

# Effect of Partial Acid Hydrolysis and Heat-Moisture Treatment on Formation of Resistant Tuber Starch

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## ABSTRACT

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Structural characteristics of resistant starch (RS) were investigated. Tuber starches, hydrolyzed with 1*N* HCl at 35°C for 8 hr followed by autoclaving-cooling treatment, were heated at 100°C for 16 hr after adjusting the moisture content to 20 or 30%. RS content of the tuber starches ranged from 5.4 to 22.7% depending on the source and type of treatment. Gelatinization parameters of RS isolated from partially acid-hydrolyzed starch with autoclaving-cooling followed by heat-moisture treatment (HMT)

showed higher enthalpy ( $\Delta H$ ) values and lower peak temperature ( $T_p$ ) compared with non-acid-hydrolyzed RS. *R* values, the difference between completion and initial temperatures, and  $\Delta H$  of RS increased by HMT. The X-ray diffraction patterns of potato and sweet potato RS isolated from partially acid-hydrolyzed starch with autoclaving-cooling showed distinct sharp peaks at 15, 25, 27, and 28°, which decreased by HMT.

In general, a fraction of the starch in starchy foods escapes complete enzyme degradation depending on granule size, amylose-to-amylopectin ratio, starch-protein interactions, amylose-lipid complexes, percentage of retrograded starch, and degree of gelatinization. This fraction is known as resistant starch (RS) (Englyst and Cummings 1985; Leloup et al 1992).

Based on decreasing scale of *in vivo* hydrolysis rate and degree of absorption, Englyst et al (1992) suggested classification of RS for nutritional purposes into three categories: RS1, physically inaccessible for digestion due to entrapment in a nondigestible matrix, e.g., whole and partly milled grains; RS2, native granule or ungelatinized starch as in raw potato and banana; and RS3, an indigestible starch fraction formed after heat-moisture treatment (HMT) of the starch that may be present in cooled or cooked starchy foods. A fourth category, RS4, formed by chemical modification of the starch was recently discovered (Eerlingen and Delcour 1995; Gidley et al 1995; Thompson 2000).

During treatment of starch granules with acid, the amorphous regions are more rapidly hydrolyzed than the crystalline ones (Robin et al 1974; Biliaderis et al 1981). Degradation of starch by acid hydrolysis decreases the molecular weight of starch, with HMW components of cereal, tuber, and root starch decreasing more than LMW components (Singh and Ali 2000). Acid hydrolysis of amylopectin, whose clusters of short chains (DP 15–17) constitute the starch crystallites, preferentially occurs at the intercrystalline regions to give short segments of linear or lightly branched molecules (Biliaderis et al 1981).

Changes in starch structure and properties on HMT depend on the botanical source. For example, tuber starches are more susceptible than legume or cereal starches toward HMT (Hoover and Vasanthan 1994; Jacobs and Delcour 1998). HMT induces additional interactions between amylose-amylose and amylose-amylopectin chains (Gunaratne and Hoover 2002). Factors affecting RS formation have been ascertained by many researchers. Partial acid hydrolysis of high amylose maize before annealing or HMT has improved RS2 yield over that of annealing or heat-moisture treatment without partial acid hydrolysis (Brumovsky and Thompson 2001).

The objectives of this study were to evaluate the effects of partial acid hydrolysis and HMT on RS formation in autoclaved-cooled tuber starches and to investigate structural characteristics of the isolated RS.

## MATERIALS AND METHODS

Potato and sweet potato starches were obtained from Samyang Genex Co. (Daejeon, Korea). Amylose contents of these starches were  $26.7 \pm 0.2\%$  and  $29.5 \pm 0.6\%$ , respectively. Pasting properties of these starches are shown in Table I. Heat stable  $\alpha$ -amylase (Termamyl 120 L) and amyloglucosidase (AMG 300 L) used for the isolation of RS were obtained from Novozymes (Bagsvaerd, Denmark). Protease (P-3910) was purchased from Sigma Chemical Co. (St. Louis, MO).

