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A Thesis for the Degree of Master of Science

Structural and rheological properties of normal rice and potato starches with amylosucrase-treated starch added

아밀로수크레이즈 처리 전분을 첨가한 쌀과 감자 전분의 구조적 및 유변학적 특성

February, 2013

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농학석사학위논문

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위 원 이 기 원 인

A Thesis for the Degree of Master of Science

Structural and rheological properties of normal rice and potato starches with amylosucrase-treated starch added

by

Lim, Hye Jin

Advisor: Tae Wha Moon, Professor

Submitted in Partial Fulfillment of the Requirement
for the Degree of Master of Science

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Seoul National University

ABSTRACT

Rice and potato starches with low digestibility were prepared using amylosucrase(AS) from *Neisseria polysaccharea* and mixed with raw starches in different ratios (0%, 25%, 50%, 75%, and 100%). These mixtures were compeletely gelatinized and retrograded for 14 days at 4℃. The blending of unmodified raw starch and modified starch caused the changes of structural characteristics such as branch chain length distribution, X-ray diffraction and thermal properties, leading to the changes in digestibility and texture properties. The increase in the chains of DP ≥ 13 was observed in the AS-treated starches and starch mixtures, while the short chains of DP ≤ 12 decreased compared with raw starches.

Differential scanning calorimetry showed that the onset, peak and conclusion temperatures increased with an increase in the proportion of AS-treated starches. Especially, the enthalpy of starch mixtures was lower than that of native starch and laid between those of the respective raw and AS-treated starches, suggesting that the starch mixtures had a low tendency towards retrogradation compared with individual raw and AS-treated starches. After AS treatment, X-ray diffraction pattern of rice starch changed from A to B-type, whereas potato starch maintained the original B-type

pattern. All starch mixtures exhibited B-type pattern, and their relative crystallinity increased with an increase in the proportion of AS-treated starch.

The contents of SDS and RS were proportional to the amount of AS-treated starch. In texture analysis, the starch mixtures showed lower or similar gel hardness compared with the respective individual starches, unmodified starch and modified starch. The chewiness exhibited a similar tendency to by hardness and cohesiveness. Especially, chewiness and cohesiveness were related to microstructure of starch mixture gels. The samples with low values of cohesiveness and chewiness exhibited more loose structures and contained some empty spaces.

In summary, the starch mixtures showed different structural and rheological characteristics according to the amount of AS-treated starch added. These mixtures maintained low digestibility and reduced the rheological disadvantages of AS-treated starches.

Keywords: slowly digestible starch, amylosucrase, starch blend, digestibility, retrogradation, texture

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ABBREVIATIONS

AS: amylosucrase

AS 0 (25, 50, 75, and 100)% : starch mixtures with 0 (25, 50, 75, and 100)% amylosucrase-treated starches added

DP: degree of polymerization

short chain: the chains of DP ≤12

medium chain: the chains of DP 13-24

long chain: the chains of DP 25-36

very long chain: the chains of DP ≥37

RDS: rapidly digestible starch

SDS: slowly digestible starch

RS: resistant starch

CONTENTS

List of figures

List of tables

INTRODUCTION

Starch is a major carbohydrate source for humans. It consists of two polymers: amylose and amylopectin. Amylose is a linear molecular chain composed of α-$_D$-(1,4) linked glucose. Amylopectin is a highly branched polymer consisting of α-$_D$-(1,4) linked glucose segments with α-$_D$-(1,6) branch linkages (Sajilata et al., 2006).

The viscosity of starch increases when heated with water. After cooling, it forms gel, which is generally used as a food additive, and viscosity agent. It is referred to as retrogradation that the changes in texture by rearrangement of released amylose and crystallinization of amylopectin.

Rice is one of the primary crops in the world. More than 50% of the world's population depends on rice as their major carbohydrate source (FAO, 2001). Especially, rice starch is used as baby foods, soups, and bakery products due to its small-size granules and soft mouth feel (Mitchell, 2009).

Potato is also one of the important crops and widely used in food industry. Potato starch is preferred over other starches in food applications because it has a low gelatinization temperature, excellent flexible film formation and binding power (FAO, 1998). It has been reported that potato starch contains a high portion of resistant starch (Sajilata et al., 2006).

In aspect of the nutrition, starch is classified into three fractions, rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS), depending on the rate and extent of starch digestion *in vitro* (Englyst et al., 1992). RDS is digested rapidly in the small intestine, and increases the blood glucose level. SDS is digested slowly but completely in small intestine, inducing slow increase of blood glucose level. RS is the fraction that cannot be digested in small intestine, but is fermented in the large intestine and considered as dietary fiber. SDS and RS are called low digestible starches that have a resistance against enzymatic digestion in the small intestine (Zhang et al., 2006). They have physiological benefits such as a decrease in blood lipid level, and prevention of diabetes and insulin resistance (Shin et al., 2010). Therefore, increasing the contents of SDS and RS in foods is an interesting subject for the food industry.

To increase the fraction of low digestible starch, physical, chemical and enzymatic modifications have been employed. Among these methods, enzymatic modification is safer and can reduce by-products (Le et al., 2009). Karim et al. (2000) reported the preparation of RS from isolated pea starch using a thermally stable α-amylase. Recently, digestion-resistant starches with increased SDS and RS have been produced by the reaction of amylosucrase (Ryu et al., 2010).

Amylosucrase (EC 2. 4. 1. 4., AS) from *Neiserria polysaccharea,* one of

the glucoside hydrolases, has an unusual specificity for sucrose hydrolysis and synthesizes an amylose-like polymer (Jung et al., 2009). It elongates the non-reducing ends of amylopectin and amylose, and produces (1→4)-α-glucans using glucose from sucrose, while releasing fructose (Rolland-Sabaté et al., 2004). A previous study showed that amylosucrase-treated starch has longer branch chain length than raw starch (Shin et al., 2010). It could induce rearrangement of chains during reaction and decrease the susceptibility to digestive enzymes, resulting in higher SDS and RS contents. However, it is difficult to make processed food products by using only amylosucrase-treated starches, because they have decreased water holding capacity, reduced gelling properties, and are unstable to heat (Ryu et al., 2010; Shin et al., 2010).

Starch blending is used to improve the properties of starches without any chemical treatment. Morris (1990) reported that the mixing of starches retarded retrogradation. Ozcan et al. (2005) found that mixtures of extruded and raw corn starches could make new products with desirable functionality. Also, the mixture of chemically modified starch and high-amylose starch was used to make noodles with desired characteristics (Jane et al., 1999). Nevertheless, previous studies have reported only the behaviors of starch mixture of two raw starches. There are no studies about the properties of mixtures using enzymatically modified starches and unmodified starches.

In the present study, rice and potato starches were modified by amylosucrase, and then they were mixed with raw starches in different ratios. For perfect mixing, starches were gelatinized by autoclaving and recrystallization was induced by storing at 4℃ for 14 days. This step was similar to the gelatinization and retrogradation phenomena occurring in starch-based food process. Digestibility, physicochemical properties, and rheological properties of starch mixtures were examined.

MATERIALS AND METHODS

1. Materials

1-1. Starch

Rice starch (Sigma, St. Louis, MO, USA) and potato starch (KMC, Brande, Denmark) were used. Rice and potato starches contained 11.6% and 20.0% moisture, 0.55% and 0.10% protein, and 1.00% and 0.27% lipid (data from Sigma and KMC). Sucrose was purchased from Junsei Chemical (Tokyo, Japan).

1-2. Enzyme

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

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).

2. Methods

2-1. Assay of amylosucrase activity

The gene of amylosucrase from *Neiserria polysaccharea* was cloned and expressed in *Escherishia coli.* The amylosucrase was purified using the method of Jung et al. (2009) by affinity chromatography with Ni-NTA (nickel-nitrilotriacetic acid) resin (Quigen, Hombrechtikom, Switzerland).

Enzyme activity was measured using the method of van der Veen et al. (2004) with some modifications. The mixture (0.25 mL of 0.1 mM sodium citrate buffer (pH 7.0), 0.1 mL of 4% sucrose, 0.1 mL of 1% glycogen, and 0.05 mL of diluted enzyme) was reacted at 30℃ and 80 rpm for 10 min in a shaking water bath. The released fructose was quantified using the DNS (dinitrosalicylic acid) method (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 starches

Starch (2%, w/w) and 100 mM sucrose were suspended in 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℃. After then, amylosucrase (20,000U/30 mL) was added to the starch suspension and

incubated in a water bath at 30℃ for 24 hr. The reaction was terminated by adding three-fold ethanol to the suspension. The AS-treated starch was precipitated by centrifugation at 10,000 ×g for 10 min, and the pellet was washed three times with distilled water by centrifugation as before. The pellet was freeze-dried and ground to pass through a 100-mesh sieve.

2-3. Determination of amylopectin branch chain distribution by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

The branched chain length distribution of starches before and after amylosucrase treatment were determined by debranching the starches with isoamylase (Megazyme). Starch (15 mg) was dispersed in 90% dimethylsulfoxide (DMSO, 3 mL) and boiled for 20 min. Ethanol (15 mL) was added to the starch suspension and centrifuged at 10,000 ×g for 10 min to precipitate starch. Distilled water (1.5 mL) was added to pellet and boiled for 10 min. After boiling, 1.5 mL of 50 mM sodium acetate buffer (pH 4.3) and 30 μL of isoamylase were added, and the sample was reacted in a water bath at 45℃ and 50 rpm for 2 hr. To stop the reaction, samples were boiled for 10 min. 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 (Dionex). The sample was eluted with a gradient of 600 mM sodium acetate in 150 mM NaOH, and 150 mM NaOH was used for column equilibration with flow rate of 1 mL/min. The gradients of sodium acetate used were as follows : increasing from 0-20 % for 0-5 min, 21-45 % for 6-30 min, 46-55 % 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 the degree of polymerization (DP) from 1 to 7 were designated using a mixture of maltooligosaccharides (DP 1-7, Sigma Chemical) as standard. The peak areas was calculated using the PeakNet software (version 5.11, Dionex)

2-4. Starch digestibility

To analyze the starch digestibility of AS-treated starches retrograded for different periods, the AS-treated starches were stored for 1, 3, 5, 7, 14 and 21 days in a refrigerator at 4℃ and their *in vitro* digestibilities were determined.

