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Action of α-Amylase and Acid on Resistant Starches Prepared from Normal Maize Starch

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Abstract Effects of acid and α-amylase on resistant starches including retrograded RS3 and cross-linked RS4 prepared from normal maize starch were investigated. Acid and α-amylase hydrolytic patterns of RS3 were similar, while those of native starch and RS4 differed. Acid hydrolysis rate of RS3 was markedly higher at initial stage, then slowly decreased up to 20 days, whereas that of RS4 increased continuously. The sizes of acid- and α-amylase-treated RS3 residues decreased, but those of RS4 remained unchanged. X-ray patterns of all treated residues did not change; however, the peak intensities increased. Swelling power of RS3 increased to 150% at 95°C, whereas that of RS4 differed depending on the treatment condition. Swelling power of acid-treated RS4 residue increased markedly, but that of α-amylase-treated one remained constant. Gel filtration chromatography profiles of untreated RS3 and RS4 residues were similar, whereas that of acid-treated RS4 residue was different from them. RS showed different hydrolytic behavior by acid and a-amylase depending on the type, and susceptibility of RS3 was higher than that of RS4.

Keywords: cross-linked RS4, retrograded RS3, acid, α-amylase, hydrolytic pattern

Introduction

Starches with differing hydrolytic rates have different nutritive values and physiological properties, which are dependent on enzyme type and digestive conditions. Although generally hydrolyzed by amylase and acid, they sometimes resist digestion by amylase.

Resistant starches (RS) include starch and products of starch degradation not absorbed by the small intestines of healthy individuals (1). They are classified into four main types (2, 3): RS1, physically inaccessible starch; RS2, granular starch with B type crystals; RS3, retrograded starch; and RS4, chemically modified starch.

Some modified starches, such as substituted and cross-linked starches, cannot easily be hydrolyzed by amylases. The susceptibility of starch degradation by α-amylase depends on sources of the starch and α-amylase (4).

The alteration of starch granule surfaces caused by heating and cross-linking is related to the irregular or granular structure of RS with/without pores (5, 6), which enable enzymatic hydrolysis. Moreover, crystallinity, internal structure with respect to the disposition of amylose and amylopectin, chain conformation, membrane structure, and pore size of the starch also influence enzyme susceptibility (7, 8), resulting in the formation of different hydrolysis patterns when modified starches are used (9-11). The acid hydrolysis of starch involves two phases; the first phase is attributed to the relatively fast hydrolysis of the amorphous region and the second phase to the slow hydrolysis of crystalline region (12). Several studies have been performed on the effects of reactions between hydrochloric acid and α-amylase on the structure of starch granule (7, 13-16).

The objectives of this study were to evaluate the effects of hydrolysis with 1 M HCl and α-amylase on the structure and stability of autoclaving/cooling-cycled and cross-linked resistant starches and to investigate the morphologies, RS levels, molecular profiles and crystallinities of the acid- and α-amylase-treated residues of RS3 and RS4 during hydrolysis.

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Materials and Methods

Materials Normal maize starch (amylose content, 28%) used for enzymatic hydrolysis was obtained from Samyang Genex Co., Korea, and α-amylase (from *Bacillus* species, Cat No. A-6380) and pancreatin (from Porcine Pancreas, Cat No. P-7545) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Pullulanase (Promozyme ID # 115193) was purchased from Novo Nordisk, Denmark.

Preparation of retrograded RS3 RS3 was prepared according to the method of Berry (17) by autoclaving-cooling normal maize starch. Briefly, starch (100 g) was mixed with 350 mL of water, and the starch suspension obtained was autoclaved at 121 for 1 hr. The resulting starch paste was cooled to room temperature, then stored at 4°C overnight. The sample was subjected to two autoclaving-cooling cycles, dried at 40°C in an incubator, and ground into fine particles (<150 μm) in a food mixer (JAM-505. Jewoo, City, Korea).