### Starch Pasting Properties

Starch pasting properties of potato and sweet potato starches were measured with a Rapid Visco Analyser ((RVA-3D, Newport Scientific Pty. Ltd., Warriewood, Australia). Double measurements using a 13-min controlled heating and cooling profile with constant shear were used, wherein the sample was held for 1 min at 50°C, heated at 12°C/min from 50 to 95°C, held for 2.5 min at 95°C, cooled at 12°C/min to 50°C, and held for 2 min at 50°C. In each case, 2 g of starch and 25 mL of distilled water were added to the RVA sample canister.

### Preparation of Partially Acid-Hydrolyzed Starch

Partial acid hydrolysis of starch was conducted using the method of Biliaderis et al (1981) with slight modification. Raw starch (10 g) was incubated with 400 mL of 1*N* HCl in a 35°C water bath for 8 hr, washed six times with distilled water then twice with 80% ethanol; it was adjusted to pH 7.0 with 1*N* NaOH and dried at room temperature.

### Autoclaving-Cooling Treatment

Autoclaved-cooled starches were prepared by the method of Sievert and Pomeranz (1989). Raw or partially acid-hydrolyzed starch was suspended in distilled water at starch-to-water ratio of 1:3.5. The suspension was autoclaved for 1 hr at 121°C, cooled to room temperature, and kept for 24 hr at 4°C. The autoclaving-cooling cycle was repeated four times. The sample was freeze-dried and pulverized to pass through a 140-mesh sieve.

### Heat-Moisture Treatment

Heat-moisture treatment was conducted using the method of Hoover and Vasanthan (1994). The autoclaved-cooled starch sample was weighed into a glass container, and the moisture content was adjusted to 20 or 30% by adding appropriate amount of distilled water. The container was sealed, kept for 24 hr at room temperature, and heated in a dry oven for 16 hr at 100°C, then the container was opened. The sample was air-dried at room temperature to  $\approx 10\%$  moisture content and ground to pass through a 140-mesh sieve.

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## Isolation of Resistant Starch

The enzymatic-gravimetric method (AOAC 1995) for determination of insoluble dietary fiber was used with slight modification to isolate RS. A 0.5-g sample in 25 mL of phosphate buffer (pH 6.0) was incubated with 0.2 mL of Termamyl in a 95°C water bath for 30 min, and cooled in an iced water bath. Amyloglucosidase (0.5 mL) was added after adjusting the sample to pH 4.5 with 0.275N HCl. The sample was incubated in a 60°C water bath for 30 min, and cooled in an iced water bath. After adjusting to pH 7.5 with 0.325N NaOH, the sample was incubated with protease solution (40 mg of protease dissolved in 50 mL of phosphate buffer) in a 60°C water bath, and centrifuged at 3,000 × g for 10 min. The pellet was washed twice with each 80% ethanol and distilled water, and filtered through a glass filtering crucible. The yield of RS was estimated as

$$\text{RS content (\%)} = [\text{wt of insoluble residue (g)/wt of sample (g)}] \times 100$$

## Gelatinization Parameters

Thermal properties of the RS samples were investigated with a differential scanning calorimeter (DSC 120, Seiko Instrument, Chiba, Japan). An indium standard was used for temperature and energy calibration. The sample (5 mg) was weighed into a Seiko high pressure stainless steel pan, into which 15 µL of distilled water was added. The sample pan was sealed and kept at room temperature for 1 hr. A pan with Al<sub>2</sub>O<sub>3</sub> and water represented the reference that kept the heat capacity to prevent deviation of baseline. The sample pan was heated from 30 to 220°C at 5°C/min.

## X-ray Diffraction

X-ray diffraction analysis was performed with an X-ray diffraction image processor (Dip 2030, Indigo 2, Oxford Model, HX-200w/c, Mac Science, Tokyo, Japan) (50 kV and 90 mA, CuK radiation  $\lambda = 1.541$ , nickel filter). The sample was scanned through 2 $\theta$  at 3–30°.

## RESULTS AND DISCUSSION

### Yield of RS

RS contents of tuber starches had a range of 5.4–22.7%, depending on the type of treatment and starch source (Fig. 1). RS content of potato starch was higher than that of sweet potato starch. However, when starches were subjected to partial acid hydrolysis, sweet potato starch showed higher RS content than potato starch in proportion to the moisture level. In addition, more

RS was formed in partially acid-hydrolyzed starch as compared with the tuber starch without partial acid hydrolysis. It has been reported that acid-pretreated starch produced higher RS values than the starch without acid treatment (Vasanthan and Bhatta 1998; Brumovsky and Thompson 2001).