Starch fractions were measured according to the method of Brumovsky and Thompson (2001) with a slight modification.

Pancreatin (2 g) was dissolved in distilled water (24 mL) and stirred well for 10 min. It was centrifuged at 1,500 ×g for 10 min, and 20 mL of supernatant was mixed with 0.4 mL of amyloglucosidase and 3.6 mL of distilled water. This enzyme solution was incubated at 37℃ for 10 min in a

water bath.

A sample (30 mg) and a glass ball were placed in a 2 mL-microtube and 0.75 mL of 100 mM sodium acetate buffer (pH 5.2) was added. After mixing each microtube, it was equilibrated in a shaking incubator (37℃, 240 rpm) for 10 min. Then, 0.75 mL of the enzyme solution was added to each microtube and the sample was reacted for 10 min and 240 min. The reaction was stopped at 10 min and 240 min after enzyme reaction by boiling for 10 min. The hydrolyzed glucose was obtained in the supernatant from centrifugation (5,000 ×g, 5 min) Glucose content was measured using a GOD-POD kit (BCS Corporation).

Starch fractions were classified based on the rate of hydrolysis. Rapidly digestible starch (RDS) was measured by the quantity of glucose after enzyme reaction for 10 min. Slowly digestible starch (SDS) was the fraction digested between 10 and 240 min. Resistant Starch (RS) was the undigested fraction after 240 min.

2-5. Preparation of starch mixtures

Raw starch and amylosucrase-treated starch were mixed at R.S:AS.S ratios 100:0, 75:25, 50:50, 25:75 and 0:100 to a final mass of 10 g. The mixtures were dispersed with 40 mL distilled water (20% solid content) and gelatinized using an autoclave at 121℃ for 30 min to mix completely. After

then, the starch gels were cooled to room temperature, hermetically sealed, and then stored at 4℃ in a refrigerator for 14 days. The samples were then freeze-dried, ground and passed through a 150- μm sieve.

2-6. X-ray diffraction patterns and relative crystallinity

X-ray diffraction analysis was performed using a powder X-ray diffractometer (Model New D8 Advance, Bruker, Karlsruhe, Germany) analyzing at 40 kV and 40 mA. Starch sample scan was conducted 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 crystallinity was determined using the equation below.

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

2-7. Thermal properties determined by differential scanning calorimetry (DSC)

Thermal properties of raw starches, amylosucrase-treated starches and starch mixtures were investigated using a differential scanning calorimeter (Diamond DSC, Perkin-Elmer, Waltham, MA, USA). Each sample (10 mg)

was weighed in a hermetic aluminum pan (Seiko, Tokyo, Japan), and 40 μL of distilled water was added. The sample pans were sealed and kept at room temperature overnight for equilibrium. DSC scan was made as the samples were heated from 30℃ to 180℃ at 5℃/min. An empty aluminum pan was used as a reference.

2-8. Texture analysis

Gel texture was evaluated at room temperature using the Texture Profile Analysis (TPA) procedure, two bite test. The starch (4 g) was mixed with distilled water (15 mL) and poured into a cyrindrical bottle (30 mm diameter and 10 mm height). This paste was gelatinized in an autoclave for 30 min and stabilized at room temperature for 30 min. The tests were performed on starch gels (during storage period from 0 to 14 days at 4℃) using a Texture Analyzer (TA-XT plus, Stable Micro System, Godalming, UK.) at a constant speed of 1.7 mm/s with a strain of 40%. The gels were compressed twice with a cylinder probe, 20 mm in diameter.

From the force-time curve of the texture profile, textural parameters including hardness, adhesiveness, springiness, cohesiveness, and chewiness were determined. Hardness is the height of the first peak, and adhesiveness is the negative area for the first bite. Springiness or elasticity is the ratio between recovered height after the first bite and the original gel height.

Cohesiveness is the ratio between the positive area of the second peak and the positive area of the first peak. Chewiness is the product of hardness, cohesiveness, and springiness. These parmeters were calculated using the equations of Bourne (1968) and Peleg (1976).

2-9. Energy-filtering transmission electron microscopy (EFTEM)

Microstructures of starch mixture gels were analyzed using Energy-filtering transmission electron microscopy. Gels were fixed in 0.05 M sodium cacodylate buffer (pH 7.2) containing 2% paraformaldehyde and 2% glutaraldehyde at 4℃ for 2 h and washed three times in 0.05 M sodium cacodylate buffer (pH 7.2) at 4℃ for each 10 min each. Then post-fixation was performed using 0.05 M sodium cacodylate buffer (pH 7.2) containing 1% osmium tetroxide at 4℃ for 2 h. Samples were washed two times at room temperature using distilled water. En bloc staining was then performed in 0.5% uranyl acetate at 4℃ for 30 min. Ethanol dehydration was done using a series of solutions of increasing ethanol concentration. Transition was conducted using 100% propylene oxide two times at room temperature for 10 min. Each sample was embedded using a mixture of Epon resin and propylene oxide. For polymerization, samples were placed in the oven at 70℃ for 24 h. Then samples were sectioned with an ultramicrotome (MT-X,

RMC, Tucson, AZ, USA) and stained with 2% uranyl acetate and Reynolds' lead citrate. Specimens were observed with an energy-filtering transmission electron microscope (LIBRA 120, Carl Zeiss, German).

2-10. Statistical analysis

All the experiments were done in triplicate, and data were expressed as mean±standard deviation. Mean separations was performed by the Duncan's multiple range 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. *In vitro* starch digestibility of AS-treated starches affected by storage time

As shown in Figure 1, the digestibilities of the AS-treated starches were significantly influenced by the storage time. Generally, as the retrogradation period passed, the RDS content decreased and the SDS increased. However, the RS content did not show a notable change during retrogradation.

In case of rice starch, the RDS and SDS contents changed dramatically between 7 and 14 days, and were maintained after 14 days. However, in potato starch, the RDS content decreased gradually, while the SDS content revealed a slightly increase after 5 days. After 14 days, any significant changes were not observed. Leman et al. (2005) reported that starch retrogradation is a process of getting ordered structure of gelatinized starch (amorphous region) by reassociation of starch molecules. This phenomenon may be affected by starch concentration, storage temperature, and starch source and so on (Orford et al., 1987; Slade et al., 1987). In general, long-linear amylose molecules have a greater tendency to reassociate than amylopectin molecules do.

The digestibilities of samples stored for 14 and 21 days showed no

significant differences (p>0.05). On the basis of these results, retrogradation period of starch mixtures was set 14 days that showed the highest content of low digestible starches.

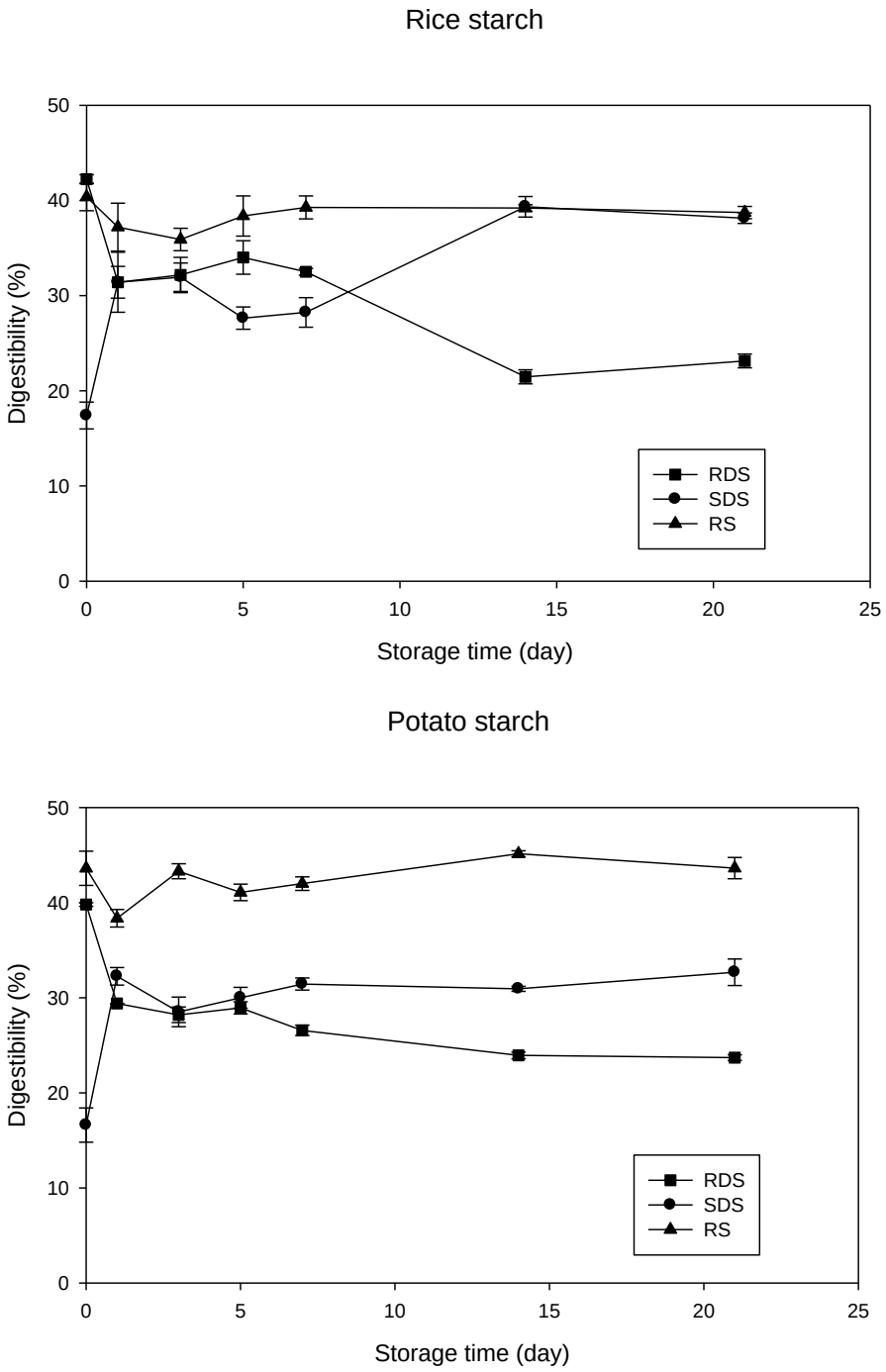

Figure 1. ***In vitro*** **starch digestibility patterns of AS-treated starches by storage time**

