JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Production of an in Vitro Low-Digestible Starch via Hydrothermal Treatment of Amylosucrase-Modified Normal and Waxy Rice Starches and Its Structural Properties

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Supporting Information

ABSTRACT: We investigated dual modification of normal and waxy rice starch, focusing on digestibility. Amylosucrase (AS) was applied to maximize the slowly digestible and resistant starch fractions. AS-modified starches were adjusted to 25−40% moisture levels and heated at 100 °C for 40 min. AS-modified starches exhibited a B-type crystalline structure, and hydrothermal treatment (HTT) significantly ($p < 0.05$) increased the relative crystallinity with moisture level. The thermal transition properties of modified starches were also affected by the moisture level. The contents of rapidly digestible starch fraction in AS-modified normal and waxy starches (43.3 ± 3.9 and 18.1 ± 0.6%) decreased to 13.0 ± 1.0 and 0.3 ± 0.3% after HTT, accordingly increasing the low digestible fractions. Although the strengthened crystalline structures of AS-modified starches by HTT were not stable enough to maintain their rigidity under cooking, application of AS and HTT was more effective in waxy rice starch than normal rice starch when lowering digestibility.

KEYWORDS: *amylosucrase, dual modification, hydrothermal treatment, resistant starch, slowly digestible starch*

INTRODUCTION

Rice is a major food crop around the world, and more than 50% of the world's population depends on rice as their primary carbohydrate source.[1] Rice flour is used in commercial food products including rice cakes, noodles, and breads. In addition, rice can be used in a variety of gluten-free products because it is a gluten-free cereal that is hypoallergenic, colorless, and bland.[2]

For nutritional purposes, starch is generally classified into three major fractions depending on the rate and extent of in vitro digestion: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS).[3] RDS rapidly increases postprandial blood glucose and insulin levels. SDS induces a slow increase in postprandial blood glucose levels, and RS is not absorbed in the small intestine, but can be fermented in the colon. RDS is related to high glycemic index (GI), whereas SDS and RS have physiological advantages such as improved glucose tolerance and insulin resistance, reduced blood lipid levels, and a prebiotic effect.[4,5]

Hydrothermal treatment (HTT), i.e., annealing and heat-moisture treatment, modifies the physicochemical properties and digestibility of starch. In general, digestive properties (RDS, SDS, and RS content) depend on the amylose/amylopectin ratio, the branched chain length distribution of amylopectin, the morphology of the raw starch, and applied conditions.[6,7] Alteration of the hydrolysis rate via hydrothermal treatment has been reported for various types of starches.[8−10]

Amylosucrase (EC 2.4.1.4; AS) is a glucosyltransferase that synthesizes an insoluble α-1,4-linked glucan polymer from sucrose to release fructose. This enzyme elongates nonreducing ends of amylose and amylopectin.[11] However, because of the great difference in the number of nonreducing ends between amylose and amylopectin, the structural changes in amylopectin were pronounced compared with those in amylose.[12] AS activity on α-glucans and several starches increases RS content considerably.[12−14] Though there have been a few studies on AS-modified starch, its modification combined with hydrothermal treatment has not been reported yet.

We hypothesized that HTT of AS-modified starch could alter digestive properties, and that these alterations depended on the presence of amylose and the branched chain length of amylopectin. Therefore, we prepared and characterized dual-modified normal and waxy rice starches. This study aimed to extend our understanding of the production of low digestible starch by dual modification using enzymatic and physical treatments.

Received: March 4, 2016
Revised: May 13, 2016
Accepted: May 26, 2016
Published: May 26, 2016

DOI: 10.1021/acs.jafc.6b01055
J. Agric. Food Chem. 2016, 64, 5045−5052

MATERIALS AND METHODS

Materials. Waxy rice, Hwasunchalbyeo (*Oryza sativa* L. var. *japonica*), was obtained from the experimental farm of the Rural Development Administration (Suwon, Korea). Normal rice starch was purchased from Sigma-Aldrich (St. Louis, MO, USA) which contained 21.5% amylose. Pancreatin (P7545, activity 8× USP/g), amyloglucosidase (AMG 300L, activity 300 AGU/mL), and isoamylase (activity 1,000 U/mL) were from Sigma-Aldrich, Novozymes (Bagsvaerd, Denmark), and Megazyme (Bray, Ireland), respectively. Sucrose was purchased from Junsei Chemical (Tokyo, Japan). All chemicals and reagents were of analytical grade.

