A Thesis for the Degree of Master of Science

Influence of amylosucrase-treatment on the retrogradation of waxy corn starch

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지도교수 문 태 화
이 논문을 석사학위 논문으로 제출함

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Influence of amylosucrase-treatment on the retrogradation of waxy corn starch

by

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ABSTRACT

Waxy corn starch was modified by using amylosucrase (AS) from *Neisseria polysaccharea* for 1, 3, and 6 hr and retrograded at 4°C and 30°C for 1, 2, and 3 weeks. Branch-chain length distribution, thermal properties, crystallinity, and digestibility were changed by the AS-treatment. A chains (DP ≤ 12) decreased, while both B2 chains (DP 25-36) and B3 chains (DP 25-36) increased with increasing reaction time. Thermal properties of AS-treated starch such as the onset temperature, peak temperature, conclusion temperature, and melting enthalpy increased with enzyme reaction time. The X-ray pattern of AS-treated waxy corn starch changed from A- type to B-type, and peak intensity increased as the AS-treatment time increased. Slowly digestible starch (SDS) and resistant starch (RS) increased, while rapidly digestible starch (RDS) decreased.

After the retrogradation at 4°C, the melting enthalpy of retrograded AS control and 1 hr samples was changed. In the case of the retrogradation at 30°C, only 1 hr samples showed differences in enthalpy. All retrograded AS-treated starches displayed nonsignificant difference in X-ray diffraction pattern. Only relative crystallinity of the retrograded 1 hr sample at 30°C increased from 17.1% to 17.8%. The contents of SDS and RS showed

nonsignificant differences, but the RDS of retrograded AS control at 4°C decreased from 84.8% to 79.7%. In addition, the SDS of retrograded 1 hr sample at 30°C increased from 13.5% to 20.8%.

In this study, the AS-treatment elongated branch-chain length, and influenced retrogradation of waxy corn starch. Thus, the retrogradation of AS-treated waxy corn starch was affected by branch-chain length and dimension of chains.

Keywords: amylosucrase, waxy corn starch, retrogradation, digestibility, branch-chain length

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ABBREVIATIONS

AS: amylosucrase

RDS: rapidly digestible starch

SDS: slowly digestible starch

RS: resistant starch

DP: degree of polymerization

short chain: the chains of DP $\leq$12

medium chain: the chains of DP 13-24

long chain: the chains of DP 25-36

very long chain: the chains of DP $\geq$37

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INTRODUCTION

Starch is the important carbohydrate source in human diet. It is consisted of amylopectin and amylose. Amylose is a linear polymer composed of α-(1, 4) linked D-glucose. Amylopectin is a branched polymer composed of α-(1, 4) linked glucose connected by α-(1, 6) linked branch linkages (Sajilata, Singhal, & Kulkarni, 2006).

Based on the rate of *in vitro* starch digestion, starch is classified into three fractions that are rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). RDS is digested rapidly in the mouth and the small intestine. SDS is digested slowly but completely in the small intestine. SDS might consist of less perfect crystalline regions containing small portions of double helices and amorphous region (Shin, Choi, Chung, Hamaker, Park, & Moon, 2004). RS cannot be digested in the small intestine, but is fermented in the large intestine. Generally, SDS and RS have many benefits for human health. For example, the benefits of SDS are improved glucose tolerance, and diabetes management. (Lehmann & Robin, 2007).

Amylosucrase (EC 2. 4. 1. 4., AS) from *Neiserria polysaccharea* elongates the non-reducing ends of amylose and amylopectin. It catalyzes the reaction

to produce α-(1, 4) glucans using sucrose, while releasing fructose (Rolland-Sabaté, Colonna, Potocki-Véronèse, Monsan, & Planchot, 2004). It uses the glucose by splitting sucrose to elongate glucan. (Buttcher, Welsh, Willmitzer, & Kossmann, 1997; Potocki de Montalk, Remaud-Simeon, Willemot, Sarçabal, Planchot, & Monsan, 2000). Amylosucrase-treated starches have higher SDS contents than normal starches due to the crystallites formed by elongated branch-chains of amylopectin (Shin, Choi, Park, & Moon, 2010).

Tester et al. (2004) reported that starch has semi-crystalline matrices, produced by the conversion of double helices into crystalline structure. Retrogradation of amylopectin includes a crystallization process of the outer short branches (DP 14-18). In contrast to what is observed with amylose, the crystallization of amylopectin is a slow process continuing over a period of several days or weeks. In general, crystallization, consisting of nucleation, propagation, and maturation (slow crystal growth and crystal perfection), strongly depends upon temperature. Therefore, the crystallization, and thus the retrogradation of amylopectin, can be influenced by the time and temperature conditions of storage (Eerlingen, Jacobs, & Delcour, 1994). Nucleation is favored at temperatures far below melting temperature of the crystals but above glass transition temperature, but propagation is limited under these conditions. At temperatures far above glass transition temperature but below melting temperature, propagation is favored and

nucleation is limited (Eerlingen, Crombez, & Delcour, 1993).

The objectives of this study were to prepare samples of different chain length by using amylosucrase and to investigate the effect of storage time and temperature on retrogradation of the amylosucrase-treated waxy corn starch.

MATERIALS AND METHODS

1. Materials

1-1. Starch

Waxy corn starch was obtained from Samyang Genex Corp. (Incheon, Korea).

1-2. Enzymes

Pancreatin (P7545, activity 8 x USP/g) was from Sigma Chemical Co. and amyloglucosidase (AMG 300L, activity 300 AGU/mL) from Novozymes (Bagsvaerd, Denmark). Isoamylase (activity 1000U) was obtained from Megazyme (Bray, Ireland).

Amylosucrase (AS) from *Neiserria polysaccharea* was provided by Food Microbiology and Bioengineering Laboratory of Kyunghee University.

2. Methods

2-1. Enzyme assay of AS activity

The amylosucrase was purified by affinity chromatography with Ni-NTA (nickel-nitrilotriacetic acid) resin according to the method of Jung et al (2009). Enzyme activity was determined using the method of Van der Veen et al. (2004) with a modification. The mixture of 0.1 mL of 4% sucrose, 0.1 mL of 1% glycogen, 0.25 mL of 0.1 mM sodium citrate buffer (pH 7.0) and 0.05 mL of diluted enzyme was reacted in a water bath at 30°C and 80 rpm for 10 min. The released fructose was quantified using the method of Miller (1959). One unit (U) of amylosucrase was defined as the amount of enzyme that catalyzes the release of 1 μM of fructose per min under the assay conditions.