Table 1. *In vitro* starch digestibility of AS-treated starches according to storage time

Samples	Rice			Potato		
	RDS(%)	SDS(%)	RS(%)	RDS(%)	SDS(%)	RS(%)
AS 0d[1)]	42.2±0.5[a2),3)]	17.4±1.4[d]	40.4±1.4[a]	39.8±0.2[a]	16.6±1.8[d]	43.6±1.8[ab]
AS 1d	31.4±1.7[c]	31.4±3.2[b]	37.2±2.5[c]	29.4±0.0[b]	32.3±0.9[ab]	38.3±0.9[d]
AS 3d	32.2±1.9[bc]	31.9±1.5[b]	35.9±1.2[d]	28.2±0.8[c]	28.5±1.6[c]	43.3±0.8[ab]
AS 5d	34.0±1.8[b]	27.6±1.2[c]	38.4±2.1[abc]	28.9±0.6[bc]	30.0±1.1[bc]	41.1±0.9[c]
AS 7d	32.5±0.4[bc]	28.2±1.6[c]	39.3±1.2[ab]	26.6±0.6[d]	31.4±0.6[ab]	42.0±0.7[bc]
AS 14d	21.5±0.7[d]	39.3±1.1[a]	39.2±0.3[ab]	23.9±0.4[e]	30.9±0.3[ab]	45.1±0.3[a]
AS 21d	23.1±0.7[d]	38.1±0.6[a]	38.7±0.6[abc]	23.7±0.3[e]	32.7±1.4[a]	43.6±1.1[ab]

[1)] AS-treated starches retrograded for 0 day

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

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

2. Branch chain length distributions of raw and AS-treated starches

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 the degree of polymerization (Hanashiro et al., 1996). Figure 2 and 3 show the branch chain length distributions of raw, AS-treated starches, and retrograded AS-treated starches analyzed by HPAEC-PAD. The relative percentages of peak area with degree of polymerization (DP) are summarized in Table 2 and 3.

Rice starch, an A type starch, had a relatively larger proportion of short A chains and smaller proportions of long B1, B2 and B3 chains than potato starch, a B type starch,. These results were in agreement with the previous report by Jane et al. (1999). In general, the A type staches such as rice starch have relatively plentiful short A chains, but small quantity of long B chains (Zhang et al., 2006).

After the AS treatment, the proportion of short A chains (DP ≤ 12) of all AS-treated starches decreased. On the other hand, the proportions of long B1, B2 and B3 (DP 13-24, DP 25-36 and DP ≥ 37) chains increased than those of raw starches. Shin et al. (2010) stated that AS treatment caused the increase

in the branch chain length of amylopectin and the decrease in the proportion of short chains, resulting from the elongation of external chains at non-reducing end of an acceptor molecule by amylosucrase (Bertolini et al., 2005). Therefore, the maximum DP was shifted to right compared with that of raw starches. The highest detectable DP of AS-treated starches increased by about DP 15 than those of raw starches. Elongated external branch chains could form the double helices which inhibit the access of hydrolytic enzymes (Shin et al., 2010). Thus the contents of SDS and RS of AS-treated starches could be increased compared with the raw starches.

Rice and potato starch mixtures showed similar results. The proportions of A chain (DP $\leq$ 12) were not significantly different between AS 25% and AS 50% samples. However, these values were much decreased as the amount of AS-treated starches exceeded the half of the whole. The proportions of B1 chain (DP 13-24) were the highest in AS 25% samples of both rice and potato. In conclusion, as the amount of AS-treated starches increased, the proportions of short A chains (DP $\leq$ 12) decreased, while those of long B2 (DP 25-36) and B3 (DP $\geq$ 37) increased. These results were related to that the AS-treated starches had more long chains than the raw starches did due to the elongation of external chains by amylosucrase.

The branch chain length distributions of retrograded AS-treated starches (AS.r 100% and AS.p 100%) were not significantly different compared with

those of AS-treated starches. When the starch is gelatinized at a high temperature, the disruption of molecular orders within the starch granule and the breaking of branch chains occur (Dreher et al., 1984). However, the branch chain distributions of AS-treated starches were not changed after recrystallization. This result suggested that the branch chain elongation by amylosucrase was not influenced by gelatinization and retrogradation process.