HMT affects the rearrangement of the starch molecules between amylose-amylose and amylose-amylopectin, strengthening the linkage within starch (Franco et al 1995; Gunaratne and Hoover 2002). Partial acid hydrolysis, on the other hand, produces short linear chains, enhancing the mobility of molecules (Thompson 2000). The short linear chains appear to participate in the formation of resistant portions through rearrangement and recrystallization of starch during autoclaving-cooling and HMT.

### Gelatinization Parameters of RS

Thermal properties of RS isolated from the autoclaved-cooled starch with HMT are presented in Table II. The transition temperature range showed a broad endothermic peak from ≈97 to 175°C, and peak temperature ( $T_p$ ) of ≈155–157°C, depending on the starch source and type of treatment. Gruchala and Pomeranz (1993) and Gidley et al (1995) also reported that DSC of wheat and amylomaize VII RS showed a broad endothermic transition starting 90°C and peaking at 150–160°C. In general, endothermic transition of starch can be understood in terms of the influence of interactions between amylose-amylopectin, amylose-lipid, and amylose-amylose. These endothermic transition ranges would be ≈80, 120, and >140°C for amylose-amylopectin, amylose-lipid, and amylose-amylose, respectively (Boltz and Thompson 1999), suggesting that the endothermic transition observed in the thermogram of RS was caused by the melting of amylose-amylose complexes (Table II) (Sievert and Pomeranz 1989). Initial temperature ( $T_i$ ) decreased when autoclaved starch was subjected to HMT, while  $R$  value, the difference between completion and initial temperatures, of RS increased. These variable  $R$  values among the starches are due to the degree of heterogeneity of crystallites, which have slightly different crystal strengths (Vasanthan and Bhatta 1998; Gunaratne and Hoover 2002).  $\Delta H$  and  $R$  value of RS were increased by HMT, coinciding with the result of RS content (Fig. 1) (Table II). Therefore, the heterogeneous crystallites, which were produced either in amorphous or crystalline regions during autoclaving-cooling and HMT, induced a broad endothermic transition and resisted enzymatic hydrolysis as well.

The endothermic transition ranges of RS overlapped that of amylose-lipid complex (Table II). However, it does not indicate that RS includes amylose-lipid complex, because the amylose-

TABLE I  
Pasting Properties<sup>a</sup> of Potato and Sweet Potato Starches

Sample	Peak Viscosity (RVU)	Breakdown (RVU)	Final Viscosity (RVU)	Setback (RVU)	Peak Time (min)	Pasting Temp. (°C)
Potato	300.3	91.1	243.3	34.0	4.47	73.2
Sweet potato	185.7	57.9	186.0	58.3	4.13	76.0

<sup>a</sup> Values are averages of two determinations.

TABLE II  
Thermal Properties<sup>a</sup> of Resistant Starch<sup>b</sup>

Starch	Treatment <sup>c</sup>	Non-Acid Hydrolysis					Acid Hydrolysis				
		$T_i$	$T_p$	$T_c$	$R$	$\Delta H$	$T_i$	$T_p$	$T_c$	$R$	$\Delta H$
Potato	AC	114.8 ± 0.8	155.0 ± 0.4	172.4 ± 1.1	57.6 ± 0.3	6.7 ± 0.0	102.4 ± 0.3	136.4 ± 0.2	176.4 ± 0.3	74.0 ± 0.5	7.7 ± 0.1
	HMT20	98.9 ± 1.3	155.4 ± 0.2	170.6 ± 1.1	71.7 ± 1.0	8.1 ± 0.2	100.9 ± 0.5	136.6 ± 0.1	188.2 ± 0.9	87.3 ± 1.4	12.0 ± 0.3
	HMT30	97.4 ± 2.2	155.5 ± 0.7	172.8 ± 1.2	75.4 ± 2.3	10.0 ± 0.3	102.9 ± 0.2	136.4 ± 0.2	182.3 ± 0.3	79.4 ± 0.4	11.4 ± 0.0
Sweet potato	AC	116.3 ± 2.6	156.7 ± 0.2	173.5 ± 1.7	57.2 ± 3.8	5.7 ± 0.1	114.1 ± 0.3	144.9 ± 0.1	186.9 ± 0.4	72.8 ± 0.5	8.2 ± 0.1
	HMT20	108.1 ± 1.4	155.9 ± 0.9	171.1 ± 0.9	63.0 ± 2.3	7.6 ± 0.1	111.1 ± 0.8	143.4 ± 0.3	188.3 ± 0.3	77.2 ± 0.5	8.5 ± 0.3
	HMT30	99.7 ± 1.4	156.1 ± 0.3	175.4 ± 0.7	75.7 ± 1.1	8.3 ± 0.1	111.2 ± 0.4	141.2 ± 0.3	186.3 ± 0.3	75.1 ± 0.5	11.4 ± 0.1