Preparation of cross-linked RS4 starch Cross-linked RS4 maize starch was prepared by the method of Woo and Seib (18). Normal maize starch (50 g, dry basis) was stirred in 25°C distilled water (70 mL) containing 5 g sodium sulfate [10% starch basis (sb)], and 12% mixture of sodium trimetaphosphate (STMP, 5.94 g sb) and sodium tripolyphosphate (STPP, 0.06 g sb) (99:1, w/w). The mixture was adjusted to pH 11.5 by adding 1 M sodium hydroxide solution (25 mL, 2% NaOH for sb). The slurry

was held at 45°C with continuous stirring for 3 hr, then neutralized to pH 6.0 by adding 1 M HCl (20 mL). The starch was then collected by centrifugation, washed with distilled water to remove un-reacted salt (200 mL × 6), and dried at 40°C. Finally, the starch sample was ground to a fine particle size and passed through a 100-mesh sieve.

Acid hydrolysis of starches Native starch, RS3, and RS4 were suspended in 1 M HCl (10 g/200 mL of acid) at 35°C. The suspensions were gently and continuously stirred. After 1/4, 1/2, 1, 2, 3, 7, 10, and 20 days, samples were centrifuged at 7,000 rpm for 10 min. Sugar content in the supernatants was measured using the phenol-sulfuric acid method (19). The insoluble residue was neutralized by adding 1 M NaOH at pH 6.5, washed with distilled water, dried at 40°C, ground, and passed through a 100-mesh sieve. The hydrolytic rate was calculated as described by Robin *et al.* (13) as follows:

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

Enzymatic hydrolysis of starches To investigate the changes in the structural properties and stability of RS during enzyme hydrolysis, RS sample were hydrolyzed for 20 days at 37°C. Native starch and RS (4 g each) were added to 80 mL of 0.1 M phosphate buffer (pH 7.0) and 20 mL α-amylase solution (105 unit /mL), and incubated at 35°C for 10 min. Hydrolysis was stopped by adding 400 mL of 95% EtOH, and the suspension was centrifuged at 7000 rpm for 10 min. Sugar contents of the supernatants were determined by the phenol-sulfuric acid method (19). The insoluble residue obtained was dried and ground, and hydrolytic rate was calculated as for the acid treatment.

Determination of RS levels by pancreatin- gravimetric method The enzyme solution was prepared as follows: 1.0 g of pancreatin was suspended in 12 mL deionized water at 25°C in a 50-mL screw-capped centrifuge tube (Nalgene Cat. No. 3139-0050) (20). After vigorous stirring with a magnetic bar (ϕ3.2 × 13 mm) for 10 min and centrifuging for 10 min at 3,500 rpm, an aliquot of the supernatant (10 mL) was mixed with 0.2 mL of promozyme and 1.8 mL of deionized water. Sodium acetate buffer solution (0.1 M, pH 5.2) was prepared by adding 13.6 g of sodium acetate to 250 mL of saturated benzoic acid solution, pH of the solution adjusted with acetic acid to 5.2, and 4 mL of 1 M $CaCl_2$ was then added.

RS levels of residues after hydrolysis were analyzed using the pancreatin-gravimetric method (20). Starch (1.0 g, dry basis) was placed in a 50-mL centrifuge tube containing a magnetic bar (ϕ3.2 × 13 mm) and dispersed in 20 mL sodium acetate buffer solution (pH 5.2). The tube was then placed in a boiling water bath for 1 hr, and cooled immediately to 37°C. After adding the enzyme solution (2.0 mL), the reaction mixture was incubated at 37°C for 16 hr in a temperature-controlled water bath. After 16 hr digestion, the reaction mixture was transferred to a 250-mL beaker containing 117 mL of 95% EtOH to produce 80% EtOH, and allowed to stand for ca 1 hr at room temperature to precipitate the residue. The insoluble residue was collected onto a tared sintered glass crucible (porosity No. 2) over a dried bed of acid-washed celite filter aid. Dry weight of the insoluble residue was measured after drying at 105°C overnight. A blank without starch was run five times, and the average value was used to correct assay values.

$$\text{RS level (\%)} = \frac{\text{Insoluble residue (g) after drying}}{\text{Weight of starch sample (g)}} \times 100$$

Scanning electron microscopy Native, RS3, and RS4 starches, and the acid and α-amylase hydrolysis residues of these starches were placed on double-sided Scotch adhesive tape pieces mounted on specimen stubs and coated with a thin layer of gold under vacuum. Scanning electron microscope (JEOL JSM-5400, Japan) observations were made at an accelerating potential of 20 kV with a photo time of 85 sec at × 2,000.