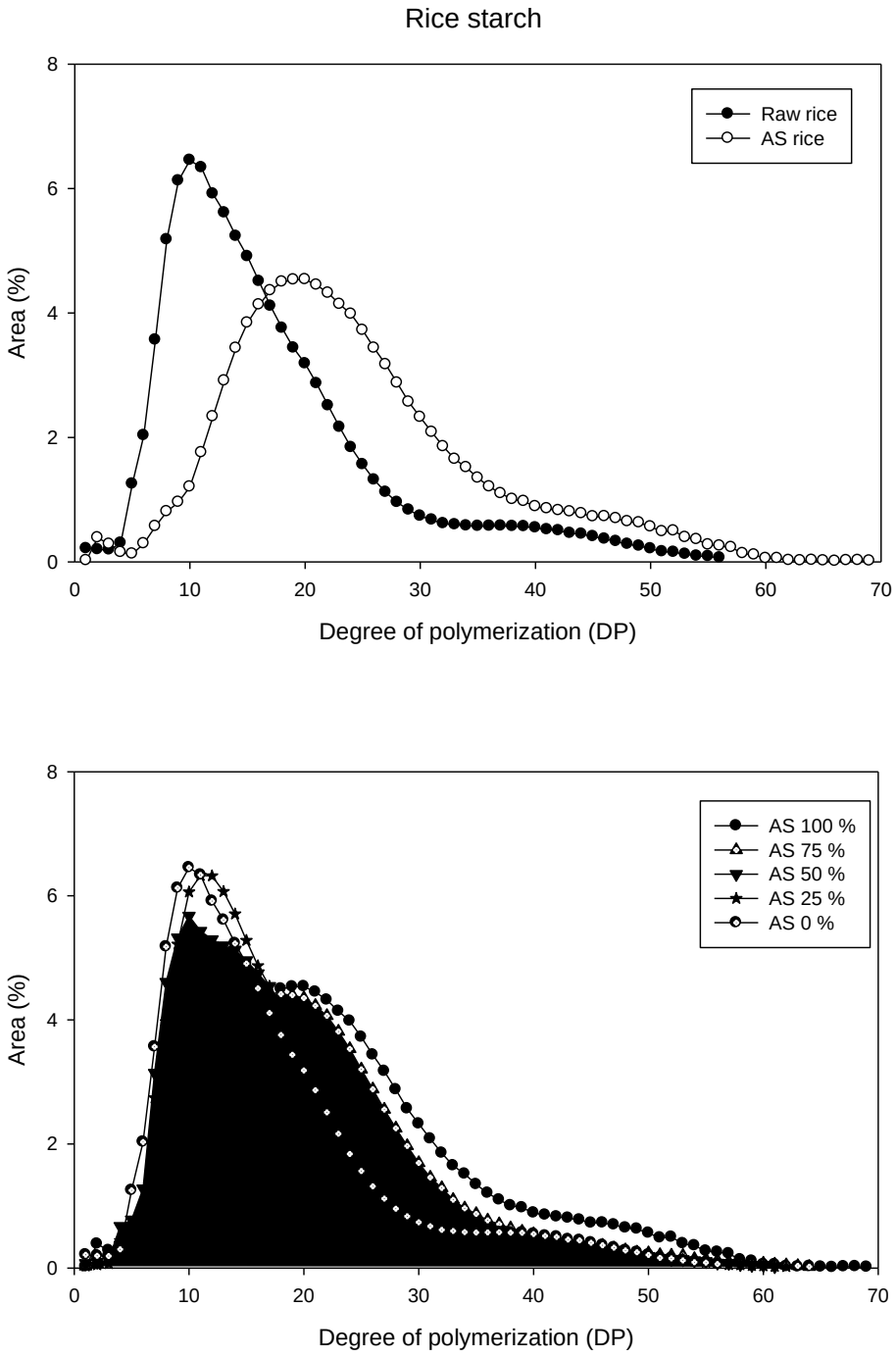

Figure 2. Branch chain length distributions of rice starch mixtures

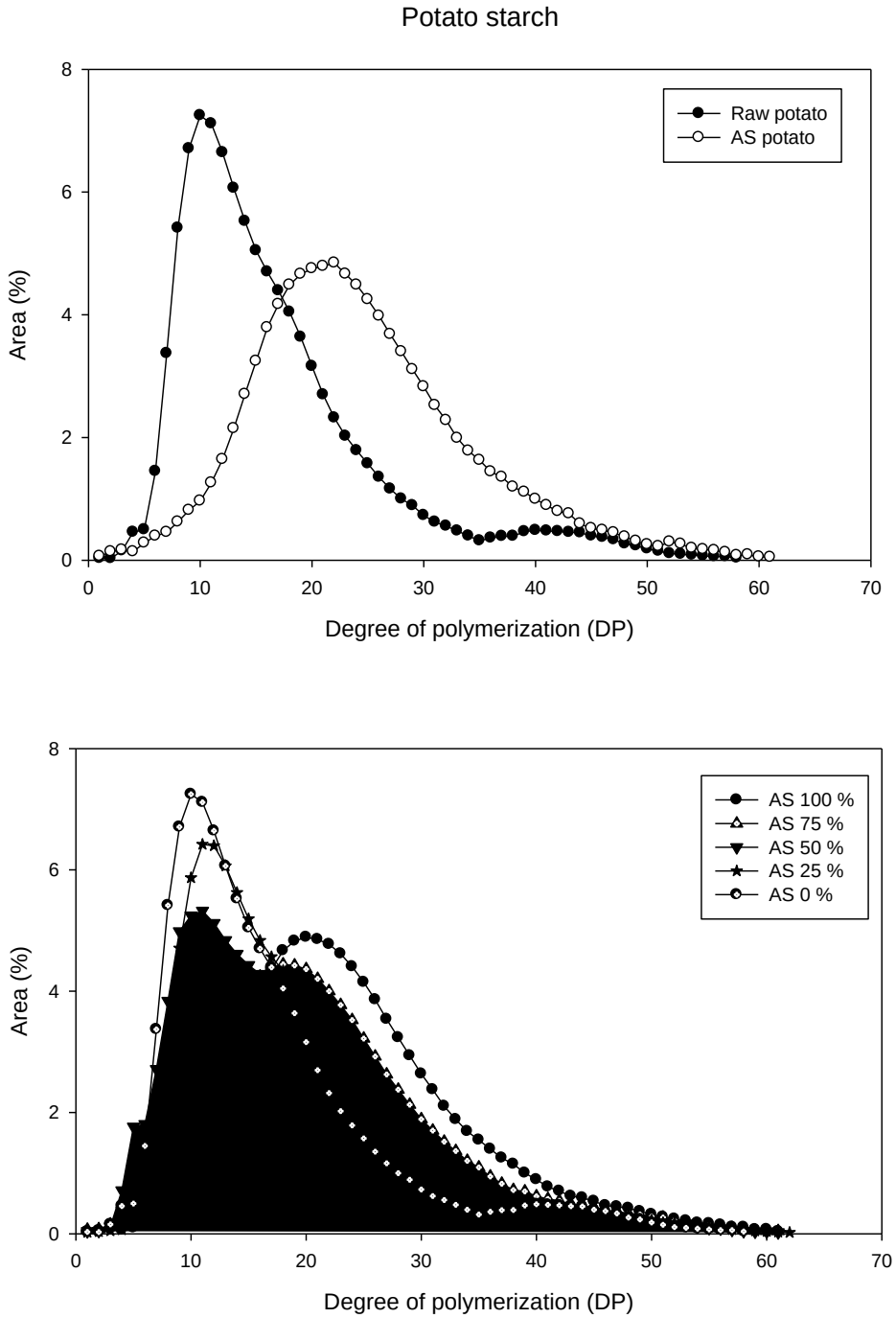

Figure 3. Branch chain length distributions of potato starch mixtures

Table 2. Percent distributions of branch chain length of rice starch mixtures

Samples	Percent distribution			
	DP [1)]6-12	DP 13-24	DP 25-36	DP ≥ 37
Raw rice	46.8±2.9[a,2),3)]	39.3±1.6[c]	7.9±1.5[e]	6.3±0.8[bc]
AS rice	8.7±0.5[d]	49.0±1.0[b]	26.8±0.0[a]	15.3±1.4[a]
AS 0%[4)]	46.1±2.5[a]	39.9±2.5[c]	7.7±1.2[e]	6.3±0.9[bc]
AS 25%	29.6±0.9[b]	52.3±1.2[a]	12.5±2.0[d]	5.6±0.4[c]
AS 50%	28.7±2.7[b]	49.0±1.3[b]	16.2±1.5[c]	6.1±0.3[bc]
AS 75%	23.0±2.2[c]	49.6±0.2[ab]	20.4±1.1[b]	7.0±0.2[b]
AS 100%	8.9±0.3[d]	49.1±0.8[b]	26.6±0.1[a]	15.4±0.4[a]

[1)] DP, degree of polymerization.

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

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

[4)] The starch mixture contained 0% of AS-treated starch.

Table 3. Percent distributions of branch chain length of potato starch mixtures

Samples	Percent distribution			
	DP [1)]6-12	DP 13-24	DP 25-36	DP ≥ 37
Raw potato	39.1±1.8[a 2),3)]	45.4±3.7[b]	9.1±0.9[e]	6.4±0.2[bc]
AS potato	7.4±3.6[d]	50.2±4.4[a]	29.9±3.4[a]	12.5± 2.5[a]
AS 0%[4)]	41.4±0.0[a]	47.2±1.1[ab]	8.2±0.9[e]	6.2±0.3[c]
AS 25%	29.4±2.0[b]	50.3±0.9[a]	14.0±0.2[d]	6.3±0.4[c]
AS 50%	28.5±1.6[b]	47.8±0.4[ab]	17.2±0.4[c]	6.5±0.1[bc]
AS 75%	19.7±0.9[c]	48.9±0.2[ab]	23.0±0.8[b]	8.4±0.2[b]
AS 100%	6.9±1.7[d]	48.7±2.7[ab]	31.4±2.3[a]	13.0±2.1[a]

[1)] DP, degree of polymerization.

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

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

[4)] The starch mixture contained 0% of AS-treated starch.

3. Thermal properties

The thermal properties of starches was determined using differential scanning calorimetry (DSC) (Sahai et al., 1999). The gelatinization onset (T_o), peak (T_p) and conclusion (T_c) temperatures and melting enthalpy (ΔH) are shown in Table 4 and 5.

The peak temperature (T_p) indicates structural stability. The onset temperature(T_o) and conclusion temperature(T_c) are related with melting of the weakest crystallites and strongest crystallites, respectively (Barichello et al., 1990; Biliaderis et al., 1980).

Raw rice and potato starches showed typical endothermic peaks in the range from 57.1℃ to 67.2℃ and from 59.1℃ to 69.2℃, respectively, which was similar with a previous study (Shin et al., 2010). In the AS-treated rice and potato starches, ΔH slightly decreased, whereas T_o, T_p and T_c increased. The increased gelatinization temperature indicated that longer chain was related with retrogradation. Cho et al. (2009) and Shin et al. (2010) suggested that the enzyme-treated starch shows a higher gelatinization temperature and wider endothermic peak compared with that of raw starch because of the retrogradation.

All starch mixtures showed shallower and broader endothermic peaks compared with that of raw starches. As the content of AS-treated starch increased, thermal transition parameters (T_o, T_p, and T_c) increased (Table 4 and 5). Retrograded starch mixtures had the thermal transition temperature ranges from approximately 50℃ to 90℃. According to a previous study, thermal transition parameters are associated with the melting of short-chain double helix structures (Gidley et al., 1995). Srichuwong et al. (2007) also stated that short A chains might require more less energy to melt because short A chains of amylopectin are too short to form double helices. That is, the existence of many short A chains of amylopectin (DP 6-12) induces the low T_o, T_p, and T_c (Noda et al., 1998). As shown before (Table 2 and 3), the mixtures containing a small quantity of AS-treated starches had many short A chains and showed low T_o, T_p and T_c.

Gelatinization enthalpy of retrograded starch mixtures was lower than those of raw starches. It could be explained though a partial crystalline polymer structure is rearranged during retrogradation, the original molecular order is not regained perfectly (Cameron et al., 1991). It might result from improper arrangement of the starch chains during reassociation forming less ordered or less stable crystalline structures than those existing in raw starches (Srichuwong et al., 2005).

Although the T_o, T_p and T_c were positively correlated with the amount of the AS-treated starches, there were no correlation between ΔH and the amount of AS-treated starches. The melting enthalpies of AS 25%, AS 50%, and AS 75% lay between the enthalpy of AS 0% and that of AS 100%. This results were in agreement with a previous studies (Gunaratne et al., 2007). Srichuwong et al. (2005) also reported a result similar to this study, suggesting that it might be due to differences in the total content of double helices which are concerned with the complexity of molecular order. Furthermore, Obanni et al. (1997) stated that low enthalpy of starch blends means low tendency to retrogradation. This result could be explained by the insufficient amounts of amylose to recrystallization due to the association of amylose molecules in starch blends with amylopectin.

The DSC gelatinization endotherm provides a crystallite quality (double helix length) from peak temperature (T_p) and overall crystallinity (quality and quantity) from melting enthalpy (ΔH) (Richard et al., 1990). Moreover, McPherson et al. (1999) demonstrated that the variations in ΔH represent differences in bonding forces between the double helices in the amylopectin crystallites, resulting in different alignment of hydrogen bonds within starch molecules. Consequently, ΔH might be affected by not only the amount of double helices but also the degree of molecular orderness.

In case of the rice starch mixtures, AS 0%, AS 25% and AS 50% showed

the second peak around 99℃, indicating the melting of amylose-lipid complexes. An additional peak at 20° was also observed in x-ray diffraction, corresponding to a V-type pattern. Type 1 amylose-lipid complex melts at 100 ± 15℃, and type 2 complex, crystalline, has a higher transition temperature compared with amorphous type 1 complex (Biliaderis et al., 1985; Biliaderis et al., 1989; Eliasson, 1986)

Generally, normal cereal starches contain approximately 1.00% lipids. Starches with endogenous lipids, such as cereal starches, show an additional melting transition which is attributed to the melting of amylose-lipid complexes (McKenna, 2003). Normal rice starch used in this study has more lipids than that of potato starch, and therefore, the amylose-lipid complex melting was observed only in rice starch mixtures. Furthermore, potato starch naturally contains phosphate groups and the absence of endogenous amylose-lipid complexes are associated with strong swelling capacity of potato starch (McKenna, 2003).

AS 75% and AS 100% samples showed the second peak at around 160℃ and 155℃ in rice and potato starches, respectively. This result could be derived from the interaction among the amylose-like long chains elongated by AS. These samples had more long chains (Table 2 and 3) than those of the other samples, which could contribute to new molecular associations amongst amylose chains during retrogradation. The crystalline regions

composed of amylose fractions show an endothermic peak at 145℃ to 153℃ (Sievert et al., 2006).

Hoover et al. (1996) described that the first peaks indicate the melting of amylopectin crystallites formed by aggregation between adjacent double helices during retrogradation and the second peaks mean the melting of the complex within elongated long linear chains.