<sup>a</sup>  $T_i$ , initial temperature;  $T_p$ , peak temperature;  $T_c$ , completion temperature;  $R$ , difference between completion and initial temperatures;  $\Delta H$ , enthalpy.

<sup>b</sup> Mean values of three replicates ± standard error.

<sup>c</sup> AC, autoclaving-cooling; HMT20 and HMT30, heat-moisture (20 and 30%, respectively) treatment after autoclaving-cooling.

amylopectin and amylose-lipid interactions are destroyed during any autoclaving cycle (Boltz and Thompson 1999; Thompson 2000). Furthermore, whereas cereal starches have  $\approx 1\%$  lipid content, tuber starches are virtually fat-free (Becker et al 2001); thus the endothermic transition of tuber RS was not affected.

Thermal properties determined by DSC of RS isolated from partially acid-hydrolyzed starch with autoclaving-cooling followed by HMT, referred to as partially acid-hydrolyzed RS hereafter, are summarized in Table II. The partially acid-hydrolyzed RS showed a broad endothermic peak at  $\approx 100\text{--}188^\circ\text{C}$ .  $T_p$  was observed at  $136\text{--}137^\circ\text{C}$  in potato starch, and  $141\text{--}145^\circ\text{C}$  in sweet potato starch depending on the moisture level, and was lower than that of RS isolated from tuber starch without acid hydrolysis. The difference in  $T_p$  between the starches can be attributed to starch composition (Vasanthan and Bhatta 1998; Gunaratne and Hoover 2002). Acid-hydrolyzed starches have many short chain fractions (Robin et al 1974; Biliaderis et al 1981), which form short double helices, requiring low temperature to dissociate completely (Yamin et al 1997). Therefore, the partially acid-hydrolyzed RS showing a lower peak transition temperature than RS isolated from starch without partial acid hydrolysis and a broad endothermic peak range could be attributed to these short chains.

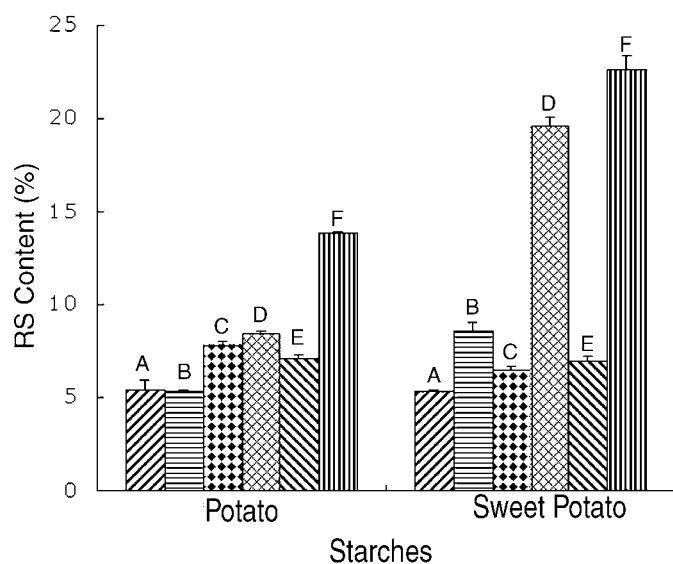
$R$  values and  $\Delta H$  of partially acid-hydrolyzed RS were higher than those of RS isolated from tuber starch without partial acid hydrolysis, and increased more than those of RS isolated from partially acid-hydrolyzed starch without HMT. In general,  $\Delta H$  values reflect mainly the loss of double helices rather than the crystalline order (Gunaratne and Hoover 2002). Therefore, the increment of  $\Delta H$  in partially acid-hydrolyzed RS could indicate the extent of increase in double helix. However, partially acid-hydrolyzed RS displayed lower  $T_p$  than RS isolated from starch without partial acid hydrolysis. This phenomenon can be responsible for the change in the amorphous region compactness (Jaspreet and Narpinder 2001). The increment of  $\Delta H$  and the decrement of  $T_p$  values indicate that the increase in double helical content is greater in the amorphous region than the crystalline one. Consequently, partially acid-hydrolyzed RS showed an increase in  $\Delta H$  values and a decrease in  $T_p$ . Increased  $R$  values suggested that