X-ray diffractometry Crystallinity of the powdered residues of native starch, RS3, and RS4 treated with α-amylase or acid were examined by X-ray diffractometry (D/Max-1200, Rigaku Co., Japan) under the following conditions: target, Cu-Kα; filter, Ni; scanning speed 8°/min; diffraction angle (2θ) 40-5°; 20 mA; 40 kV.

Swelling power Swelling powers of the insoluble residues obtained by treating RS with 1 M HCl and α-amylase were measured at 30 and 95°C as described by Medcalf and Gilles (21).

Gel permeation chromatography Gel permeation chromatography as described by Juliano *et al.* (22) was performed. Briefly, a sample (50 mg) was heat-dispersed in 5 mL of 90% dimethyl sulfoxide (DMSO) in a boiling water bath. The dispersed sample was filtrated using Whatman No 42 filter paper, and 1 mL of this filtrate was loaded onto the column (ϕ 2.3 × 95 cm, Sepharose CL-2B, Pharmacia Co.), which was eluted with a downward flow of degassed 0.01 M NaOH containing 0.02% sodium azide at room temperature. The flow rate was maintained at 20 mL/hr/cm^2, and fractions of 6 mL were collected. Total sugar content was measured using a spectrophotometer at 490 nm by the phenol-sulfuric acid method (19). The alternative distribution coefficient (Kav) was measured using the following equation:

$$\text{Kav} = \text{Ve-Vo} / \text{Vt-Vo},$$

where Ve, Vo, and Vt are the elution, void, and total volumes, respectively. Vo of the column was estimated from Ve of a 5×10^4 –40×10^4 dextran series (Sigma Co.). Dextran T-10 (Mw 1×10^4), dextran T-40 (Mw 4×10^4), and dextran T-500 (Mw 50×10^4) were used to determine molecular weights of the fraction. It is known that the elution peak of amylopectin fraction shows at void volume by using gel filtration chromatography with Sepharose 2L-CB, because molecular weight of amylopectin is higher to pass it through.

Statistical analysis Experiments were performed in triplicates, and data were expressed as means±SD using

the SAS system for Window (version 6.12). Duncan multiple range test was applied.

Results and Discussion

Resistant starch hydrolysis The hydrolytic changes caused by α-amylase and 1 M HCl on the native and resistant starches prepared from normal maize starch are shown in Fig. 1. The acid and enzymatic degradations of granular starches provide information on the physical and chemical structure of the starch granules (13, 15). RS3, which consisted of gelatinized (amorphous) and recrystallized (crystalline) fractions, was highly hydrolyzed during all hydrolysis periods examined. In contrast to RS3, the hydrolytic pattern of RS4 was similar to that of the native starch, which showed a slightly increased hydrolytic rate, although RS4 was not digested by α-amylase. Biliaderis *et al.* (23) reported that the acid hydrolysis of legume starch with 2.2 N HCl showed a biphasic hydrolysis pattern, i.e., a relatively fast initial hydrolysis rate followed by a slower rate. Rice starch showed a three-step hydrolysis pattern after the hydrolysis with 2.2 N HCl (24), whereas, similar to our results, mild hydrolysis of native starch with 1 M HCl instead of 2.2 N HCl showed different patterns (25). After 20 day hydrolysis, the hydrolytic rates of native, RS3, and RS4 were 47.4, 82.7, and 50.3%, respectively, and that of RS3 approximately 2 times higher than those of others. The degree of α-amylase hydrolysis was 74.2% for RS3, whereas that of RS4 was only 3.1% during the same 20-day period. Acid and α-amylase, when used with native starch, showed different hydrolytic patterns, although their hydrolytic rates were similar. In comparison with the native starch, RS4 showed similar degradation to acid, but was more resistant to α-amylase.

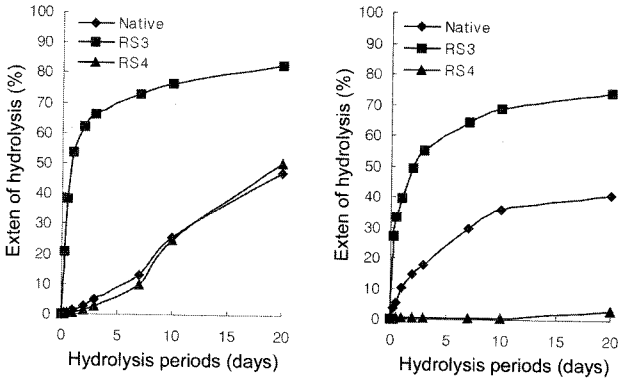

Fig. 1. Hydrolytic patterns of native (-◆ -), RS3 (-■ -), and RS4 (-▲ -) maize starches treated with 1 M HCl (left) or α-amylase (right) at 35°C for 20 days.