Table 4. DSC parameters of rice starch mixtures

Samples	Peak 1				Peak 2			
	T_o (℃)[1]	T_p (℃)	T_c (℃)	ΔH (J/g)[2]	T_o (℃)	T_p (℃)	T_c (℃)	ΔH (J/g)
Raw rice	57.1±0.0[d6),7)]	63.0±0.1[c]	67.2±0.2[c]	14.2±1.4[a]	89.3±0.9[e]	96.9±1.7[c]	102.2±0.3[c]	1.6±0.0[c]
AS rice[4)]	83.2±3.0[b]	94.6±0.5[a]	107.6±4.3[a]	13.0±1.8[a]			N.D[3)]	
AS.r 0%[5)]	47.3±1.0[e]	53.7±0.6[e]	60.4±0.0[d]	6.5.±0.9[b]	93.5±0.1[d]	99.0±0.1[c]	103.2±0.3[c]	1.9±0.2[c]
AS.r 25%	47.3±0.4[e]	54.4±0.2[d]	60.5±0.1[d]	3.5±0.3[c]	94.8±1.3[cd]	99.1±0.6[c]	103.7±0.3[c]	1.5±0.3[c]
AS.r 50%	48.3±0.1[e]	54.5±0.3[d]	59.9±0.6[d]	2.9±0.7[c]	95.4±0.4[c]	98.4±0.0[c]	103.2±1.9[c]	0.9±0.4[d]
AS.r 75%	79.7±0.2[c]	89.8±0.2[b]	100.9±0.1[b]	5.6±0.5[b]	150.3±0.1[b]	157.4±0.8[b]	164.9±1.8[b]	2.4±0.2[b]
AS.r 100%	92.0±0.3[a]	95.1±0.0[a]	104.8±0.2[a]	6.4±0.8[b]	155.8±1.0[a]	163.5±0.8[a]	178.4±2.7[a]	2.9±0.2[a]

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

[2)] ΔH indicates the melting enthalpy.

[3)] Not detected

[4)] AS rice; AS-treated rice starch

[5)] The starch mixture containing 0% of AS-treated starch.

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

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

Table 5. DSC parameters of potato starch mixtures

Samples	Peak 1				Peak 2			
	T_o (℃)[1]	T_p (℃)	T_c (℃)	ΔH (J/g)[2]	T_o (℃)	T_p (℃)	T_c (℃)	ΔH (J/g)
Raw potato	59.1±0.0[d6),7)]	62.6±0.3[d]	69.2±0.6[d]	27.1±1.6[a]			N.D[3)]	
AS potato[4)]	75.9±0.7[c]	85.2±0.1[c]	97.9±0.7[c]	6.9±0.6[c]			N.D	
AS.p 0%[5)]	51.3±0.4[e]	59.4±0.5[e]	67.6±0.8[e]	6.9±0.1[c]			N.D	
AS.p 25%	52.6±1.9[e]	59.8±1.5[e]	67.7±0.8[e]	4.3±0.2[d]			N.D	
AS.p 50%	52.6±0.8[e]	59.5±0.2[e]	68.7±0.2[de]	3.3±0.5[d]			N.D	
AS.p 75%	81.9±0.9[b]	90.2±0.4[b]	100.5±1.0[b]	6.9±0.8[c]	140.9±0.5[b]	154.7±0.1[a]	163.2±0.7[b]	9.3±1.0[a]
AS.p 100%	90.2±0.2[a]	95.2±0.5[a]	108.1±0.3[a]	8.7±0.2[b]	144.6±1.1[a]	155.6±1.7[a]	164.9±1.1[a]	11.4±1.9[a]

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

[2)] ΔH indicates the melting enthalpy.

[3)] Not detected

[4)] AS potato; AS-treated potato starch

[5)] The starch mixture containing 0% of AS-treated starch.

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

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

4. X-ray diffraction patterns and relative crystallinity

The X-ray diffraction patterns and relative crystallinities of the raw, AS-treated starches and starch mixtures are shown in Figure 4 and 5 and Table 6 and 7, respectively. The raw rice starch showed strong peaks at 15.1°, 17.1°, 18.0°, and 22.9°, and weaker peaks at 10.1°, 20.0° and 26.6°. The raw potato starch showed strong peaks at 5.5°, 17.1° and 22.1°, and weaker peaks at 15.0°, 19.7°, 24.1° and 26°. These values corresponded to those of a typical A type and a B type pattern, respectvely (Hanashiro et al., 1996; Hizukuri et al., 1980; Shin et al., 2010).In general, cereal starches are referred to as A type, whereas tuber starches tend to show a B type pattern (Hoover et al., 1985). After AS treatment, the X-ray diffraction patterns of starches changed to a weak B type, showing strong peaks at 17.0°, 20.0°, and 22.1°. Shamai et al. (2003) reported that the peak at 5.5° in B type originated from the raw tuber starch, and therefore this peak was not shown in recrystallized B type crystalline structure. By the AS reaction, the X-ray diffraction pattern of rice starch changed from A type to B type, while potato starch showed a similar pattern. However, the peak intensity of AS-treated potato starch was lower than that of raw potato starch. These changes were related to the elongation of branch chain length and the retrogradation during enzyme reaction at

30℃ for 24 hr. Kim et al. (2009) reported that retrograded starch showed a B type pattern, which was induced from the regions composed of double helices in a hexagonal structure. Whether starch has an A or B type pattern in the nature, formed starch gels formed develop a B type pattern on retrogradation (Katz et al., 1934). It has been known that short chains of amylopectin induce an A type pattern, and long chains of amylopectin are involved in the formation of a B type pattern (Pohu et al., 2004). Long branched chains in the AS-treated starches could act like long amylose (Rolland-Sabaté et al., 2004). These amylose-like long chains could contribute to the formation of B type pattern.

The starch mixtures also showed a weak B type pattern, influenced by the retrogradation during 14 day storage at 4℃. The peak intensity increased slightly with an increase in the portion of AS-treated starches. Rice starch mixtures exhibited a peak at 20°, a general pattern shown by the presence of an amylose–lipid complex (Bultosa et al., 2003). However, this peak disappeared as the portion of AS-treated starches increased, i.e., that of rice starch containing free lipid decreased.

The relative crystallinity of the starches could be influenced by 1) crystal size, 2) amylopectin chain length, 3) extent of interaction between double helices and 4) orientation of the double helices within the crystalline domains (Hoover et al., 2002). The raw rice starch exhibited a higher relative

crystallinity than that of potato starch (Table 6). Generally, relative crystallinity for the A type starch is higher than those for B and C type starches. It is caused by the cereal starches (A type starches) being more densely packed in helical structures and also the amylopectin of A type starches containing a higher portion of shorter branched chains than B type starches do (Shamai et al., 2003; Srichuwong et al., 2005). As shown in Table 2, rice starch has more short chains (DP $\leq$ 12) than potato starch does, whereas potato starch has more intermediate and long chains.

The relative crystallinity of AS-treated starches decreased compared with that of raw starches due to the crystalline disruption during the gelatinization before the enzymatic treatment. The elongated branch chains formed double helices, which could contribute to the crystalline structure in the AS-treated starch. In general, the long chain has more tendency to rearrange, and these double helices might increase the relative crystallinity (Mua et al., 1998). Thus, the relative crystallinity increased as the portion of AS-treated starch increased.

In the case of AS 25% and AS 50% samples, the relative crystallinity of these samples tended to increase according to the portion of the AS-treated starches, but there was no significant difference between AS 25% and AS 50%. However, the samples with more than half of AS-treated starch showed significantly higher relative crystallinities compared with the AS-treated

starch samples. Especially, the AS 100% sample showed a much higher relative crystallinity compared with the other samples, indicating that its crystalline structure was more densely packed than that of the other samples due to enhanced retrogradation.

Consequently, the extended chains by AS treatment were more favored to form the crystallites. The elongation of branch chain length and retrogradation induced the increase of the relative crystallinity of starch mixtures resulting in the increases in the fraction of low digestible starches (SDS and RS) and gelatinization temperature.