short linear chains, produced by partial acid hydrolysis, formed various small crystallites during autoclaving-cooling and HMT. These small crystallites showed a broad endothermic transition.

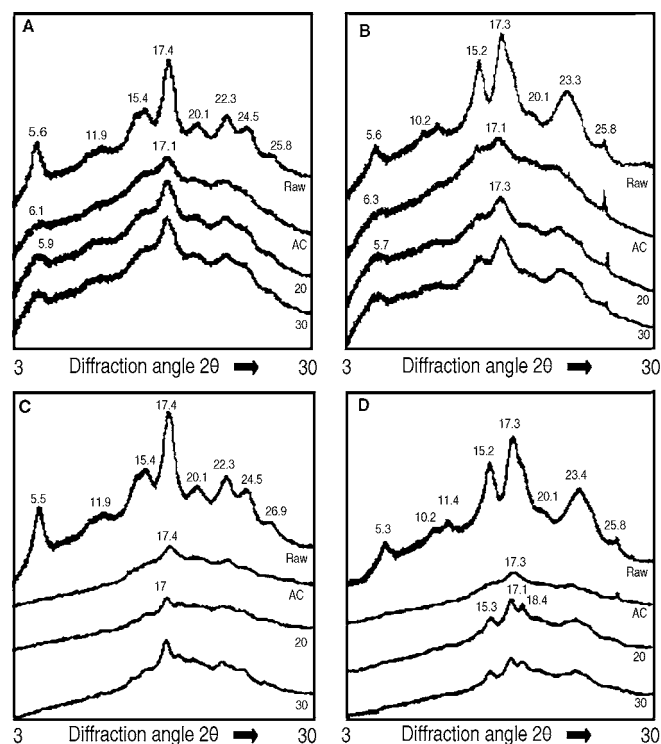
### X-ray Diffraction of Potato and Sweet Potato Starches

X-ray diffractograms of tuber starches and their partially acid-hydrolyzed starch are presented in Fig. 2. Raw potato and raw sweet potato starches showed B- and C-type patterns, respectively (Hizukuri 1996). Peaks of autoclaved-cooled starch were less intense than those of raw starch. Autoclaved-cooled potato starch showed a broad peak at  $23^\circ$  that covered the unresolved doublet region  $22.8\text{--}24.5^\circ$ . Apart from the broadening of the peaks, the peaks at  $17.1$  and  $6.1^\circ$  differed slightly from their apparent counterparts in raw potato starch (Fig. 2A). In addition, because enlarged lattice can be formed between  $17$  and  $6^\circ$  (Sievert et al 1991) due to the imperfect nature of the crystallites, which, in turn, could induce a broadening and slight overlapping of the  $15$  and  $17^\circ$  peaks (Fig. 2A and B), the peaks of autoclaved-cooled sweet potato starch shifted at  $17.3$  and  $5.6^\circ$  of raw starch (Fig. 2B) (Sievert et al 1991).