Starch Isolation. Waxy rice was milled with a laboratory milling tester (SYTRM92, SSangyoung Machine Industry, Incheon, Korea), and waxy rice starch was isolated using an alkaline steeping method with a slight modification.[15] Briefly, after 6 h of steeping in 0.2% sodium hydroxide, the rice was ground in a laboratory blender (LB10S, Waring Products Corp., New Hartfort, CT, USA) at maximum speed for 1 min and filtered through 35-, 50-, and 100-mesh sieves. The starch suspension was centrifuged at 6350*g* for 10 min. The supernatant and the top yellowish layer were removed repeatedly to eliminate protein. After rinsing with distilled water, the starch slurry was dried at 30 °C in a forced-air oven, ground, and passed through a 100-mesh sieve.

AS Activity Assay. AS from *Neisseria polysaccharea* was purified from cell extracts by affinity chromatography with Ni-NTA (nickel nitrilotriacetic acid) resin as described previously.[16] AS activity was measured as described previously[17] with some modifications. A mixture of 0.1 mL of 4% sucrose, 0.1 mL of 1% glycogen, 0.25 mL of 100 mM sodium citrate buffer (pH 7.0), and 0.05 mL of diluted enzyme was reacted for 10 min. The released fructose was quantified using the dinitrosalicylic acid method.[18] One unit (U) of AS was defined as the amount of enzyme that catalyzes the consumption of 1 μM of sucrose per min under assay conditions.

AS-Modified Starch Production. Starch suspension (2%, w/v) in a 100 mM sodium acetate buffer (pH 7.0) containing 100 mM sucrose was prepared to reach a final volume of 30 mL. This suspension was boiled for 30 min and cooled to 30 °C for enzyme accessibility. AS (18,700 U/mL starch suspension) was added to the suspension and incubated at 30 °C for 13 h 40 min. This condition for AS modification was established in our preliminary experiments to obtain highest content of SDS and RS in modified waxy rice starch, using response surface methodology considering the unit of AS and treatment time (data not shown). It was also applied to the normal rice starch. To terminate the reaction, three volumes of ethanol were added and centrifuged at 10000*g* for 10 min. Then, the pellet was washed three times by suspending it in distilled water and then centrifuging at 10000*g* for 10 min. The insoluble fraction was freeze-dried. Dry starch was ground and passed through a 100-mesh sieve. The control was prepared using the same method, except for AS addition.

Hydrothermal Treatment of AS-Modified Starch. To prepare AS-HTT-modified starch, the moisture content was measured according to the AACC 44-15A method.[19] AS-modified starches were put in glass containers, adjusted to 25, 30, 35, and 40% moisture levels by adding distilled water, and labeled as AS-HTT 25%, AS-HTT 30%, AS-HTT 35%, and AS-HTT 40%, respectively. The sealed glass containers were placed at ambient temperature overnight to allow sample moisture to equilibrate. Hydrated samples were heated at 100 °C for 40 min and dried in a 40 °C forced-air oven until the moisture content of the samples reached about 10%.

In Vitro Starch Digestibility. The starch fraction was determined as described previously[20] with slight modifications.[21] Pancreatin (2 g, P-7545, activity 8× USP/g, Sigma-Aldrich) was dissolved in distilled water (24 mL), stirred for 10 min, and centrifuged at 1500*g* for 10 min, and then 20 mL of supernatant was mixed with 3.6 mL of distilled water and 0.4 mL of amyloglucosidase (AMG 300L, activity 300 AGU/mL, Novozymes, Bagsvaerd, Denmark). This enzyme solution was kept in a water bath at 37 °C for 10 min.

A sample (30 mg) and a glass bead were placed in a 2 mL microtube, and 0.75 mL of 100 mM sodium acetate buffer (pH 5.2) was added. The microtube was kept in a shaking incubator at 37 °C for 10 min with a stroke speed of 240 rpm. Then, 0.75 mL of the prepared enzyme solution was added to the tube and incubated for 10 and 240 min. The reaction was stopped by boiling for 10 min. The hydrolyzed glucose content in the supernatant obtained after centrifugation (5000*g*, 5 min) was measured using a GOP-POD kit (BCS, Anyang, Korea). To test cooked starches, the microtube was boiled for 20 min and cooled to 37 °C before enzyme addition. Starch fractions were classified based on the extent of hydrolysis. The RDS fraction was measured by the value of glucose after a 10 min reaction. SDS was defined as the fraction that was digestible between 10 and 240 min. RS was defined as the fraction undigested after 240 min. This procedure gave almost the same results as the method of Englyst et al.[3] did.