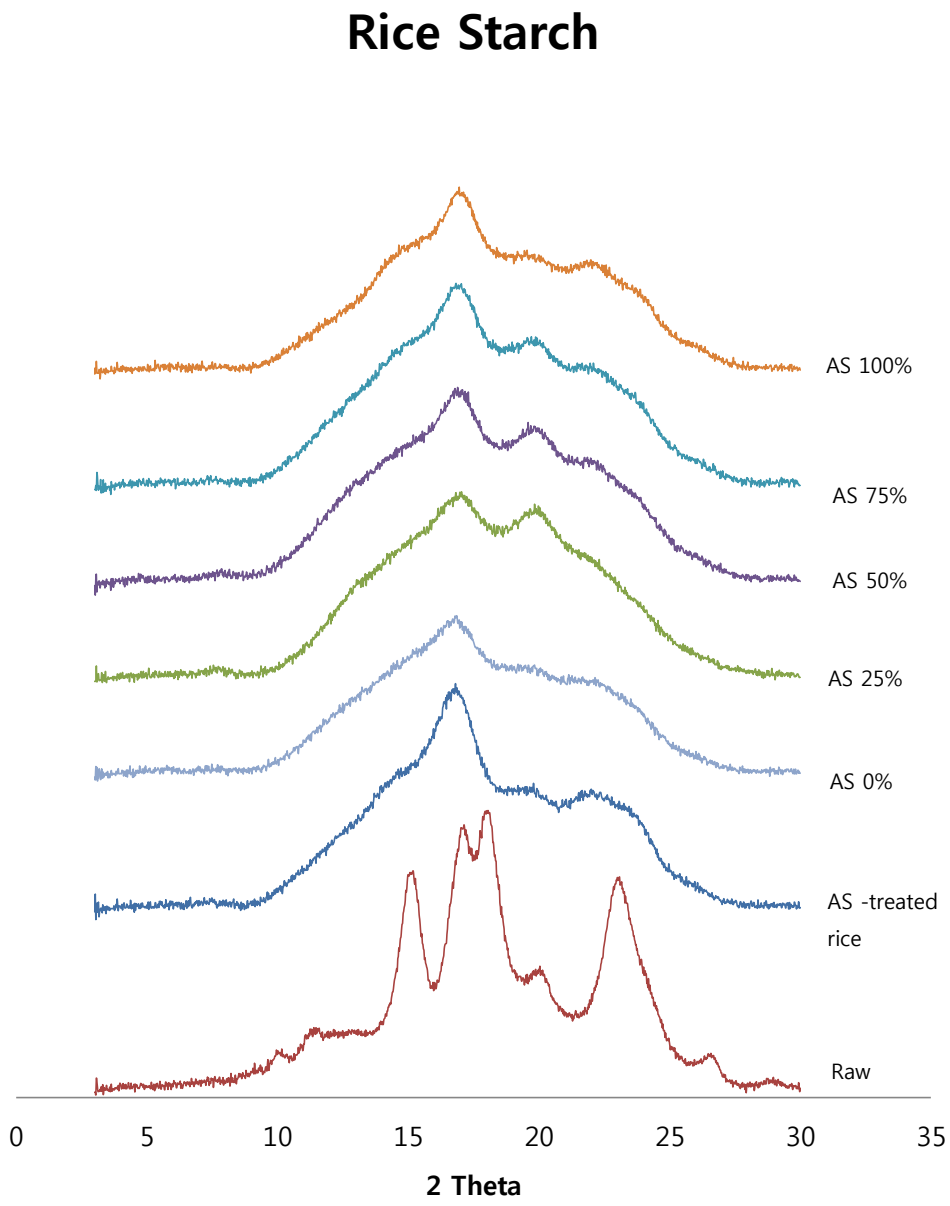

Figure 4. X-ray diffraction patterns of rice starch mixtures

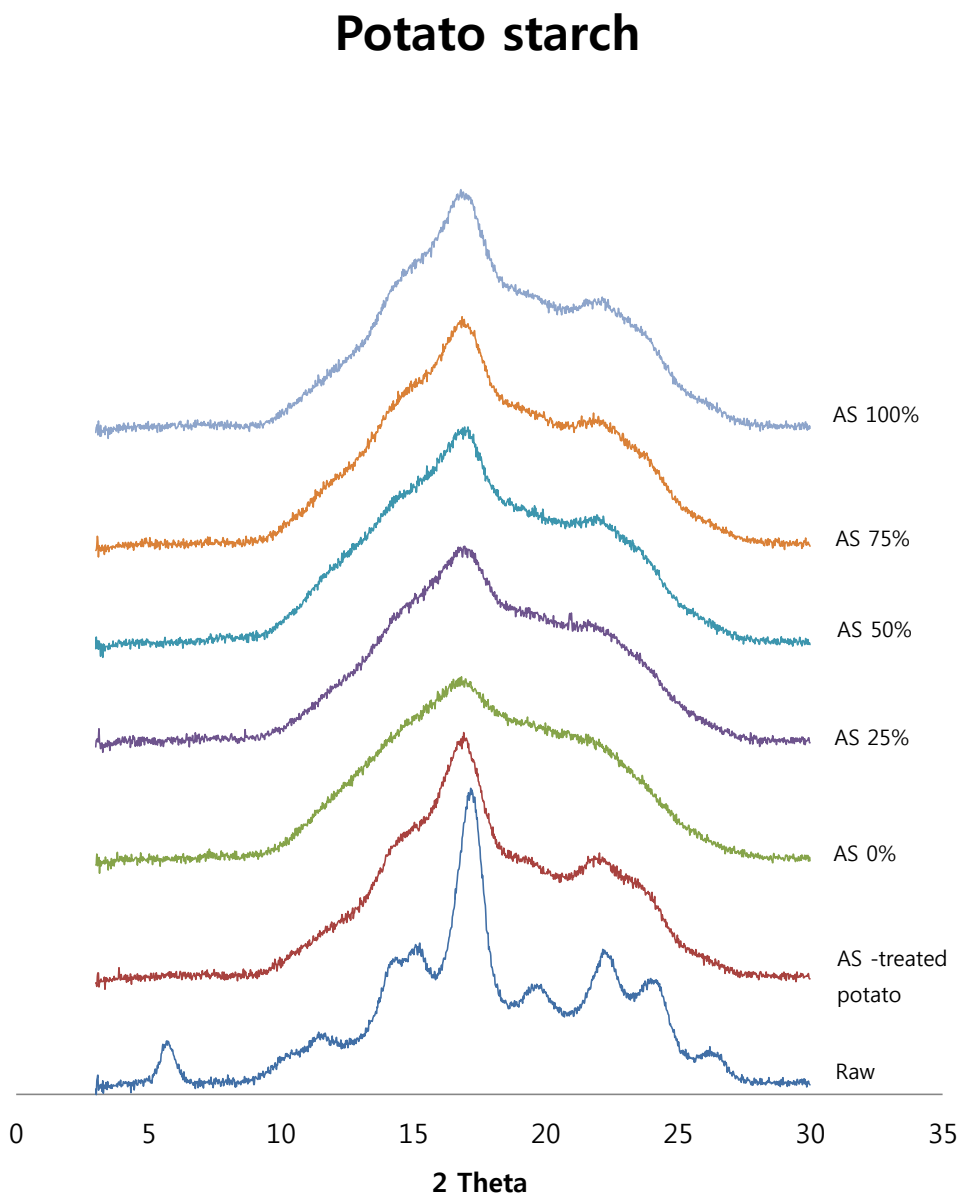

Figure 5. X-ray diffraction patterns of potato starch mixtures

Table 6. Relative crystallinity of rice starch mixtures

Samples	Relative crystallinity
Raw rice	47.1±0.1[a 1),2)]
AS rice	38.6±0.6[b]
AS 0%[3)]	23.6±0.9[f]
AS 25%	26.0±0.3[e]
AS 50%	29.5±0.3[d]
AS 75%	30.1±0.6[d]
AS 100%	32.5±0.9[c]

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

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

[3)]The starch mixture contained 0% of AS-treated starch.

Table 7. Relative crystallinity of potato starch mixtures

Samples	Relative crystallinity
Raw potato	41.8±0.1[a 1),2)]
AS potato	37.3±0.7[b]
AS 0% [3)]	22.4±1.6[f]
AS 25%	24.2±0.5[e]
AS 50%	28.1±0.3[d]
AS 75%	31.5±1.6[c]
AS 100%	35.7±1.1[b]

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

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

[3)]The starch mixture contained 0% of AS-treated starch.

5. Determination of digestibility of starch mixtures

Table 8 presents the *in vitro* digestibility of rice and potato starch mixtures. According to the Englyst assay, starch is divided into RDS, SDS and RS by digestion time (Englyst et al., 1992). It is well known that in vitro digestibility of starches by α-amylase is affected by such factors as molecular associations between starch components (Dreher et al., 1984), crystalline structures (Planchot et al., 1997) and granule size (Vandeputte et al., 2003).

The RDS contents of cooked rice and potato starches were 72.9% and 69.0%, respectively. It suggested that the semicrystalline structure of raw starch granules seemed to be destroyed by gelatinization, resulting in a decrease of SDS and an increase of RDS (Cousin et al., 1996; Zhang et al., 2006). In the AS-treated rice and potato starches, RDS contents decreased from 72.9% to 41.0% and from 69.0% to 39.8%, respectively, while SDS and RS contents increased.

The decrease of susceptibility to enzymatic hydrolysis is associated with branch chain length distribution and relative crystallinity (Srichuwong et al., 2005). By the elongation mechanism of AS, enzymatically modified starches had longer branched chain length than did raw starches (Table 2 and 3). Those results were in agreement with previous reports (Rolland-Sabaté et al.,

2004; Shin et al., 2010).

The shortest A chains may disturb the formation of an ordered crystalline structure, while the longer B chains tend to form double helices which constitute crystalline region. Shorter chains form short or weak double helices that would produce imperfect crystalline structures (Jane et al., 1999). Digetive enzymes are easier to access amorphous regions than crystalline regions (Zhang et al., 2006). As a result, rice starch having more shorter chains showed a high proportion of RDS. Shin et al. (2010) also reported no difference in the starch fractions between starch sources. Furthermore, long-branched chains of starch were rearranged by retrogradation during enzyme reaction at 30℃ for 24 hr. It also may cause a decrease of enzyme susceptibility. Kalichevsky et al. (1990) and Silverio et al. (2000) suggested longer branch chains increase the extent of retrogradation.

In case of mixture samples, with more AS-treated starch contents, SDS and RS fractions increased, but RDS fraction decreased. All mixture samples were gelatinized and recrystallized for 14 days, and so their granules were disrupted. The samples containing more AS-treated starches had high portions of long–branched chains (Table 2 and 3). Moreover, the relative crystallinity also increased with increasing portion of AS-treated starches (Table 4 and 5). As mentioned before, these result come from the amount of longer branched chains that were elongated by AS. Zhang et al. (2006)

reported that the starch with more long chains (DP≥13) had more SDS content.

Compared with the AS-treated starches, the AS 100% samples showed higher SDS fractions and lower RDS fractions. It indicates that the increase of SDS and RS fractions in starch mixtures resulted from not only the elongation of branch chain in amylopectin by AS but also the retrogradation during the 14 days storage at 4℃.

In conclusion, the AS 100% samples which contain much AS-treated starches had high fractions of SDS and RS as a result of decreased susceptibility to enzymatic hydrolysis by long branched chains and retrogradation.