X-ray diffractogram of partially acid-hydrolyzed starches with autoclaving-cooling showed that the peaks of raw starch disappeared or broadened. Subsequent HMT slightly increased the intensity of peaks at  $15^\circ$  (sweet potato) and  $17^\circ$  (Fig. 2C and D), although the intensity was lower than that of tuber starch without partial acid hydrolysis. In general, X-ray diffraction diagram of acid-hydrolyzed starch shows higher resolution and sharper peaks than that of raw starch (Biliaderis et al 1981). However, partially acid-hydrolyzed starch with autoclaving-cooling showed lower intensity than raw starch (Fig. 2). These differences in X-ray diffraction among the starches can be attributed to the disruption of starch granules caused by autoclaving-cooling (Sievert and Pomeranz 1989), and partial acid hydrolysis and a decrease in



**Fig. 1.** Yield of resistant starch (RS). **A**, RS isolated from autoclaved-cooled starch; **B**, RS isolated from autoclaved-cooled starch subjected to heat-moisture treatment (HMT) (20%); **C**, RS isolated from autoclaved-cooled starch subjected to HMT (30%); **D**, RS isolated from partially acid hydrolyzed starch subjected to autoclaving-cooling; **E**, RS isolated from partially acid hydrolyzed starch subjected to autoclaving-cooling and HMT (20%); **F**, RS isolated from partially acid hydrolyzed starch subjected to autoclaving-cooling and HMT (30%).



**Fig. 2.** X-ray diffraction patterns of tuber starches subjected to partial acid hydrolysis, autoclaving-cooling, and heat-moisture treatment. **A**, potato starch without acid hydrolysis; **B**, sweet potato starch without acid hydrolysis; **C**, potato starch with acid hydrolysis; **D**, sweet potato starch with acid hydrolysis; Raw, raw starch; AC, autoclaving-cooling; 20, heat-moisture (20%) treatment after autoclaving-cooling; 30, heat-moisture (30%) treatment after autoclaving-cooling.

average chain length (Biliaderis et al 1981), which influences the crystalline structure during autoclaving-cooling. In other words, short linear chains became the main components in partially acid-hydrolyzed starch, which lost more crystalline structure than non-acid-hydrolyzed starch. Therefore, the intensity of peaks of partially acid-hydrolyzed starch with autoclaving-cooling decreased and some peaks disappeared during autoclaving-cooling.

### X-ray Diffraction of RS Residues

Diffraction patterns of RS isolated from autoclaved-cooled starch are presented in Fig. 3. Potato RS were characterized by weak diffraction lines at 5.6° and broad peaks at 17.1 and 23° (Fig. 3A), similar to B-type pattern. Potato RS showed increases in the intensity and resolutions of the 5.6, 17.1, and 23° peaks by HMT, showing results similar to those reported by Sievert et al (1991) (Fig. 3A). Sweet potato RS displayed a profile similar to C-type pattern, which showed distinctive peaks at 5.6, 15, 17, and 22–25° (Hizukuri et al 1996) (Fig. 3B), but showed more peaks at >25°. This RS pattern was somewhat different from the poor B-type pattern reported previously (Sievert et al 1991; Englyst et al 1992; Gidley et al 1995). Englyst et al (1992) suggested that X-ray patterns of B- or C-type tend to be more resistant to digestion by pancreatic amylase. Raw sweet potato starch has a C-type pattern and thus requires drastic treatment conditions to achieve proper modification (Kawabata et al 1994). Therefore, sweet potato RS maintained the C-type pattern. In addition, it produced novel sharp peaks during autoclaving-cooling and HMT.

RS isolated from the autoclaved-cooled tuber starch with partial acid hydrolysis showed a C-type pattern with sharp peaks (Fig. 3C and D). In particular, potato RS isolated from the autoclaved-

cooled starch with partial acid hydrolysis exhibited a novel X-ray pattern with two sharp peaks at 15 and 25° (Fig. 3C). Partial acid hydrolysis produces many short linear chains that form double helices with different strengths (Robin et al 1974; Yamin et al 1997; Biliaderis et al 1981). Probably, double helices were formed from these chains present in the amorphous or weak crystallites (Fig. 2C and D). The movement of double helices during autoclaving-cooling could change the crystallite orientation, which would explain the observed X-ray diffraction of potato RS isolated from partially acid-hydrolyzed starch subjected to autoclaving-cooling. Sweet potato RS showed a few sharp peaks at 15, 25, 27, and 28°, similar to the non-acid-hydrolyzed sample (Fig. 3D). Acid-treated rice and potato starches are reported to have sharp diffraction peaks (Robin et al 1974; Biliaderis et al 1981). Sievert and Pomeranz (1989) reported that incubation of starch with amylase and amyloglucosidase removed degradable portion in the starch structure and hence the apparent concentration of RS on treated starches. Therefore, the characteristic structure of tuber RS manifested in X-ray diffraction pattern must have been formed during acid hydrolysis with autoclaving-cooling and enzyme treatment.