2-2. Preparation of AS-treated starch

Starch suspension (2%, w/w) was prepared by mixing starch, 100 mM sucrose, and 100 mM sodium acetate buffer (pH 7.0) to reach the final volume of 150 mL. The starch suspension was boiled for 30 min and cooled to 30°C. Amylosucrase (10,000U/30 mL) was added to the starch suspension. After then, the starch suspension including amylosucrase was incubated in a

water bath at 30 °C for 1, 3, and 6 hr. The enzyme reaction was stopped by adding 450 mL of ethanol to the suspension. The AS-treated starch was precipitated by centrifugation at 10,000 xg for 10 min, and the supernatant was removed. The pellet was washed with 450 mL of distilled water by centrifugation at 10,000 xg for 10 min. The precipitate was freeze-dried, ground and passed through a 100-mesh sieve. AS-control was prepared according to the same method for the AS-treated starch preparation without the amylosucrase addition.

2-3. Preparation of retrograded starch

Starch suspensions (40%, w/w) were prepared and gelatinized using an autoclave at 121°C for 30 min. After then, the starch gels were cooled to room temperature, and hermetically sealed. The starch gels were stored at 4°C in a refrigerator and 30°C in a water bath for 1, 2, and 3 weeks, respectively. The samples were then freeze-dried, ground and passed through a 100-mesh sieve.

2-4. Preparation of debranched starch

Starch (15 mg) was dispersed in 90% DMSO (3 mL) and boiled for 30

min. Ethanol (15 mL) was added to the starch suspension to precipitate starch and centrifuged at 10,000 xg for 10 min. Then distilled water (1.5 mL) was added to the pellet and boiled for 15 min. After boiling, 1.5 mL of 50 mM sodium acetate buffer (pH 4.3) was added and boiled for 20 min. Isoamylase (30 μL, 1000 U/mL, Megazyme) was added to the starch dispersion and the sample was incubated at 45°C, 30 rpm for 2 hr in a water bath. Enzyme reaction was stopped by boiling for 10 min.

2-5. Determination of amylopectin branch chain distribution by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

The branch chain-length distribution of starches was determined after debranching the starch. Debranched sample was filtered through a 0.45 μm membrane filter and analyzed using HPAEC-PAD on a Carbo-pack PA1 anion-exchange column (4x250 mm, Dionex, Sunnyvale, CA, USA) with a pulsed amperometric detector. The sample was eluted with a gradient of 600 mM sodium acetate in 150 mM NaOH with a flow rate 1 mL/min. The gradients of sodium acetate used were as follows: increasing from 0-20 % for 0-5 min, 20-45 % for 6-30 min, 45-55 % for 31-60 min, 56-60% for 61-80 min, 61-65 % for 81-90 min, 66-80 % for 91-95 min, and 81-100 % for 96-

100 min. The values of degree of polymerization (DP) were designated using a mixture of maltooligosaccharides (DP 1-7, Sigma Chemical) as standard. PeakNet software (version 5.11, Dionex) was used for calculation of peak areas.

2-6. Measurement of thermal properties

Thermal properties of the samples were investigated using a differential scanning calorimeter (Diamond DSC, Perkin-Elmer, Waltham, MA, USA). Each sample (8 mg) was weighed in a hermetic aluminum pan (Seiko, Tokyo, Japan), and 32 μL of distilled water was added. The sample pan was sealed and kept at room temperature overnight for equilibrium. An empty aluminum pan was used as a reference. DSC scan was performed from 30°C to 160°C at 10°C/min.

2-7. X-ray diffraction patterns and relative crystallinity

X-ray diffraction was analyzed using a powder X-ray dffractometer (Model New D8 Advance, Bruker, Karlsruhe, Germany) at 40 kV and 40 mA.

Starch sample scan was performed through 2θ range from 3° to 30° with a 0.02° step size and a count time of 2 sec. The area was calculated using a

software developed by the instrument manufacturer (EVA, 2.0). The relative crystallinity was determined by the equation below.

$$\text{Degree of crystallinity (\%)} = \left(\frac{\text{Area of the peaks}}{\text{Total curve area}}\right) \times 100$$

2-8. Starch digestibility

Starch digestibility was determined by the method of Brumovsky and Thompson (2001) with slight modification. Pancreatin (2 g) was dissolved in distilled water (24 mL) and stirred well for 10 min. It was precipitated by centrifugation at 1,500 xg for 10 min. A 20 mL aliquot of supernatant was mixed with 0.4 mL of amyloglucosidase and 3.6 mL of distilled water, and incubated at 37°C for 10 min.

A starch sample (30 mg) was dispersed in a 2 mL-microtube with sodium acetate buffer (0.75 mL, 0.1 M, pH 5.2) with one glass bead. After mixing each microtube, it was equilibrated in a shaking incubator (240 rpm at 37°C) for 10 min. Then, 0.75 mL of the prepared enzyme solution was added to the tube, and the starch sample was incubated in a shaking incubator (240 rpm at 37°C) for 10 min and 240 min, respectively. The reaction was stopped by boiling for 10 min. The glucose released under hydrolysis of starch was

obtained in supernatant after the centrifugation at 5,000 xg for 10 min. The glucose content was measured using a GOD-POD kit (BCS Co., Anyang, Korea).

Starch fractions are classified based on the rate and degree of hydrolysis. RDS was measured by the quantity of glucose after reaction for 10 min. SDS was the fraction digested between 10 and 240 min. RS was the unhydrolyzed fraction after 240 min.

2-9. Statistical analysis

All the experiments were done in triplicate, and data were expressed as mean±standard deviation. Analysis of variance (ANOVA) was conducted and the mean separations were done by the Tukey's HSD test ($p<0.05$). All the statistical analyses described above were conducted using SPSS (version 12.0.1, Chicago, IL, USA).

RESULTS AND DISCUSSION

1. Branch-chain length distributions of AS-treated starches

The branch-chain length distributions and the relative percentages of peak area with degree of polymerization of starches are shown in Figure 1 and Table 1, respectively. Amylopectin branch-chains are classified into A chain (DP 6-12), B1 chain (DP 13-24), B2 chain (DP 25-36) and B3 chain (DP ≥ 37) depending on degree of polymerization (Hanashiro, Abe, & Hizukuri, 1996).

A difference was observed in branch-chain length distributions between the AS-treated starches and AS control. The waxy corn starch, one of A type starches, had a larger proportion of short chains such as A chain but smaller proportions of longer chains (Zhang, Venkatachalam, & Hamaker, 2006).

Shin et al. (2010) reported that amylosucrase treatment induced the increase in the chain length of amylopectin, and accordingly the decrease in the proportions of short chains. After the AS treatment, A chains (DP ≤ 12) decreased while both B2 chains (DP 25-36) and B3 chains (DP 25-36) increased with the increase of enzyme reaction time. These results were due to the elongation of external chains by amylosucrase.