Determination of Branched Chain Length Distribution. Starch (15 mg) was dispersed in 90% DMSO (3 mL) and boiled for 15 min. Ethanol (15 mL) was added to the starch suspension to precipitate starch. The suspension was centrifuged at 10000*g* for 10 min. Distilled water (1.5 mL) was added to the 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 (activity 1,000U/mL, Megazyme, Bray, Ireland) were added, and then the sample was incubated in a water bath at 45 °C and 50 rpm for 2 h. After reaction, samples were boiled for 10 min. The debranched sample was filtered through a 0.45 μm membrane filter and analyzed using high-performance anion-exchange chromatography with a pulsed amperometric detector (HPAEC-PAD; Dionex, Sunnyvale, CA, USA) equipped a CarboPac PA1 anion exchange column (250 × 4 mm; Dionex). This analysis was performed using 150 mM NaOH for column equilibration and 600 mM sodium acetate in 150 mM NaOH for sample elution, with flow rate of 1 mL/min. The gradients of sodium acetate used were as follows: linear gradients from 0 to 20% for 0 to 5 min, 20 to 45% for 5 to 30 min, 45 to 55% for 30 to 60 min, 55 to 60% for 60 to 80 min, 60 to 65% for 80 to 90 min, 65 to 80% for 90 to 95 min, and 80 to 100% for 95 to 100 min. A degree of polymerization (DP) from 1 to 7 was designated using a mixture of maltooligosaccharides (DP 1−7, Sigma-Aldrich) as a standard.

X-ray Diffraction and Relative Crystallinity. X-ray diffraction analysis (XRD) was performed using an X-ray diffractometer (D5005, Bruker, Karlsruhe, Germany). Operating conditions were 40 kV and 40 mA with Cu Kα radiation of 0.15406 nm (nickel filter; time constant, 4 s). The scan was conducted through a 2θ range from 3 to 30°. The relative crystallinity was calculated as described previously[22] using Origin 8.1 (Micro Cal, Northampton, MA, USA).

Thermal Transition Properties. A differential scanning calorimeter (Diamond DSC, PerkinElmer, Shelton, CT, USA) was used to investigate thermal transition properties of starch samples. Each sample (10 mg) was weighed into a hermetic pan, and 40 μL of distilled water was added. Sample pans were sealed and kept at room temperature overnight for equilibrium. DSC scan was performed as the sample was heated from 30 to 160 °C at a scan rate of 5 °C/min. An empty pan was used as a reference.

Statistical Analysis. All experimental data were analyzed using analysis of variance (ANOVA) and expressed as the mean ± standard deviation of repeated measurements. Significant differences among mean values were compared using Duncan's multiple range test ($p < 0.05$). Statistical analysis was conducted with IBM SPSS statistics version 21.0 (IBM, Armonk, NY, USA).

RESULTS AND DISCUSSION

Branched Chain Length Distributions of AS-Modified Starches. Figure 1 shows the branched chain length distributions of AS controls and AS-modified starches, and Table 1 shows the relative percentage of peak area. The branched chain length distributions of raw starches were very similar to those of AS controls (data not shown). Hanashiro et al.[23] categorized branched chains into four fractions named A, B_1, B_2, and B_3 and longer chains, which correspond to chain