Differences in the rates and extents of hydrolysis have been attributed to differences in the extents of starch chain interactions within the amorphous and crystalline domains of the starch granules (26), the extent of phosphorylation (27), α-1,6 linkage levels (28), and the extent of distribution of α-1,6 glycosidic linkages between amorphous and crystalline starch domains (27). Factors affecting acid hydrolysis include the amorphous to crystalline region ratio, amylose-lipid complex level, amylose content, amount of α(1-6) linkage, granule size, and presence, numbers, and size of pores on granule surfaces. In addition, susceptibility of starch granules to degradation by α-amylase depends on the sources of the starch and the enzyme, and morphology and structure of starch including surface pore. Because H_3O+ and enzyme can directly infiltrate starch through the surface pores, they are important factors in terms of hydrolysis control. Access into the granules through the pores is limited by increased starch cross-linking, cross-linking between starch molecule and the protein of the granular membrane, and pore clogging during the cross-linking process (29). Penetration into RS4 by α-amylase was harder than by acid, because enzyme has higher molecular weight than H_3O+. However, in the case of RS3, α-amylase could easily penetrate and attack the starch molecule, because the protein membrane was denatured and deformed during the autoclaving process.

The degree of hydrolysis was affected by the acid- or α-amylase-combining site on the starch molecule. Acid can randomly hydrolyze α-1,4 and α-1,6 glycosidic linkages, while α-amylase hydrolyzes only the α-1,4 linkage in starch. In addition, the specificity of α-amylase depends on its source, optimal condition of digestion, and subsite specificity (30, 31). *Bacillus* α-amylase has a combining site of nine contiguous α-1,4-linked glucosyl units. These results suggest that the action of acid or α-amylase on RS was affected by several factors related to the starch itself, including modified condition for RS preparation, acid concentration, and α-amylase source.

RS levels of acid or α-amylase treated RS residues Table 1 shows the RS levels of residues treated with 1 M HCl or α-amylase at 35°C for 1/2, 1, 2, 3, 7, and 20 days. RS

Table 1. RS level of untreated native, RS3 and RS4 maize starches and their residues treated with 1 M HCl or α-amylase at 35°C for 20 days (unit: %)

Treatment	Sample	Hydrolysis periods (days)						
		0	1/2	1	2	3	7	20
1 M HCl	Native	4.9±1.6^{y}	^{b}7.0±1.2^{z}	^{b}8.0±0.9^{z}	^{b}7.8±0.7^{z}	^{b}7.1±0.4^{z}	^{b}5.7±1.1^{z}	^{b}5.7±0.6^{y}
	RS3	17.4±0.4^{x}	^{b}27.6±1.6^{x}	^{b}33.0±1.5^{x}	^{b}40.5±1.9^{x}	^{b}43.5±2.2^{x}	^{b}44.5±2.5^{x}	^{b}45.1±0.6^{x}
	RS4	17.2±0.1^{x}	^{b}16.4±2.0^{y}	^{b}15.8±1.4^{y}	^{b}15.3±0.5^{y}	^{b}15.0±1.6^{y}	^{b}14.1±0.0^{y}	^{b}6.3±0.5^{y}
α-amylase	Native	4.9±1.6^{y}	^{a}14.1±0.3^{z}	^{a}15.8±1.9^{z}	^{a}17.6±1.1^{z}	^{a}18.6±2.1^{z}	^{a}24.6±2.3^{y}	^{a}26.6±2.4^{y}
	RS3	17.4±0.4^{x}	^{a}49.1±1.8^{x}	^{a}51.7±1.2^{x}	^{a}53.4±1.8^{x}	^{a}55.6±2.1^{x}	^{a}62.8±1.2^{x}	^{a}64.2±1.6^{x}
	RS4	17.2±0.1^{x}	^{a}25.9±1.8^{y}	^{a}26.3±1.8^{y}	^{a}27.1±1.6^{y}	^{a}26.7±1.5^{y}	^{a}25.5±0.8^{y}	^{a}23.7±2.8^{y}

