected by the acid concentration, as suggested by the previous study (Wang et al., 2003), but native starch, RS3 and RS4, hydrolyzed with 1 N

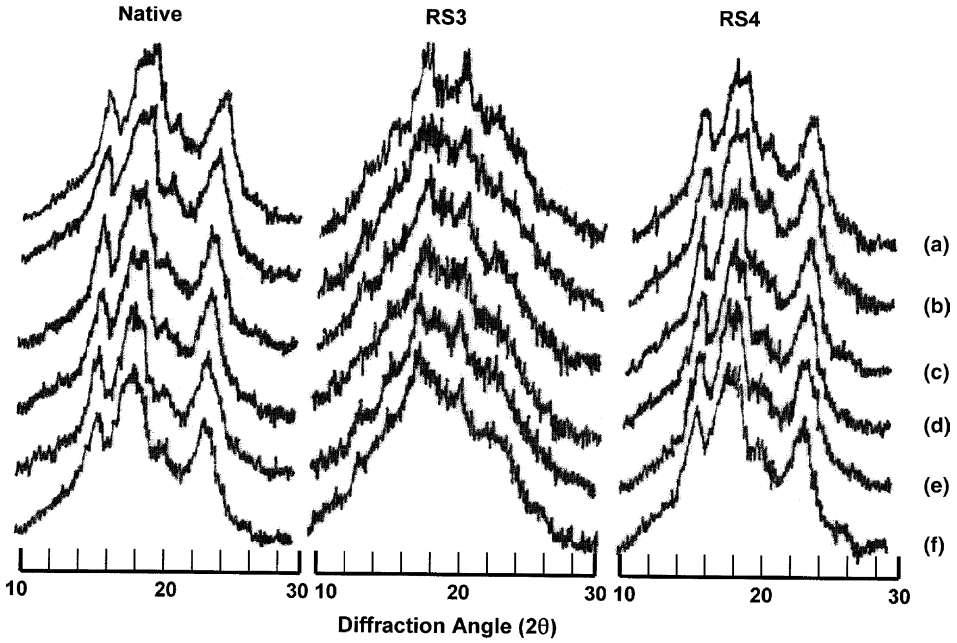

Fig. 2. X-ray diffractograms of native starch, RS3, and RS4 hydrolyzed with 0.1 M HCl at 35 °C. A, without treatment; B, 6 h; C, 2 days; D, 10 days; E, 20 days; F, 30 days.

HCl, did not show any changes in the crystalline form as reported by Lim et al. (2004).

4. Conclusion

The RS has diverse physicochemical properties, depending on types, and can be added to develop low-fat and low-calorie foodstuffs such as dressings or sauces. These have low pH, and subsequently, we studied the RS stability to mild acid by measuring acid hydrolysis rate, molecular weight, and crystallinity of residues after the hydrolysis. The acid hydrolysis rate of RS3 was shown to be the highest (44.1%) and also the molecular weight was decreased significantly after hydrolysis with 0.1 M HCl. However, RS4 showed less than 5% of hydrolysis, and the crystallinity of RS4 did not show any significant change with 0.1 M HCl. Accordingly, RS4 might be desirable as a resistant starch to be used for food of low pH.

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