Therefore, the highest peak was shifted to the right with the increasing reaction time compared with that of AS control. On the other hand, the branch-chain length distributions of AS-treated starches showed nonsignificant differences after 6 hr of reaction. Potocki de Montalk et al. (1999) reported that amylosucrase elongates branch-chain length of amylose and amylopectin by catalyzing the attachment of 12 to 18 glucosyl units at non-reducing ends. In this study, the elongation of the branch-chain length might be completed within 6 hr of enzyme reaction.

Double helices formed by elongated branch-chains could inhibit the access of hydrolytic enzymes. The increased long branch-chain and the decreased short branch-chain in amylopectin could induce the formation of perfect crystallites, which resist starch-digestive enzymes (Shin, Choi, Park, & Moon, 2010). Therefore, the proportion of SDS and RS of AS-treated starches could be increased compared with the AS control.

During retrogradation, only the rearrangement of starch chains occurred without changes in branch-chain length distributions. For this reason, only the branch-chain length distributions of nonretrograded AS-treated starches were displayed in this study.

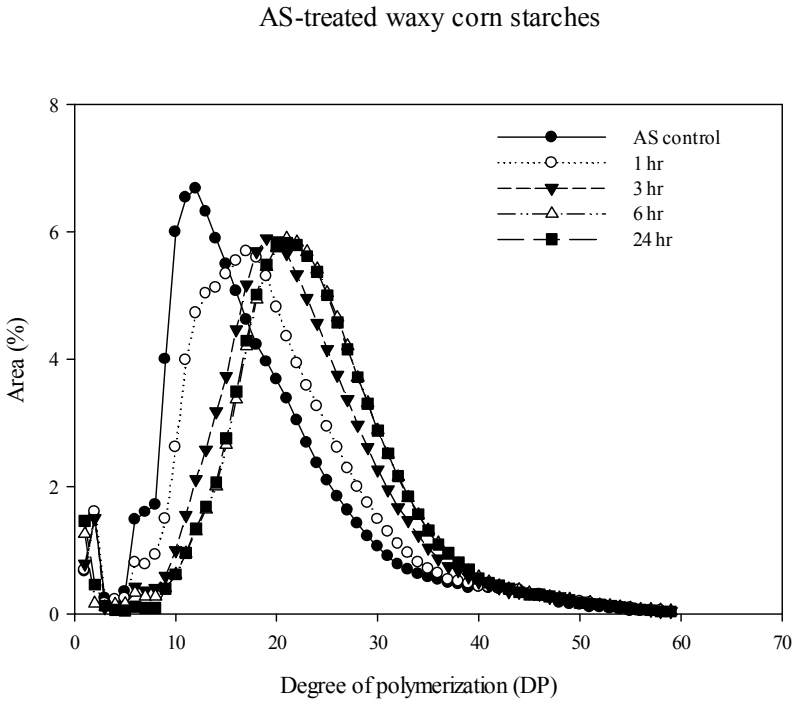

Figure 1. Branch-chain length distributions of AS-treated starches

Table 1. Percent branch-chain length distributions of AS-treated starches

Sample	Percent distribution (%)				
	DP[1] ≤ 5	DP 6-12	DP 13-24	DP 25-36	DP ≥ 37
AS control[2]	2.3±1.1[a 3), 4)]	26.7±1.5[a]	50.6±0.7[b]	14.5±0.8[d]	5.8±0.3[b]
1 hr	2.2±1.1[a]	14.0±3.0[b]	57.5±0.7[a]	20.0±2.0[c]	6.3±0.6[b]
3 hr	2.6±0.1[a]	5.7±1.7[c]	55.6±1.1[a]	28.9±1.9[b]	7.2±0.7[ab]
6 hr	2.9±0.3[a]	3.5±0.9[c]	50.5±1.8[b]	35.3±2.5[a]	7.9±0.2[a]
24 hr	2.7±0.5[a]	3.4±1.0[c]	50.6±1.9[b]	35.4±2.4[a]	7.9±0.7[a]

[1] DP, degree of polymerization.

[2] AS control; no enzymatic treatment after gelatinization.

[3] Data are expressed as average value and standard deviation.

[4] The values with different superscripts in the same column are significantly different ($p<0.05$).

2. Thermal properties

Thermal properties of AS control and AS-treated starches were determined by differential scanning calorimetry (DSC). Onset (T_o), peak (T_p), and conclusion (T_c) temperatures, and melting enthalpy (ΔH) are presented as thermal properties.

The peak temperature (T_p) means the structural stability. The onset temperature (T_o) and the conclusion temperature (T_c) are associated with melting of the weakest crystallites and the strongest crystallites, respectively (Barichello, Yada, Coffin, & Stanley, 1990; Biliaderis, Maurice, & Vose, 1980).

The endothermic peak of AS control did not appear, due to the disruption of double helices by gelatinization. The onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and ΔH gradually increased with the increasing reaction time. The AS-treated starches had increased branch-chain length, and so made interchain association easier. Thus, the recrystallization was promoted, leading to more resistance to digestive enzymes (Ryu et al., 2010). Due to the elongation of branch-chains by the AS treatment, gelatinization temperatures could be higher with melting of the crystallites with longer branch-chains of amylopectin (Han, Ao,

Janaswamy, Jane, Chandrasekaran, & Hamaker, 2006). Liu and Thompson (1998) also reported that the peak presented at a high temperature was due to double helices formed by long chains. These studies explained why T_o, T_p and T_c of AS-treated starch increased with increasing reaction time. Moreover, thermal properties such as T_o, T_p and T_c were the lowest in the 1 hr sample due to a larger proportion of short chains. Noda et al. (1998) stated that low T_o, T_p and T_c indicate the high amount of short chains. The short chains of amylopectin are hard to form double helices, and therefore might require less energy to melt during gelatinization (Chung, Liu, & Hoover, 2010; Srichuwong & Jane, 2007).

All AS-treated starches displayed broader endothermic peaks due to the elongated branch-chain length. The enthalpy (ΔH) gradually increased with increasing reaction time because of the formation of new double helices by the AS treatment. It reflected an increase of crystalline stability. Lopez-Rubio et al. (2008) reported that the enthalpy is connected to melting of imperfect crystals based on amylopectin, which contribute to both crystal packing and helix melting enthalpies.

These results indicated that the thermal properties of AS-treated starch were influenced by the formation of stable and more perfect crystallites resulting from the AS treatment.

Table 2. DSC parameters of raw and AS-treated starches

Sample	T_o (°C)[1)]	T_p (°C)	T_c (°C)	ΔH (J/g)
AS control[3)]	N.D.[2)]			
1 hr	52.2±1.6[b 4)]	61.9±0.5[c]	79.6±0.9[b]	6.3±0.6[b]
3 hr	53.8±0.2[b]	66.3±0.7[b]	83.8±1.1[b]	10.5±1.1[a]
6 hr	61.0±4.1[a]	78.9±1.4[a]	96.4±4.4[a]	11.4±0.5[a]

[1)] T_o, T_p and T_c indicate the onset, peak and conclusion temperatures of melting, respectively.