Data were presented in average±SD.
^{a,b}means within treatments of same sample with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range tests.
^{x,y,z}means within samples under same treatment with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range tests.

levels of untreated native, RS3, and RS4 maize starches were 2.0, 17.4, and 17.2%, respectively. After acid hydrolysis, the RS level of native starch remained constant, whereas that of RS3 increased from 17.4 to 45.1%. Hydrolysis rate of RS3 increased rapidly on the 2nd day, then slowly thereafter. RS level of RS4 during acid hydrolysis decreased slightly from 17.2 to 14.1% between day 0 and day 7, but decreased sharply to 6.3% on day 20. Amorphous region of RS3 increased from 60% in native starch to 80% through autoclaving/cooling process. The amorphous fraction (80%) of RS3 was degraded easily by acid, resulting in the increased RS level of the residue. The retrograded amylose (crystalline) fraction of RS3 melted at approximately 155°C by DSC, while not at 100°C during RS assay. RS4 was prepared by cross-linking starch molecules with STMP and STPP in the amorphous regions of native starch granules. The amorphous fraction of RS4 remained constant compared to the native starch, and some parts of the amorphous region were cross-linked between starch molecules. The di-starch phosphate bond in cross-linked starch inhibits the mobility of starch channel and is not hydrolyzed by α-amylase (32). In addition, increasing degree of cross-linking decreased the hydrolysis rate, while the RS level did not increased. In contrast to RS4, the RS level of RS3 increased up to 2.5 times by hydrolysis with amylase for 6 hr and by acid for 3 days.

Scanning electron microscopy The shapes of the hydrolyzed residues obtained by the acid and enzyme treatment of RS3 and RS4 during hydrolysis are shown in Fig. 2. Autoclaving-cooling-cycled RS3 had an irregular shape, whereas cross-linked RS4 showed a poly-gonal shape similar to that of the native starch granules. The shape of the acid- and α-amylase-treated residues of RS3 remained irregular, although small particles were observed on the surfaces. On day 2 of hydrolysis, shapes of acid- and α-amylase-treated residues of native and RS4 remained granular, similar to that of the native starch, and some pores were observed on the granule surfaces (A2, A4, C2, C4). Native starch granules were markedly eroded after hydrolysis for 20 days, regardless of acid or α-amylase treatment (A3, A5). RS4 starch granules were eroded by the acid treatment for 20 days (C3), while shape of α-amylase-treated RS4 residue remained granular with some pits observed on the granule surface (C5).

X-ray diffraction pattern X-ray diffractograms of acid- and α-amylase-treated residues of native, RS3, and RS4 maize starches are shown in Fig. 3. Native and RS4 starches showed A-type crystallinity with peaks of 2θ = 15, 17, and 23°, while RS3 had B type crystallinity with a strong peak at 2θ =16.7°, which is a distinctive peak for retrograded starch and V type of crystallinity with peak at 2θ = 20° for formation of amylose-lipid complex during heating. Although all treated residues kept the same crystalline types of initial samples, their peak intensities increased with hydrolysis time due to the acid- and α-amylase-cleaved starch chains in the amorphous region. The cleavage of starch molecules allows reordering of the chain segments into crystalline structure of the starch (14). These trends were stronger in acid treatment than α-amylase treatment of native and resistant starches at similar hydrolysis rates, indicating that the structures of crystalline regions in starch granules were altered in different ways by acid or enzyme. Kainuma and French (14) reported that acid attacks boundary region between amorphous and crystalline regions, but cannot penetrate the crystalline region of starch granules; thus, crystalline regions retain their crystallinity. Wang *et al.* (33) investigated residue of corn starch, which was hydrolyzed at different HCl concentrations, and x-ray diffractometry results show its crystalline type remained unchanged and crystallinity