Table 8. *In vitro* digestibility of starch mixtures

Samples	Rice			Potato		
	RDS(%)	SDS(%)	RS(%)	RDS(%)	SDS(%)	RS(%)
Cooked starch	72.9±0.8[a 2),3)]	8.3±0.5[d]	18.8±0.4[e]	69.0±1.2[a]	5.1±0.7[f]	25.9±0.8[d]
AS-treated starch	41.0±1.3[e]	17.0±1.5[b]	42.0±0.7[a]	39.8±0.2[e]	16.6±1.8[bc]	43.6±1.8[a]
AS 0% [1)]	72.0±0.2[a]	9.8±1.0[d]	18.3±1.2[e]	71.3±0.9[a]	9.0±1.5[e]	19.7±0.7[e]
AS 25%	61.8±0.3[b]	14.4±0.8[c]	23.8±0.6[d]	63.4±0.3[b]	11.4±0.4[de]	25.3±0.7[d]
AS 50%	52.1±0.3[c]	18.4±0.4[b]	29.5±0.7[c]	54.6±2.0[c]	13.5±2.8[cd]	31.9±1.1[c]
AS 75%	46.1±0.5[d]	18.7±1.1[b]	35.2±1.6[b]	45.8±1.2[d]	18.7±2.3[b]	35.5±1.4[b]
AS 100%	21.2±0.1[f]	38.1±1.2[a]	40.7±1.3[a]	28.3±2.7[f]	30.0±1.8[a]	41.7±0.9[a]

[1)] The starch mixture contained 0% of AS-treated starch.

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

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

6. Gel textural properties

The gel textural properties before and after retrogradation of starch mixtures determined using the texture analyzer are shown in Table 9 and 10. The texture properties of starch gels are influenced by various factors, including the rigidiy and volume fraction of the gelatinized starch granules, the rheological characteristics of the amylose matrix as well as the interactions between dispersed and continuous phases of the gel (Biliaderis, 1998).

The starch mixtures exhibited lower or similar hardness compared with the respective individual starches, raw and AS-treated starches. In particular, the hardness of AS 25% samples decreased. However, as the amount of AS-treated starch increased, the other starch mixtures (AS 50% and AS 75%) had higher values of the hardness. As mentioned in "thermal properties" section, the melting enthalpies of retrograded starch mixtures (ΔH) were also generally between the values of their individual components. Presumably, the hardness of starch gels, like melting enthalpies of retrograded starch mixtures, might be concerned with the arrangement of starch chains and the amount of double helices.

The increase in hardness depending on the amount of AS-treated starches

(AS 100%) resulted from the elongated long chains by AS treatment. As the amount of AS-treated starches increased, the proportions of long chains (DP 25-36 and DP ≥ 37) increased (Table 2 and 3). Mua et al. (1997) suggested that the harder starch gels tend to have higher amylose content and longer amylopectin chains. The gel hardness is mainly caused by retrogradation of starch, which is associated with the crystallization of amylopectin and syneresis of water resulting in firmer gels (Miles et al., 1985). The long and linear amylopectin fraction has a tendency toward rapid retrogradation due to its structural similarity to amylose (Hizukuri et al., 1989). Likewise, the amylose-like long chains elongated by AS treatment induced a harder gel, and also the hardness increased after storage for 14 days at 4℃ because of retrogradation.

The low hardness of starch mixtures than those of the individual raw and AS-treated starches suggested a less influence of retrogradation. This results were in agreement with a previous study (Puncha-arnon et al., 2008). Obanni et al. (1997) stated that a mixture of starches have the additional property of low retrogradation tendency. They also mentioned that the amylose molecules in these blends have associate with amylopectin, and therefore are not available for recrystallization.

Adhesiveness is a surface characteristic and the strength necessary to pull the probe away from the sample (Pons et al., 2007). In case of starch

mixtures, adhesiveness decreased with an increase in the portion of AS-treated starch (Table 10). Adhesiveness has an inverse linear relationship with amylose molar mass in gels. In addition, this value decreases when starch has a high percentage of long chains (Karam et al., 2005). Consequently, the AS 100% samples containing a high proportion of long B chains (Table 2 and 3) showed low adhesiveness.

Springiness did not show significant differences among the samples, and it did not change after retrogradation for 14 days at 4℃. Cohesiveness, the strength to maintain the original form, showed a similar tendency with adhesiveness. Huang et al. (2007) mentioned that the high viscoelasticity and cohesiveness created the gel with high adhesiveness.

Chewiness is the energy required for masticating a sample to a steady state of swallowing (Karam et al., 2005). The raw and AS-treated starches formed relatively firmer gels, showing high values of chewiness. It suggested that with an increase in the amount of AS-treated starch, more energy was needed to chew the gel on the whole. However, the AS 75% sample of rice starch mixtures showed a low chewiness value due to its low springiness and cohesiveness. In short, chewiness was related to not only hardness but also springiness and cohesiveness. A firm gel is easy to swallow, if it has low tendency to cohere itself. Meullenet et al. (1998) stated that the chewiness is most highly correlated with cohesiveness.

According to a previous study, the low hardness and high adhesiveness of rice food has a good taste (Guo et al., 2006). In this study, the AS 25% sample tended to exhibit a similar property like a previous study. This result suggests that it could be used as a functional ingredient in the food industry.

Table 9. Textural properties of mixed rice starch gels

Samples	Hardness (g)	Adhesiveness (g·sec)	Springiness	Cohesiveness	Chewiness
AS 0% [1)]	2936.6±118.4^{b} [3),4)]	-555.8±39.1^{c}	0.97±0.01^{a}	0.45±0.03^{a}	1366.0±59.1^{b}
AS 25%	2505.1±91.9^{c}	-1773.2±57.5^{a}	0.98±0.01^{a}	0.44±0.05^{a}	1104.4±61.7^{c}
AS 50%	2892.9±81.5^{b}	-1600.3±48.7^{b}	0.99±0.00^{a}	0.36±0.00^{b}	1042.3±15.4^{c}
AS 75%	2906.7±86.3^{b}	-255.4±41.3^{d}	0.80±0.07^{b}	0.13±0.00^{c}	279.2±8.5^{d}
AS 100%	7689.9±300.6^{a}	-280.9±20.5^{d}	0.98±0.01^{a}	0.41±0.04^{ab}	3231.8±53.8^{a}
AS 0% (r) [2)]	8509.6±103.1^{b}	-215.1±30.4^{c}	0.99±0.01^{a}	0.23±0.02^{a}	2559.0±140.9^{a}
AS 25% (r)	4479.9±92.9^{e}	-543.2±36.6^{a}	1.00±0.01^{a}	0.15±0.02^{b}	863.6±48.9^{b}
AS 50% (r)	5427.3±139.5^{d}	-282.5±7.0^{b}	0.99±0.01^{a}	0.15±0.02^{b}	846.2±10.3^{b}
AS 75% (r)	6001.5±86.3^{c}	-236.4±21.6^{c}	0.91±0.01^{b}	0.15±0.02^{b}	777.8±16.3^{b}
AS 100% (r)	9654.2±450.0^{a}	-146.5±15.1^{d}	0.97±0.06^{a}	0.20±0.00^{a}	2637.3±179.3^{a}

[1)] The starch mixture contains 0 percent of AS-treated starch.

[2)] The retrograded starch mixture contains 0 percent of AS-treated starch.

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

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

Table 10. Textural properties of mixed potato starch gels

Samples	Hardness (g)	Adhesiveness (g·sec)	Springiness	Cohesiveness	Chewiness
AS 0% [1)]	2888.0±36.1^{c} [3),4)]	-1420.3±319.2^{b}	0.92±0.03^{b}	0.67±0.01^{a}	1783.9±56.3^{c}
AS 25%	1427.3±92.7^{d}	-2188.1±28.2^{a}	0.97±0.02^{ab}	0.39±0.07^{c}	574.9±16.8^{e}
AS 50%	3140.6±27.8^{c}	-451.9±12.0^{c}	0.97±0.03^{ab}	0.44±0.01^{c}	1143.6±92.3^{d}
AS 75%	4013.1±354.9^{b}	-234.2±46.0^{c}	0.99±0.01^{ab}	0.52±0.04^{b}	2490.6±160.6^{b}
AS 100%	8696.2±246.1^{a}	-444.6±30.0^{c}	0.99±0.00^{a}	0.57±0.01^{b}	3151.4±34.6^{a}
AS 0% (r) [2)]	13760.6±310.0^{c}	-1486.6±160.9^{c}	0.99±0.00^{ab}	0.40±0.02^{c}	5320.7±104.5^{c}
AS 25% (r)	3091.3±134.8^{e}	-2626.1±139.2^{a}	0.99±0.00^{ab}	0.14±0.02^{e}	393.5±14.0^{d}
AS 50% (r)	12565.0±700.9^{d}	-2262.6±197.6^{b}	0.99±0.01^{ab}	0.32±0.01^{d}	4241.7±151.0^{c}
AS 75% (r)	21347.0±231.1^{b}	-298.3±1.3^{d}	1.00±0.00^{a}	0.45±0.02^{b}	10049.0±1475.9^{b}
AS 100% (r)	32777.2±624.6^{a}	-387.1±6.4^{d}	0.96±0.03^{b}	0.53±0.01^{a}	20609.9±1350.5^{a}

[1)] The starch mixture contains 0 percent of AS-treated starch.

[2)] The retrograded starch mixture contains 0 percent of AS-treated starch.

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

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

7. Microstructures of starch mixture gels

Figure 6 and 7 are energy-filtering transmission electron micrographs (EFTEM) of starch mixture gels. At this level of magnification (10,000×), the microstructure of gels showed that the tangled structures were agglomerated and the empty space was formed.

As the amount of AS-treated starches increased, denser structures were formed but the starch mixture gels (AS 25%, AS 50%, and AS 75%) exhibited less dense structure than the AS 0% and AS 100% samples. The AS 0% and AS 100% samples had more homogenous structure. Especially, the AS 100% samples showed irregularly structure and pores within the particles. In case of the high solution viscosity of amylopectin also showed like this microstucture(Greenwood, 1956). Yamaguchi et al. (1979) stated that the amylopectin has a strong tendency to form aggregates and created more compact structure than others.

The overall results were consistent with the previously reported gel texture properties data. The high value of cohesiveness means that the inner structure of gels are combined tightly, so the samples which showed high cohesiveness exhibited denser. Moreover, the fact that there were a lot of empty spaces means that it could break easily when it is bitten. As shown in

the Figure 6 and 7, the AS 100% samples showed the most compact structures and had very few empty spaces. These results were consistent with the high values of chewiness (Table 9 and 10).