[2)] Not detected within the temperature range.

[3)] AS control; no enzymatic treatment.

[4)] The values with different superscripts in the same column are significantly different ($p<0.05$).

3. X-ray diffraction patterns and relative crystallinity

X-ray diffraction patterns and relative crystallinities of the AS control and AS-treated starches are represented in Figure 2 and Table 3, respectively. In the previous studies, the raw waxy corn starch showed major peaks at 15°, 17°, 18° and 23°, presenting a typical A type pattern (Hizukuri, Takeda, Usami, & Takase, 1980; Shin, Choi, Park, & Moon, 2010). The AS control mainly composed of the amorphous regions, and so did not show any significant peaks (Shin, Choi, Park, & Moon, 2010). The AS-treated starches changed from a A type to a weak B-type X-ray pattern with the peaks at 5.5°, 17°, 19.3°, 22° and 23°. By increasing AS treatment time, the peak intensity of AS-treated starches increased.

After the AS treatment, amylopectin showed a B-type X-ray diffraction pattern and the amylopectin with elongated branch-chains behaved like long amylose (Rolland-Sabaté, Colonna, Potocki-Véronèse, Monsan, & Planchot, 2004). Moreover, the retrograded starches also showed a B-type pattern, presumably due to the aggregation of the longer chains (Hizukuri, Takeda, Usami, & Takase, 1980; Pohu, Planchot, Putaux, Colonna, & Buleon, 2004).

Table 3 shows the relative crystallinity of the samples. The relative crystallinity of AS control was the lowest due to the gelatinization prior to

the enzyme reaction. The relative crystallinity of AS-treated starch gradually increased from 17.1 to 21.6 % with increasing reaction time. The elongated branch chains favored to form double helices, which could lead to the crystalline structure in the AS-treated starch. The crystallinity of starch could be influenced by 1) amylopectin content 2) average amylopectin chain length 3) orientation of double helices within the crystallites to the X- ray beam, and 4) crystallite size (Chung, Hoover, & Liu, 2009).

In sum, the elongated branch chains by the AS treatment favored to form double helices which induced formation of crystallites during enzyme reaction, and therefore increased the relative crystallinity of AS-treated starch.

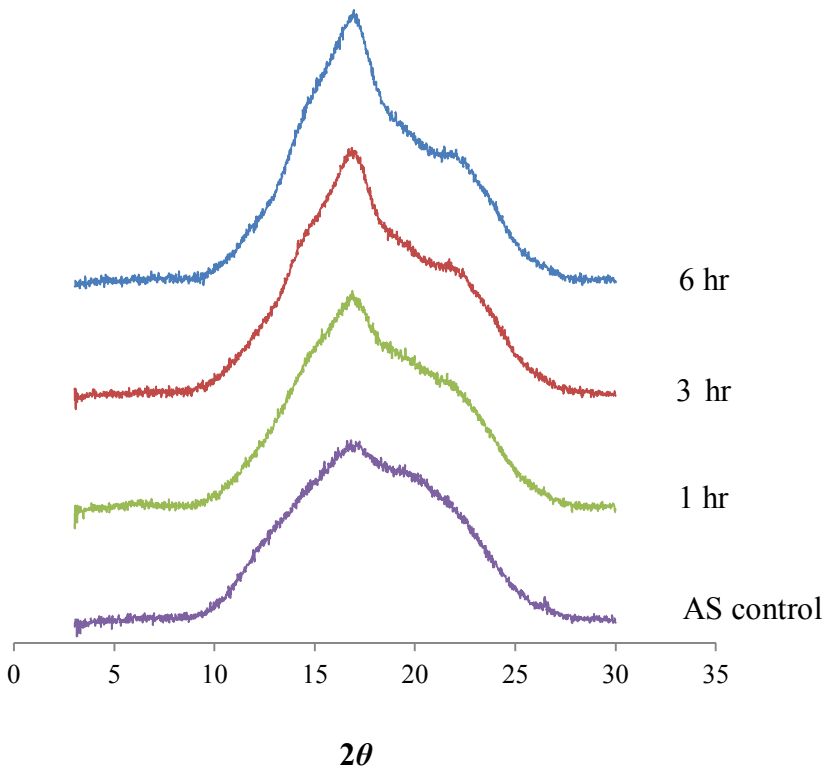

Figure 2. X-ray diffraction patterns of AS-treated starches

Table 3. Relative crystallinity of AS-treated starches

Sample	Relative crystallinity (%)
AS control	15.6±0.5[d 1), 2)]
1 hr	17.1±0.4[c]
3 hr	18.2±0.2[b]
6 hr	21.6±0.1[a]

[1)] Data are expressed as average value with standard deviation.

[2)] The values with different superscripts in the same column are significantly different (p<0.05).

4. Starch digestibility

Table 4 shows the *in vitro* digestibility of AS control and AS-treated starches. Starch is divided into three fractions by reaction time according to the Englyst assay (Englyst, Kingman, & Cummings, 1992). The AS control had the highest RDS content. Because the gelatinization process destroys the semicrystalline structure of raw starch granules, gelatinized starches had a higher RDS content compared with raw starches (Cousin, Cuzon, Guillaume, & Aquacop, 1996; Zhang, Venkatachalam, & Hamaker, 2006). The contents of RDS of the AS-treated starches decreased with increasing reaction time. After the AS treatment for 1, 3, and 6 hr, the SDS and RS contents increased from 1.9 to 34.9 % and from 13.3 to 20.5 %, respectively, while the RDS content decreased from 84.8 to 44.5 %.

The resistance to enzymatic hydrolysis can be described with branch-chain length distribution and relative crystallinity. By the AS treatment, the branch-chain length of AS-treated starches became longer than that of AS control (Rolland-Sabaté, Colonna, Potocki-Véronèse, Monsan, & Planchot, 2004; Shin, Choi, Park, & Moon, 2010). The double helices could be formed by elongated branch chains, which could contribute to the crystalline structure in the AS-treated starch. The crystalline regions have decreased susceptibility

to enzymatic hydrolysis, while the amorphous regions are easier to access by enzyme (Zhang, Venkatachalam, & Hamaker, 2006). Jane et al. (1999) informed that the long branched chains of starch contribute to retrogradation which reduces the enzyme susceptibility, while the short chains form short or weak double helices that would produce imperfect crystalline structures.

Conclusively, the elongation of branch chain length and recrystallization during enzyme reaction might increase resistance to enzymatic hydrolysis and increase both SDS and RS contents.