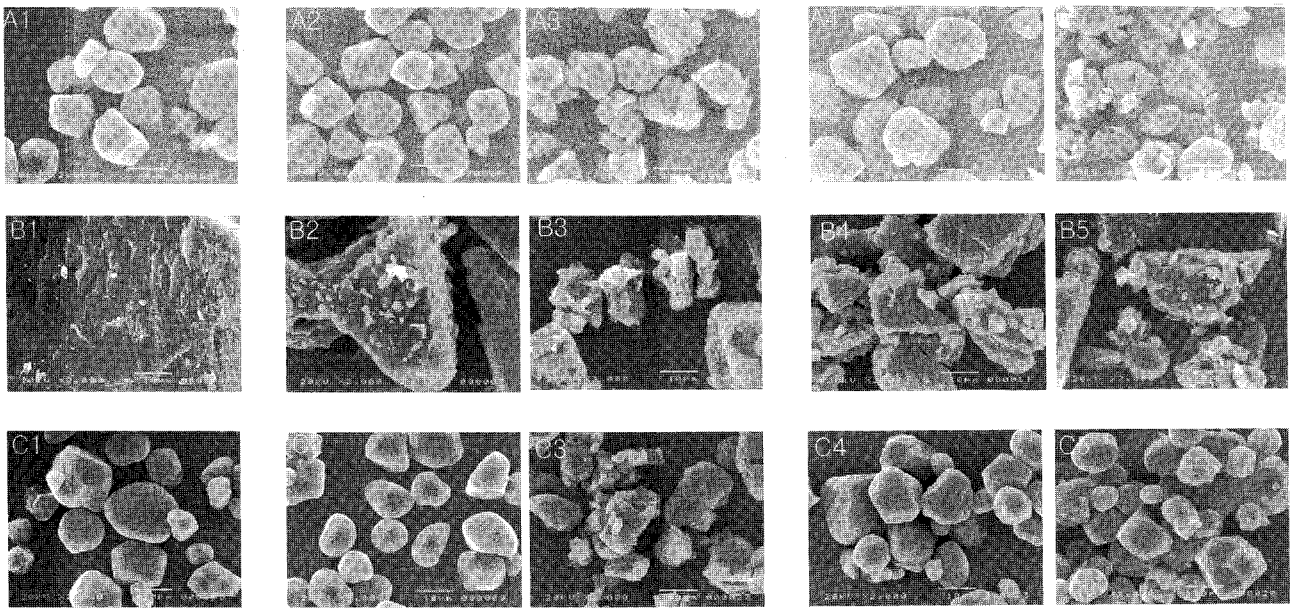

Fig. 2. Scanning electron microphotographs of untreated native (A), RS3 (B), and RS4 (C) starches and their residues treated with 1 M HCl or α-amylase at 35°C for 2 and 20 days. Untreated (1), Acid treated for 2 days (2), Acid treated for 20 days (3), α-amylase treated for 2 days (4), and α-amylase treated for 20 days (5).

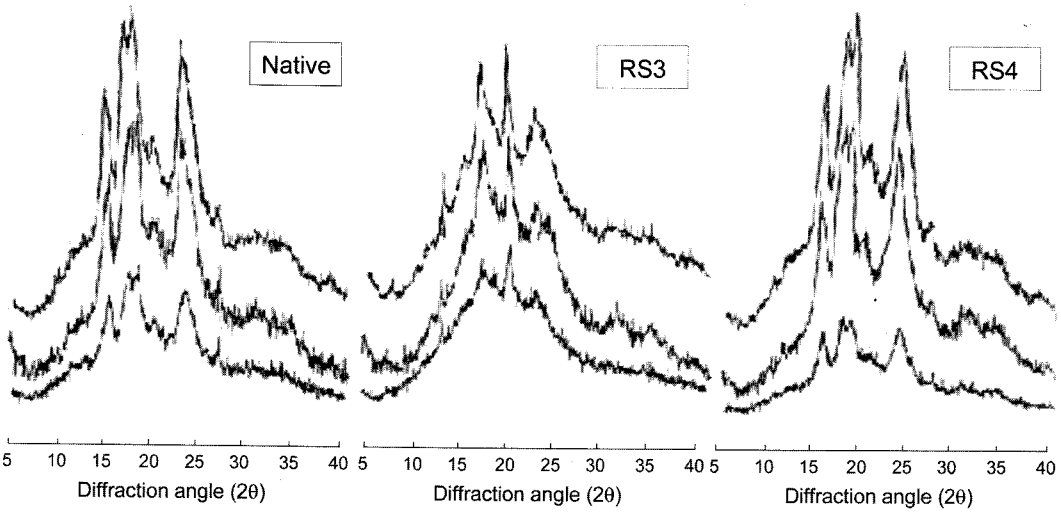

Fig. 3. X-ray diffractograms of untreated native (left), RS3 (middle) and RS4 (right) maize starches and their residues treated with α-amylase 1 M HCl or at 35°C for 20 days. Untreated (1), α-amylase treated for 20 days (2), and acid treated for 20 days (3).

increased.