The peaks of partially acid-hydrolyzed RS were decreased by HMT (Fig. 3C and D). The intensity of peaks at 15, 25, 27, and 29° decreased as compared with those of RS isolated from the autoclaved starch with partial acid hydrolysis, and a broad peak at 22–24° was formed. This phenomenon of decreased intensity coincided with DSC results, showing low  $T_p$  values and high  $\Delta H$  (Table I). Therefore, decreasing of the peaks by HMT could be attributed to the movement of a pair of double helices in the crystalline region, which causes a change in the crystalline orientation (Gunaratne and Hoover 2002).

## CONCLUSIONS

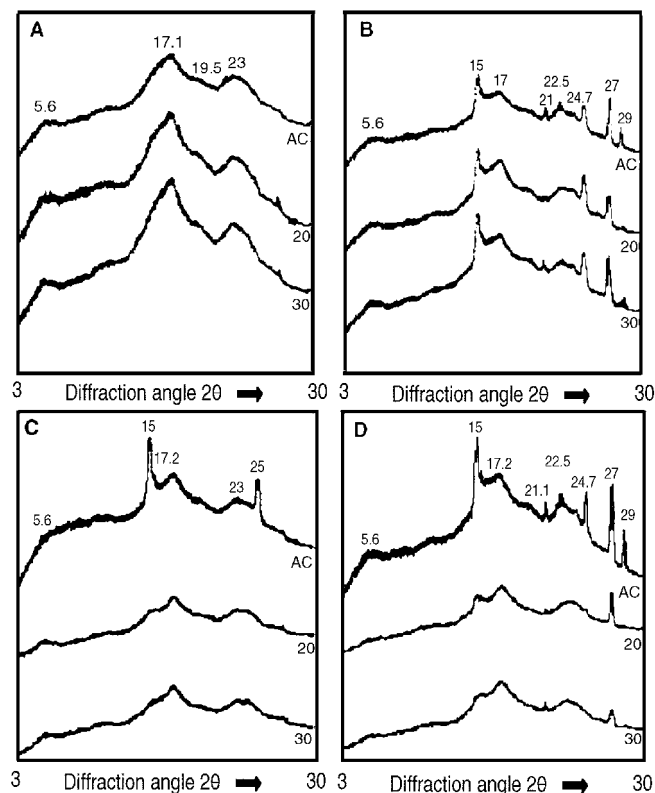
Partially acid-hydrolyzed starch with autoclaving-cooling followed by HMT showed a prominent increase in RS content. DSC analysis displayed an increase in  $\Delta H$  values and a decrease in  $T_p$  in partially acid-hydrolyzed RS compared with non-acid-hydrolyzed RS due to the increase in double helical content, which was higher in the amorphous than in the crystalline region. The X-ray diffraction pattern of potato and sweet potato RS isolated from partially acid-hydrolyzed starch with autoclaving-cooling showed sharp peaks at 15 and 25°. The sharp peaks in partially acid-hydrolyzed potato and sweet potato RS were decreased by HMT, indicating changes in the components of the starch structure during partial acid hydrolysis, autoclaving-cooling, and HMT. These results suggest that partial acid hydrolysis, autoclaving-cooling, and HMT are good methods for increasing RS content of tuber starch.

## ACKNOWLEDGMENTS

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**Fig. 3.** X-ray diffraction patterns of resistant starch (RS) isolated from tuber starches subjected to partial acid hydrolysis, autoclaving-cooling, and heat-moisture treatment. **A**, potato starch without acid hydrolysis; **B**, sweet potato starch without acid hydrolysis; **C**, potato starch with acid hydrolysis; **D**, sweet potato starch with acid hydrolysis. Raw, raw starch; AC: autoclaving-cooling; 20, heat-moisture (20%) treatment after autoclaving-cooling; 30, heat-moisture (30%) treatment after autoclaving-cooling.

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