Table 4. Contents of RDS, SDS and RS of AS-treated starches

Sample	RDS (%)	SDS (%)	RS (%)
AS control	84.8±0.3$^{a\ 1),\ 2)}$	1.9±1.1^{c}	13.3±1.2^{b}
1 hr	76.1±2.4^{b}	13.5±1.3^{b}	10.3±3.6^{b}
3 hr	57.2±1.6^{c}	34.6±3.5^{a}	8.2±3.3^{b}
6 hr	44.5±4.5^{d}	34.9±4.0^{a}	20.5±3.0^{a}

[1)] Data are expressed as average value with standard deviation.

[2)] The values with different superscripts in a column are significantly different (p<0.05).

5. Thermal properties of AS-treated starches after retrogradation

Table 5 and 6 show thermal properties of retrograded starches. After 1 week, 2 weeks and 3 weeks retrogradation, thermal properties of the AS-treated starches were slightly changed. It could be due to the formation of double helices and crystalline regions by the AS treatment.

After the retrogradation of AS-treated starches at 4°C, the enthalpy (ΔH) of AS control increased with increasing retrogradation time, while those of 3 hr and 6 hr displayed no significant differences. This result indicated that the AS control formed new double helices during retrogradation at 4°C which contributed to retrogradation. The enthalpy of 1 hr samples increased only within 1 week retrogradation, suggesting that the formation of new double helices was completed within 1 week. After 2 weeks retrogradation, no more double helices formed due to the limited dimensions of the chains. On the other hand, 3 hr and 6 hr samples could not form double helices. This result also might be due to the limited dimensions of the chains which could cause the stability of these crystallites to be lower compared with those formed from amylose (Eerlingen & Delcour, 1995).

By the retrogradation at 30°C, the endothermic peak of AS control did not

appear in all samples. This result could be due to the disruption of double helices by gelatinization, and suggests that the AS control could not form new double helices because of the temperature condition. During the retrogradation at 30°C, where propagation and maturation are favored, no double helices were formed in the AS control. Same as the retrogradation at 4°C, only the enthalpy of 1 hr sample increased slightly with the retrogradation time within 1 week due to the limited dimensions of the chains.

In the case of crystallization of amylopectin in a starch gel, the glass transition temperature is ~ -5°C and the melting temperature of the B-type crystals is ~60°C. Therefore, the crystallization of amylopectin can be influenced by the time and temperature. At 4°C, nucleation is favored; whereas at 30°C, propagation is promoted. Maturation is also favored at 30°C. In addition, because amylopectin retrogradation is a slow process, increased retrogradation is expected with the storage of the starch sample for several weeks (Eerlingen, Jacobs, & Delcour, 1994).

Table 5. DSC parameters of AS-treated starches after retrogradation (4°C)

	AS control[1)]				1 hr			
	T_o (°C)[2)]	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
0 day	N.D.[3)]				52.2±1.6[a 4)]	61.9±0.5[a]	79.6±0.9[a]	6.3±0.6[b]
1 week	50.1±1.9[a]	56.1±0.6[a]	62.9±0.0[a]	1.8±0.5[c]	51.0±0.3[a]	60.7±0.6[ab]	77.6±0.7[ab]	8.8±0.1[a]
2 weeks	48.6±0.8[a]	55.2±0.3[a]	65.7±3.8[a]	2.8±0.1[b]	50.4±0.2[a]	60.9±0.7[ab]	72.3±0.0[b]	8.4±0.2[a]
3 weeks	47.5±0.4[a]	54.9±0.7[a]	64.1±3.1[a]	3.9±0.3[a]	51.1±1.1[a]	60.4±0.3[b]	75.3±4.0[ab]	8.8±0.1[a]
	3 hr				6 hr			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
0 day	53.8±0.2[a]	66.3±0.7[a]	83.8±1.1[b]	10.5±1.1[a]	61.0±4.1[ab]	78.9±1.4[a]	96.4±4.4[a]	11.4±0.5[a]
1 week	53.3±1.1[a]	69.7±3.7[a]	91.1±4.5[ab]	11.4±1.8[a]	60.2±2.5[ab]	78.2±0.6[a]	97.5±1.0[a]	10.1±1.0[ab]
2 weeks	55.0±3.5[a]	68.2±3.1[a]	93.9±0.6[a]	10.5±1.1[a]	57.7±4.8[b]	79.0±1.3[a]	94.8±0.0[a]	9.1±0.6[b]
3 weeks	55.9±1.6[a]	72.0±0.0[a]	89.3±4.6[ab]	9.0±0.4[a]	66.8±1.0[a]	80.1±1.3[a]	95.7±5.6[a]	8.7±1.0[b]

[1)] AS control; no enzymatic treatment.

[2)] T_o, T_p and T_c indicate the onset, peak and conclusion temperatures of melting, respectively.

[3)] Not detected within the temperature range.

[4)] The value with different superscripts in a same column are significantly different (p<0.05).

Table 6. DSC parameters of AS-treated starches after retrogradation (30°C)

	AS control[1)]				1 hr			
	T_o (°C)[2)]	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
0 day	N.D.[3)]				52.2±1.6[a 4)]	61.9±0.5[c]	79.6±0.9[a]	6.3±0.6[b]
1 week					55.1±1.8[a]	63.5±1.1[bc]	75.6±4.1[a]	9.4±1.1[a]
2 weeks					56.0±3.0[a]	64.7±0.4[b]	81.3±0.8[a]	10.1±0.9[a]
3 weeks					52.9±0.1[a]	66.9±0.4[a]	79.3±1.9[a]	9.7±0.3[a]
	3 hr				6 hr			
	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
0 day	53.8±0.2[a]	66.3±0.7[b]	83.8±1.1[b]	10.5±1.1[a]	61.0±4.1[a]	78.9±1.4[a]	96.4±4.4[a]	11.4±0.5[a]
1 week	58.6±2.4[a]	72.7±0.2[a]	94.5±0.1[a]	10.8±0.1[a]	61.3±2.9[a]	78.6±0.0[a]	92.7±5.2[a]	10.4±0.6[a]
2 weeks	56.9±1.7[a]	72.2±0.3[a]	95.6±1.6[a]	9.8±0.4[a]	60.0±4.0[a]	79.3±0.0[a]	100.3±0.7[a]	10.5±0.4[a]
3 weeks	57.7±3.6[a]	72.0±0.1[a]	93.2±3.5[a]	11.2±0.7[a]	56.2±2.9[a]	78.4±0.3[a]	95.0±1.1[a]	10.7±0.8[a]

[1)] AS control; no enzymatic treatment.

[2)] T_o, T_p and T_c indicate the onset, peak and conclusion temperatures of melting, respectively.

[3)] Not detected within the temperature range.

[4)] The value with different superscripts in a same column are significantly different (p<0.05).

6. X-ray diffraction patterns and relative crystallinity of AS-treated starches after retrogradation

Figure 3, 4 and Table 7 displayed the X-ray diffraction and relative crystallinity of AS-treated starches after retrogradation. All samples exhibited the B-type X-ray pattern showing the peaks at 5.5°, 17°, 19.3°, 22° and 23°. The peak intensity of samples was not significantly changed after retrogradation. Besides, the peak intensity of retrograded starches at 4°C and 30°C had similar trends.