In certain areas, starch particles seemed to be aggregated into larger clusters. In previous study, the double helices of retrograded starch formed small ordered regions being the clusters of linear α-(1,4) glucan chains and adjacent clusters of ordered chains formed physical cross-links between amylopectin molecules (Keetels et al., 1996). The authors stated that the average length of such cross-links would affect the melting enthalpy. In this study, the AS 25%, AS 50%, and AS 75% samples showed a lower or similar values of melting enthalpied than those of AS 0% and of AS 100% samples. In conclusion, the samples with denser structure had long cross-linked cluster leading to the increase of the melting enthalpy and cohesiveness.

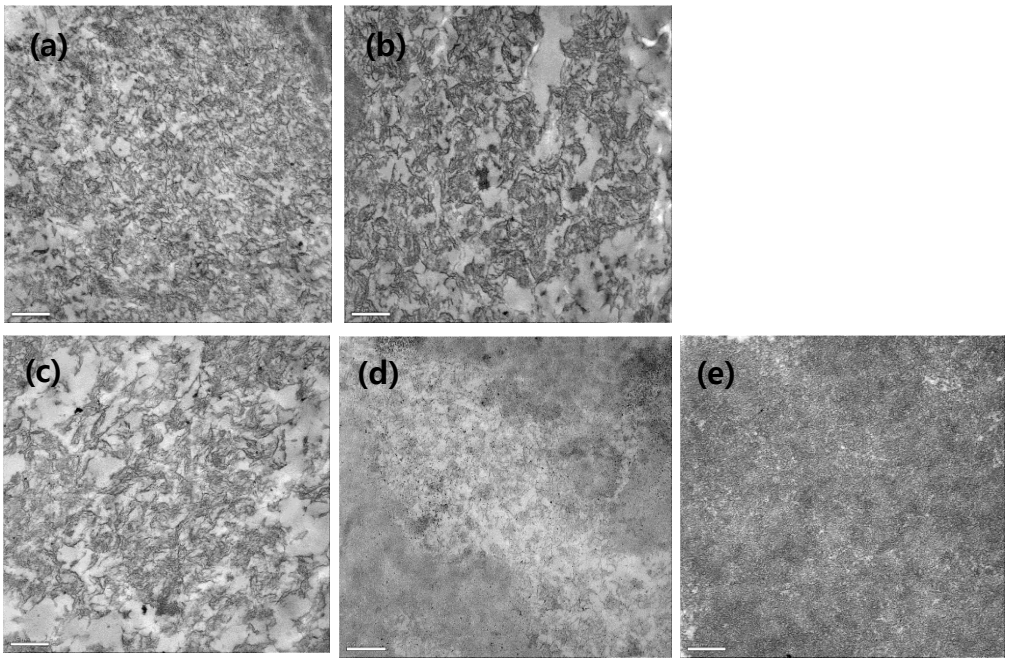

Figure 6. Representative EFTEM images (10,000×) of rice starch mixture gels : AS 0% (a), AS 25% (b), AS 50% (c), AS 75% (d), AS 100% (e).

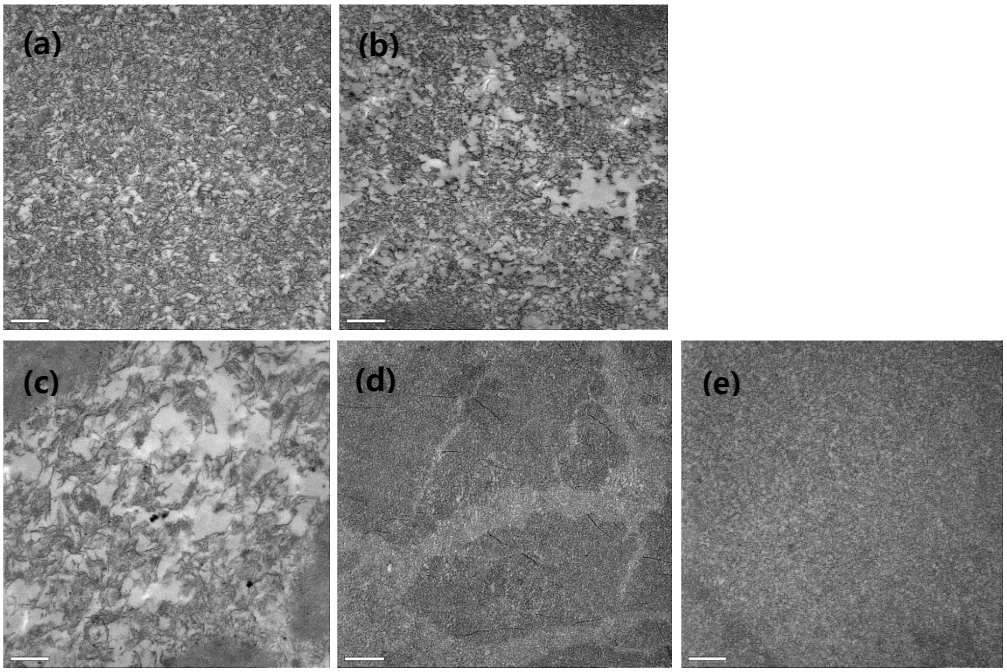

Figure 7. Representative EFTEM images (10,000×) of potato starch mixture gels : AS 0% (a), AS 25% (b), AS 50% (c), AS 75% (d), AS 100% (e).

CONCLUSION

In this study, rice and potato starches modified by amylosucrase were mixed with raw starches in different ratios, gelatinized and retrograded for 14 days at 4℃. The elongated branch chains by AS were maintained after mixing; and the long branch chains (DP 13-36, DP≥37) increased with an increase in the portion of AS-treated starch. The AS-treated starches had increased the contents of SDS and RS by elongation of branch chain length. The more starch mixtures had AS-treated starches, the more they contained SDS and RS fracions.

The mixture samples showed higher thermal transition parameters (T_o, T_p, and T_c) as the amount of AS-treated starches increased. All starch mixtures exhibited a B-type X-ray pattern because of retrogradation. And also, relative crystallinity slightly increased with an increase in the amount of AS-treated starches. Moreover, the starch mixtures containing AS-treated starch had lower or similar hardness and retrogradation enthalpy value than those of individual starches. Cohesiveness, chewiness and melting enthalpy were positively correlated to microstructure of starch mixture gels.

In summary, the starch mixtures with added the AS-treated starches added contained high SDS and RS fractions showed better texture properties than the individual components do. Therefore, the mixtures of raw and

enzymatically modified starches can provide different functionalities for starch-based food systems. This study also suggests that native starches may be flexibly formulated to obtain new properties similar to enzymatically modified starches with reduced costs. Further studies may focus on investigating the characteristics of more blends with both modified and unmodified starches.

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국문 초록

지소화성(SDS) 및 난소화성전분(RS) 은 식후 혈당 수준을 천천히 증가시켜 당뇨병과 비만 예방에 도움을 준다. 이 연구에서는 쌀전분과 감자전분에 amylosucrase(AS)를 처리하여 지소화성 및 난소화성 전분의 함량을 높이고 이를 각각의 생전분과 여러가지 비율로 섞어 그 특성 변화를 조사하였다.

전분 현탁액(2%)에 AS 20,000U를 처리하고 30℃에서 24시간동안 반응하여 변성 전분을 얻었다. 이 변성전분을 각각의 원래 생전분에 0%, 25%, 50%, 75%, 100% 비율로 섞어 완전하게 호화시킨 뒤 4℃에서 14일간 노화를 일으켰다. 이렇게 조제한 전분 혼합물을 이용하여 전분 사슬의 길이 분포, 열특성, X-선 회절 및 상대적 결정화도, *in vitro* 소화율 측정과 TA를 이용한 텍스쳐 분석을 통해 구조적 및 이화학적 특성을 규명하였다.

HPAEC-PAD를 이용하여 아밀로펙틴의 가지 사슬을 분석한 결과, AS 처리한 전분은 DP 12 이하의 짧은 사슬이 감소하였으며 DP 13 이상의 긴 사슬들이 증가하였다. 전분 혼합물의 경우, AS 처리 전분의 양이 많아 질수록 긴 사슬의 양 또한 점점

증가하였다. 즉, AS로써 연장된 사슬은 혼합 후에도 유지됨을 알 수 있었다.

시차 주사 열량계를 통해 제조된 시료의 열 특성을 살펴본 결과, 용융 엔탈피(ΔH)값이 섞기 전의 두 전분에 비해 전분 혼합물이 낮은 값을 보여, 혼합 시에 노화 억제 효과를 나타낸 것으로 사료되었다. X선 회절도형에서 쌀전분과 감자전분은 각기 A형과 B형을 나타냈고, AS 처리 전분과 혼합물은 모두 B형을 나타내었으며, 믹스쳐에서 AS 처리 전분의 양이 많아질수록 상대적 결정화도가 증가하였다. *In vitro* 소화율 측정 결과, AS 처리 전분의 양이 많아질수록 SDS와 RS가 증가하는 경향이 나타났다. 또한, 노화 전 후의 텍스쳐를 측정해 본 결과, 두 전분을 섞었을 때에 단독으로 사용 했을 때보다 낮거나 비슷한 경도를 보였고, 이러한 경도와 응집성에 따라 씹힘성도 유사한 경향을 보였다.

결론적으로, AS 처리 전분과 생전분을 섞은 시료의 경우 섞지 않았을 때의 AS 처리 전분과 비슷한 소화율을 나타냄과 동시에 AS 처리 전분이 가진 물성적인 단점을 개선하는 데에 크게 도움을 주는 것으로 나타났다. 또한 처리하지 않은 생전분과 AS 처리

전분에 비해 낮은 노화도를 보여, 각기 단독으로 사용 했을 때보다 노화가 지연됨을 알 수 있었다.

더 나아가, 이러한 전분 혼합제에서의 전분간 상호작용을 파악하여 실제 전분식품 젤의 물성을 개선하는 것은 더 나은 가공식품의 개발 및 품질 개선에 중요한 기초자료가 될 수 있을 것이다.

주요어 : 지소화성 전분, 아밀로수크레이스, 전분 블랜드, 소화율, 텍스쳐, 노화

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