Swelling power The swelling powers of acid- and α-amylase-treated residues of native starch, RS3, and RS4 are shown in Table 2. The swelling powers of those residues of native maize starch were unchanged at 30°C, and the swelling powers of acid-treated residues were not measured, because the acid-treated starch was solubilized at 100°C. Swelling power of α-amylase-treated residues decreased with increasing hydrolysis.

Swelling power of RS3 was 2.5 times higher than that of the native starch at 30°C. This is attributed to the autoclaving-cooling treatment, which converts most part of RS3 into an amorphous structure that easily imbibes water. RS4 showed slightly lower swelling power than the native starch at 30°C. In RS4, the swelling power of acid-treated residue increased rapidly from 2nd day to 7th day, while those of residues with α-amylase were not changed.

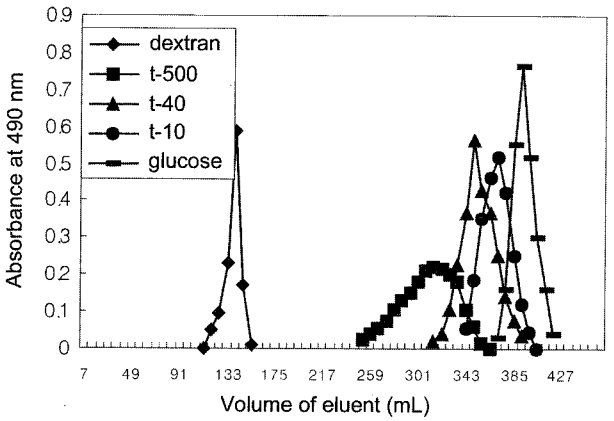

Fig. 4. Gel filtration chromatogram for standard dextran series using Sepharose CL-2B column. Dextran series used: dextran T-500, dextran T-40, and dextran T-10.

Gel permeation chromatography Chromatogram of dextran used to calibrate the gel filtration column (Sepharose CL-2B, Pharmacia Co.), and the relationship between alternative distribution coefficient (K_{av}) and log molecular weight of dextran are shown in Fig. 4. The chromatographic profiles of acid- and α-amylase-treated residues of native starch, RS3, and RS4 are shown in Fig. 5. Two peaks were observed in the native starch, representing the amylopectin peak (peak I, Fr no 110-160) and the amylose peak (peak II, above Fr no.240); amylopectin is eluted at void volume

Table 2. Swelling powers of untreated native, RS3 and RS4 maize starches and their residues treated with 1 M HCl or α-amylase at 35°C for 20 days

Sample	Temp (°C)	Acid treated (day)				α-amylase treated (day)			
		0	2	7	20	0	2	7	20
Native	30	1.8±0.0^{y}	1.9±0.0^{y}	2.3±0.2^{y}	2.5±0.1^{y}	1.8±0.0^{y}	1.5±0.2^{y}	1.6±0.1^{y}	1.6±0.3^{y}
	95	19.8±1.3^{x}	-	-	-	19.8±1.3^{x}	24.4±2.6^{x}	14.1±0.2^{x}	5. 8±0.9^{x}
RS3	30	4.7±0.6^{x}	^{b}4.1±0.2^{x}	^{b}3.7±0.2^{x}	^{b}3.5±0.^{1x}	4.7±0.6^{x}	^{a}5.9±0.3^{x}	^{a}4.9±0.2^{x}	^{a}4.5±0.2^{x}
	95	6.1±0.2^{y}	7.4±0.6^{x}	9. 6±0.8^{x}	8. 7±0.9	6.1±0.2^{y}	7.3±0.5^{y}	9.7±0.2^{y}	8.2±0.2^{x}
RS4	30	1.7±0.0^{y}	1.8±0.1^{y}	2.0±0.1^{y}	2.1±0.1^{z}	1.7±0.0^{y}	1.5±0.1^{y}	1.6±0.1^{y}	1.6±0.1^{y}
	95	3.5±0.4^{z}	^{a}5.0±0.2^{y}	^{a}22.4±1.2^{y}	-	3.5±0.4^{z}	^{b}3.0±0.0^{y}	^{b}3.2±0.3^{z}	3.1±0.0^{y}

Values are mean±SD.
- means that it is impossible to determine.
^{a,b}means within treatments of same samples with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range tests.
^{x,y,z}means within samples under same treatments with different superscripts are significantly different at $p<0.05$ by Duncan's multiple range tests.