Table 7 displays the relative crystallinity of samples. The relative crystallinities of AS-treated starches after retrogradation at 4°C showed no significant differences among samples. It seems that, during the AS-treatment, the elongation of branch chain length and the retrogradation fully occurred already. Furthermore, no more recrystallization was observed during the retrogradation at 4°C after the AS-treatment because of the limited dimensions of the chains. On the other hand, the relative crystallinity of 1 hr sample after the retrogradation at 30°C increased slightly with the retrogradation time. The AS-treatment for 1 hr elongated the branch chain length slightly, which could induce the formation of new double helices and increase the crystalline regions regardless of the limited dimensions of the chains. In addition, the retrogradation at 30°C accelerated propagation and

maturation than nucleation. For this reason, under the general condition, increased retrogradation is expected with the storage at 4°C and, subsequently, at 30°C (Eerlingen, Jacobs, & Delcour, 1994). This result could indicate that formation of double helices by the AS-treatment might be similar to nucleation like formation of critical nuclei.

The nonsignificant difference in relative crystallinity of samples after 3 hr and 6 hr retrogradation can be explained with branch-chain length distribution and thermal properties. Table 1 showed that short chains decreased, while medium and long chains increased with enzyme reaction time. The retrograded samples treated with AS for 3 hr and 6 hr had too long chains to recrystallize because of the limited dimensions of the chains in granules. Furthermore, these 3 hr and 6 hr samples retrograded fully during the AS-treatment, giving no significant changes in enthalpy.

Conclusively, the AS treatment and retrogradation increased crystalline regions influenced by branch-chain length and storage temperature.

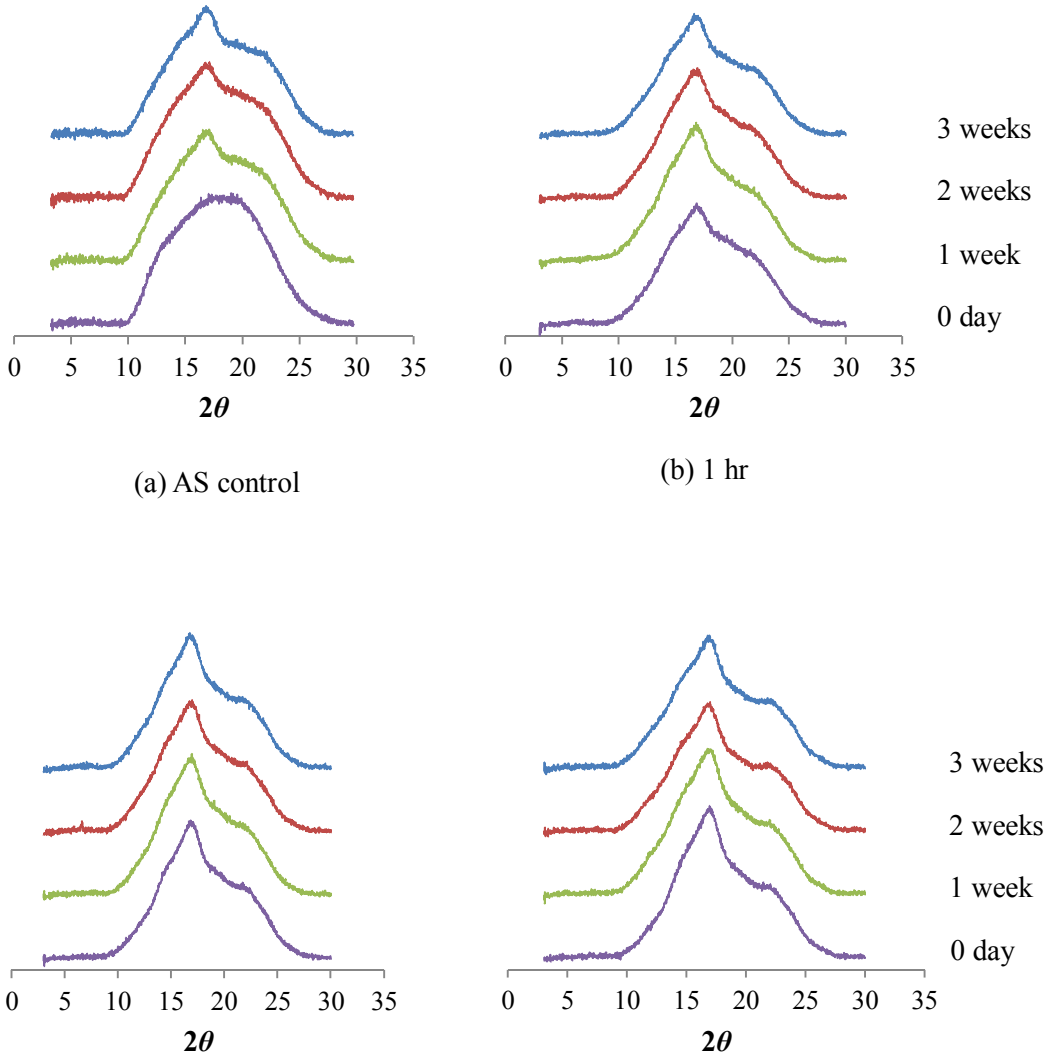

Figure 3. X-ray diffraction patterns of AS-treated starches after retrogradation (4°C)

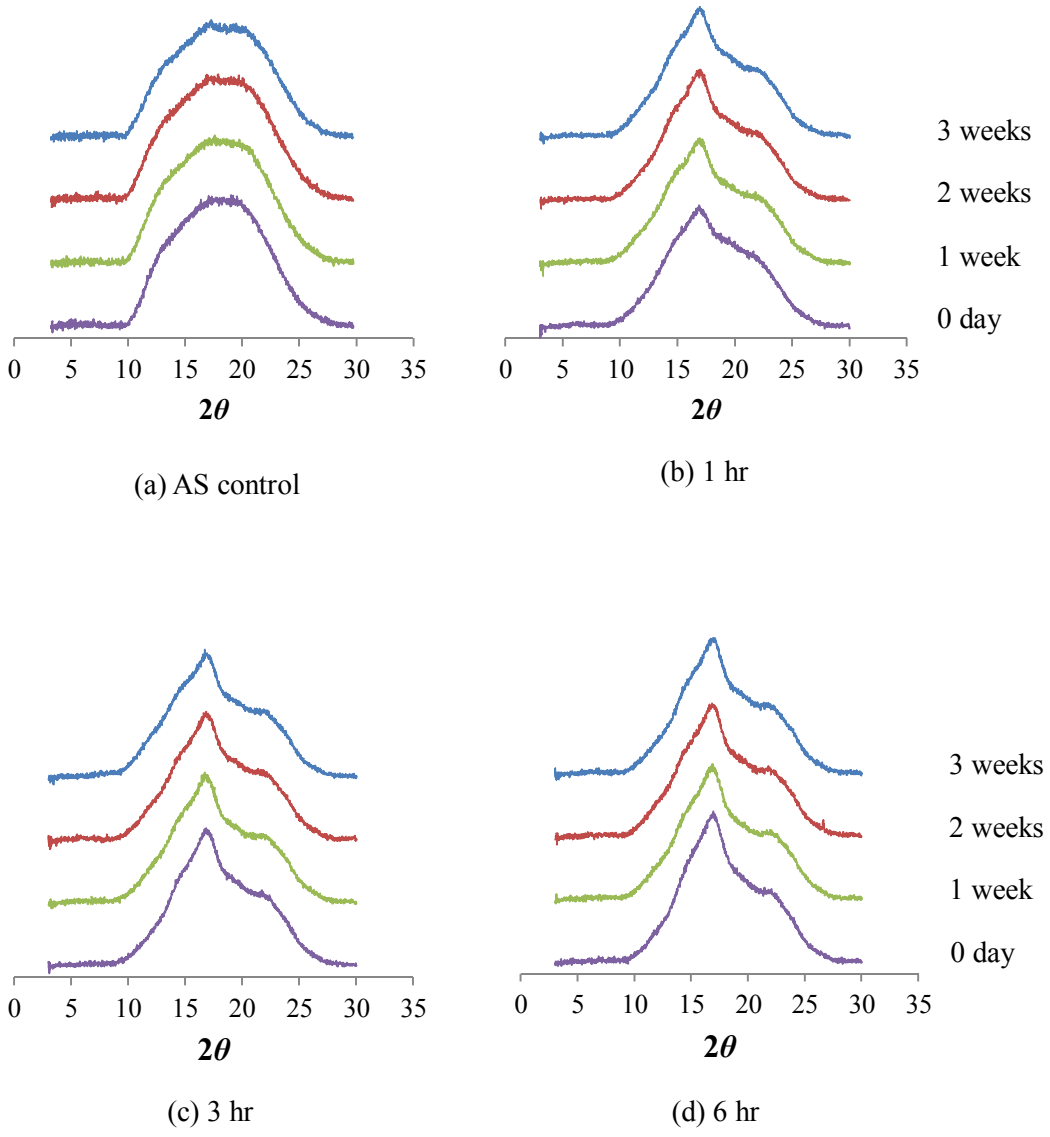

Figure 4. X-ray diffraction patterns of AS-treated starches after retrogradation (30°C)

Table 7. Relative crystallinity of AS-treated starches after retrogradation

	4°C			
	AS control[1)]	1 hr	3 hr	6 hr
0 day	15.6±0.5[a 2), 3)]	17.1±0.4[a]	18.2±0.2[b]	21.6±0.1[a]
1 week	16.0±0.5[a]	17.2±0.2[a]	18.7±0.2[a]	21.5±0.2[a]
2 weeks	15.7±0.5[a]	17.1±0.4[a]	18.2±0.2[b]	21.2±0.2[ab]
3 weeks	16.2±0.6[a]	17.1±0.3[a]	18.7±0.2[a]	21.0±0.2[b]
	30°C			
	AS control	1 hr	3 hr	6 hr
0 day	15.6±0.5[a]	17.1±0.4[b]	18.2±0.2[a]	21.6±0.1[a]
1 week	15.5±0.6[a]	17.4±0.1[ab]	18.1±0.2[a]	20.9±0.2[c]
2 weeks	15.4±0.5[a]	17.9±0.2[a]	18.3±0.2[a]	21.3±0.2[ab]
3 weeks	15.9±0.3[a]	17.8±0.2[a]	18.2±0.4[a]	21.0±0.1[bc]

[1)] AS control; no enzymatic treatment after gelatinization.

[2)] Data are expressed as average value and standard deviation.

[3)] The value with different superscripts in the same column are significantly different ($p<0.05$).

7. Starch digestibility of AS-treated starches after retrogradation

Table 8 and 9 show starch digestibility of the AS-treated starches after retrogradation at 4°C and 30°C, respectively. As stated above, starch digestibility was affected by the branch chain length and recrystallization by the AS-treatment.

After retrogradation at 4°C, only the AS control showed changes in the RDS fraction, while the other samples showed no significant differences. The RDS fraction decreased with increasing retrogradation time due to the formation of crystalline region. In the case of retrogradation at 30°C, the SDS fraction of 1 hr samples increased with increasing retrogradation time due to the formation of new double helices and recrystallization. These results can be related with thermal properties and relative crystallinity. Table 5 displays that the enthalpy of AS control increased with increasing retrogradation time due to the formation of new double helices. Table 7 shows that the relative crystallinity of retrograded 1 hr samples at 30°C slightly increased with increasing retrogradation time because of recrystallization, so did SDS fraction of 1 hr samples increase.

The retrogradation of amylopectin can be influenced by the time and temperature conditions of storage. At 4°C, nucleation is favored; at 30°C,

propagation and maturation is more preferred than nucleation. Therefore, the retrograded AS control at 4°C favored nucleation and showed an increase of enthalpy with increasing retrogradation time. The enthalpy is related to melting of imperfect crystals based on amylopectin, which contributes to both crystal packing and helix melting enthalpies (Lopez-Rubio, Flanagan, Gilbert, & Gidley, 2008). The retrograded 1 hr samples at 30°C favored propagation and maturation, supporting the assumption that the double helices formed by the AS treatmentfor 1 hr acted like critical nuclei formed by nucleation process. As a result, relative crystallinity increased with retrogradation time. Because elongated branch chain length of AS-treatment for 1 hr was not too long, 1 hr samples could form new double helices and increased the crystalline region regardless of limited dimensions of the chains.

Table 8. Starch digestibility of AS-treated starches after retrogradation (4°C)

	AS control[1)]			1 hr		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
0 day	84.8±0.3^{a} [2), 3)]	1.9±1.1^{a}	13.3±1.2^{a}	76.1±2.4^{a}	13.5±1.3^{a}	10.3±3.6^{a}
1 week	81.3±2.8^{ab}	5.0±1.8^{a}	13.7±3.5^{a}	75.0±4.3^{a}	17.7±4.7^{a}	7.3±1.8^{a}
2 weeks	79.7±2.5^{b}	6.9±3.8^{a}	13.4±3.1^{a}	76.1±1.2^{a}	12.2±3.4^{a}	11.7±3.9^{a}
3 weeks	79.9±0.8^{ab}	7.5±2.1^{a}	12.5±1.8^{a}	74.5±2.2^{a}	20.3±2.7^{a}	5.2±2.1^{a}
	3 hr			6 hr		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
0 day	57.2±1.6^{a}	34.6±3.5^{a}	8.2±3.3^{a}	44.5±4.5^{a}	34.9±4.0^{a}	20.5±3.0^{a}
1 week	60.9±3.4^{a}	27.6±2.5^{a}	11.5±1.9^{a}	47.2±1.9^{a}	34.1±0.8^{a}	18.7±1.8^{a}
2 weeks	59.8±1.2^{a}	27.1±1.7^{a}	13.2±2.9^{a}	48.5±2.5^{a}	32.2±1.1^{a}	19.3±1.5^{a}
3 weeks	60.3±3.3^{a}	27.9±4.3^{a}	11.8±1.2^{a}	49.1±1.9^{a}	30.5±1.5^{a}	20.3±1.0^{a}

[1)] AS control; no enzymatic treatment.