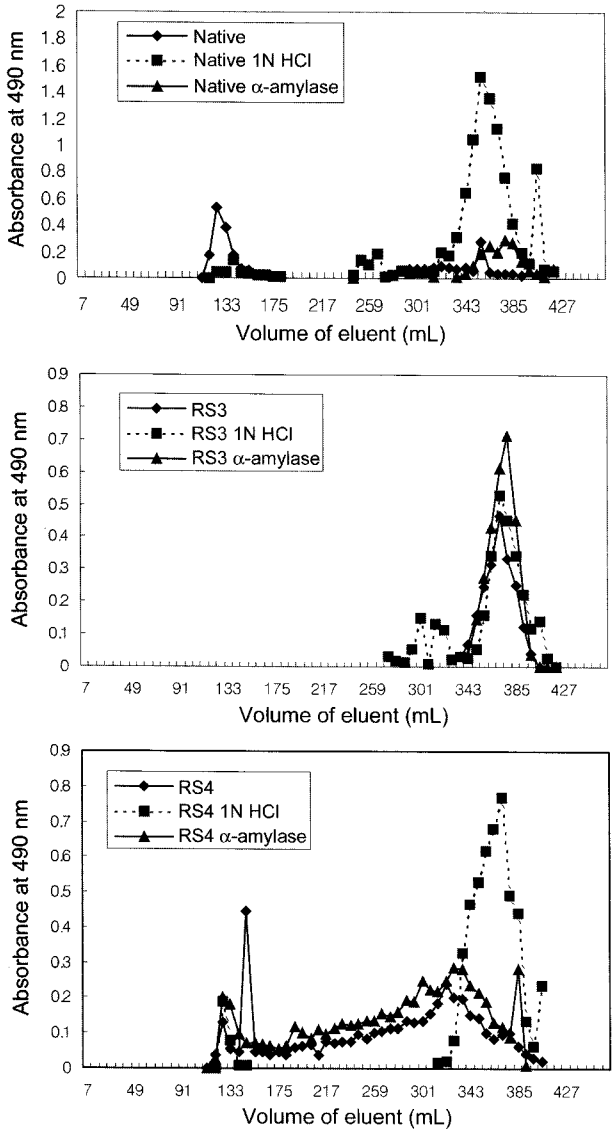

Fig. 5. Gel filtration chromatograms using Sepharose CL-2B column for native, RS3, and RS4 starches and their residues treated with 1 M HCl or α-amylase at 35°C for 7 days.

for gel filtration chromatography using Sepharose CL-2B column. Mw of peaks I and II were 4×10^7 and 4×10^4, respectively. After acid hydrolysis of the native starch, three fractions of Mw 4×10^7, 5×10^5, and 1-4×10^4 were detected, with peak II showing a larger peak area than others. The large molecular chain of amylopectin was hydrolyzed into smaller fractions, peak II and the 5×10^5 fraction. Peak I disappeared after α-amylase hydrolysis, and a peak of Mw 1×10^4 was observed, which indicates that amylopectin and amylose were also hydrolyzed into small chains by α-amylase. The acid hydrolysis pattern of the native starch residue differed from that of the residue obtained by α-amylase digestion.

Chromatogram of the retrograded RS3 showed a peak at Mw 1×10^4, and that of the residue from acid hydrolysis showed an additional peak at Mw 5×10^5, caused by the entanglement of some low-Mw fractions during hydrolysis. However, residue obtained from α-amylase digestion had a pattern similar to that of the untreated RS3.

Cross-linked RS4 showed two peaks, which were above Mw 4×10^7 and below 4×10^7 in the chromatogram. The peak fraction of Mw 4×10^4~4×10^7 disappeared after acid hydrolysis and was replaced by two peaks at Mw 4×10^7 and 4×10^4. The chromatogram pattern of residues from α-amylase digestion was similar to that of untreated RS4, suggesting that RS4 is difficult to hydrolyze with α-amylase. Because RS3 was more susceptible than RS4 to acid and α-amylase treatments, RS4 would be more desirable for RS application in acidic and α-amylase-containing foods.

Acknowledgments

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