[2)] Data are expressed as average value and standard deviation.

[3)] The value with different superscripts in the same column are significantly different (p<0.05).

Table 9. Starch digestibility of AS-treated starches after retrogradation (30°C)

	AS control[1)]			1 hr		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
0 day	84.8±0.3[a 2), 3)]	1.9±1.1[a]	13.3±1.2[a]	76.1±2.4[a]	13.5±1.3[b]	10.3±3.6[a]
1 week	81.9±2.5[a]	6.7±3.1[a]	11.5±0.6[a]	72.7±2.5[a]	20.6±1.5[a]	6.6±2.2[a]
2 weeks	83.5±4.4[a]	3.6±0.8[a]	12.9±4.7[a]	73.3±0.9[a]	17.0±3.1[ab]	9.6±2.4[a]
3 weeks	82.1±1.5[a]	5.4±2.0[a]	12.6±2.1[a]	71.7±3.3[a]	20.8±3.8[a]	7.5±2.4[a]
	3 hr			6 hr		
	RDS (%)	SDS (%)	RS (%)	RDS (%)	SDS (%)	RS (%)
0 day	57.2±1.6[a]	34.6±3.5[a]	8.2±3.3[b]	44.5±4.5[a]	34.9±4.0[a]	20.5±3.0[a]
1 week	56.7±4.7[a]	25.9±2.6[b]	17.4±2.2[a]	46.6±1.9[a]	32.6±1.3[a]	20.8±1.3[a]
2 weeks	57.0±3.8[a]	26.8±4.4[ab]	16.3±0.7[a]	46.1±1.4[a]	32.2±2.4[a]	21.7±1.8[a]
3 weeks	56.3±1.6[a]	27.1±2.2[ab]	16.6±2.4[a]	46.2±0.7[a]	30.8±2.0[a]	23.0±1.4[a]

[1)] AS control; no enzymatic treatment.

[2)] Data are expressed as average value and standard deviation.

[3)] The value with different superscripts in the same column are significantly different ($p<0.05$).

CONCLUSION

In this study, waxy corn starch was treated with amylosucrase for 1, 3 and 6 hr and retrograded at 4°C and 30°C. The AS-treatment affected branch-chain length distribution, thermal properties, relative crystallinity, and *in vitro* digestibility. After the AS treatment, short chains decreased, while both medium chains and long chains increased with reaction time. The AS-treated starches showed broader endothermic peaks, and the enthalpy increased as the reaction time increased due to the elongated branch-chain length. These results could indicate that the AS-treatment formed stable and more perfect crystallites affecting the thermal properties. The X-ray diffraction pattern of waxy corn starch changed from A type to B type, and the relative crystallinity of AS-treated starch increased with increasing reaction time. In regard of *in vitro* digestibility, the SDS and RS contents increased, while the RDS content decreased.

After the retrogradation of AS-treated starches, only the melting enthalpy of 1 hr AS-treated sample retrograded at 4°C and 30°C increased by the retrogradation for 1 week. No differences in X-ray diffraction pattern were observed. However the relative crystallinity of 1 hr samples retrograded at 30°C increased slightly. After the retrogradation at 4°C, only the AS control

showed changes in RDS fraction, while at 30°C, the SDS fraction of 1 hr samples increased.

Thus, the chains which became too long by the AS-treatment could not form double helices because of the limited dimensions of the chains in the granule. Based on the recrystallization of 1 hr samples at 30°C, the AS-treatment might be a reaction to form double helices, similar as the nucleation to form nuclei. Further studies may focus on the modification of AS-treated starches with retrogradation at other conditions.

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국문 초록

Neisseria polysaccharea 유래의 amylosucrase (AS)는 수크로오스를 기질로 하여 α-(1, 4)-글루칸을 생산하는 효소로, 수크로오스의 분해로 생긴 글루코오스를 수용체 비환원성 말단에 붙여서 사슬을 연장시키는 반응을 일으킨다. 이 연구에서는 찰옥수수 전분에 시간대별로 AS 를 처리하여 사슬 길이를 다르게 한 후, 이를 각각 4℃ 와 30℃ 에서 노화시켜 그 특성을 살펴보았다.

전분 현탁액 (2%)에 AS 10,000 U 을 처리하고 30℃ 에서 1, 3, 6 시간 동안 반응시켜 사슬 길이가 다른 전분을 얻었다. 각각의 사슬 길이를 HPAEC-PAD 를 통해 측정하여 AS 처리 시간이 증가할수록 DP ≥ 25 의 긴 사슬들이 증가함을 확인하였다. 열적 특성 및 상대적 결정화도를 분석하여 AS 처리에 의해 형성된 긴 사슬들이 결정형 영역을 이룸을 확인하였다. *In vitro* 소화율 측정 결과, SDS 와 RS 가 증가하는 경향을 보였다.

이를 4℃ 와 30℃ 에서 노화시켜 그 특성 변화를 관찰하였다. 열적 특성, 상대적 결정화도, *in vitro* 소화율을 측정한 결과, AS 처리 시간이 3 시간, 6 시간인 전분은 큰 변화를 보이지 않았다. AS

1 시간 처리 전분의 경우, 4℃ 와 30℃ 에서 노화시킨 전분 모두 용융 엔탈피(ΔH)값이 증가하는 경향을 보였고, 상대적 결정화도와 *in vitro* 소화율의 경우에는 30℃ 에서 노화시킨 전분만이 변화하는 경향을 나타냈다.

이 실험을 통해, AS 처리 전분의 사슬 길이가 얼마나 연장되었는지에 따라 노화에 대한 영향이 다름을 알 수 있었다. 일정 이상으로 길이가 길어진 경우, 오히려 노화가 저해를 받는 결과를 나타냈다. 이는 사슬 길이와 전분 내부 공간의 제약이 찰옥수수 전분의 노화에 영향을 미치기 때문이라고 유추할 수 있다.

주요어 : 아밀로수크레이스, 찰옥수수 전분, 노화, 소화율, 사슬 길이

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