DOI: 10.1021/acs.jafc.6b01055
J. Agric. Food Chem. 2016, 64, 5045−5052

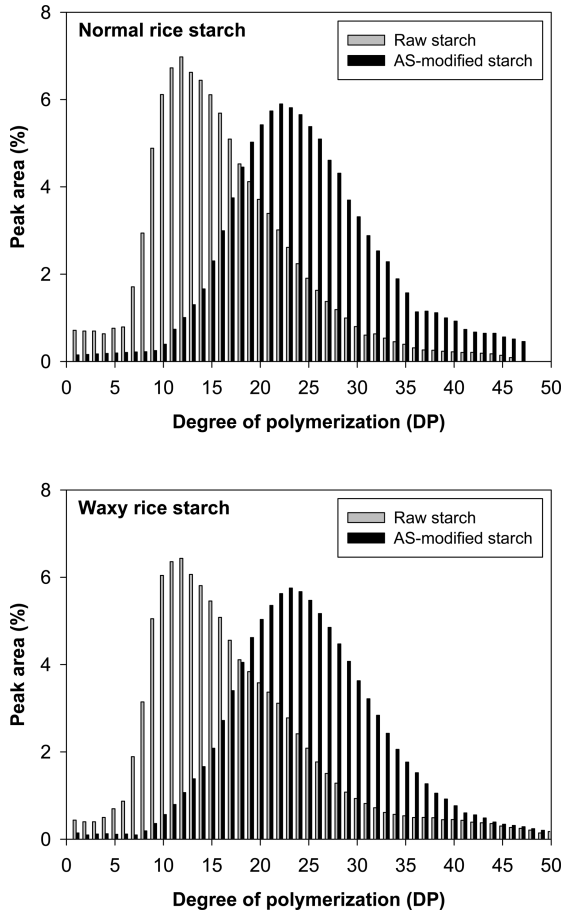

Figure 1. Amylopectin branched chain length distribution of amylosucrase-modified normal and waxy rice starches.

lengths of DP 6−12, 13−24, 25−36, and ≥37, respectively. However, since the chains of amylopectin were classified into four fractions based on their length, location in the amylopectin, and whether they carried other chains, this fractionation was no longer valid for AS-modified starches.

AS controls had a high proportion of chains with DP ≤ 24. However, AS-modified starches had smaller proportions of chains with DP ≤ 24 and larger proportions of chains with DP ≥ 25, which corresponded with the results of previous studies.[12,24] AS-modified starches had more than three times the proportion of chains with DP 25−36 than AS controls. de Montalk et al.[25] reported that AS catalyzes the elongation of some external chains of amylopectin and amylose by attachment of glucosyl units at nonreducing ends. According to the cluster model for amylopectin proposed by Hizukuri,[26] amylopectin consists of nonbranching A chains and B chains carrying A or other B chains by α-1,6-glycosidic linkages. A and B_1 chains are easily found at the exterior of amylopectin molecules. Therefore, external chains such as A and B_1 chains may have a higher probability of elongation than the B_2 and B_3 chains buried in the interior amylopectin. The small increase in the proportion of B_3 and longer chains (DP ≥ 37) in both starches after AS treatment indicated that the chains buried in the interior amylopectin were elongated to a limited extent. Long linear chains (DP ≥ 25) of AS-treated starches were anticipated as they could contribute to the formation of RS during HTT, because they could be retrograded through aggregation of side chains.[14]

X-ray Diffraction Patterns and Degree of Relative Crystallinity. The X-ray diffraction (XRD) patterns and degree of relative crystallinity (DRC, or perfectness), which is defined as the fraction of mass being strictly crystalline, of all samples are presented in Figure 2 and Table 2. Both the raw starches revealed a typical A-type pattern, having main peaks at $2\theta \approx 15$, 17, 18, and 23° and a small amount of V-type polymorph, which was recognized by a peak at $2\theta \approx 20°$.[27] No peaks were observed in the AS controls, indicating a considerable increase in amorphous regions due to the gelatinization process that occurred during boiling prior to the AS modification. The XRD pattern of AS-modified rice starches showed a change in crystalline structure from A- to B-type, which had peaks at 5, 15, 17, 22, and 24°, though a V-type crystalline peak was still observed. In general, starch retrogradation easily occurs in the presence of amylose and amylopectin long chains, and cereal starches show a B-type crystalline pattern after retrogradation regardless of their original crystalline structures.[28,29] Moreover, as the aggregation of longer linear chains could accelerate the formation of B-type crystalline structures,[30] the chain elongation by AS could also attribute to it. The B-type polymorph of AS-modified starches was reported in previous studies.[12,14] Follow-up HTT did not alter the B-type pattern of AS-treated starches, but the intensity of B-type peaks increased in a moisture content dependent manner.

Starch crystallinity is influenced by the ratio of amylose/amylopectin, average amylopectin chain length, and crystallite size.[31] The DRC values of raw normal and waxy rice starches were 45.9% and 49.8%, respectively (Table 2). In general, waxy starches have higher crystallinity than normal starches, because amylopectin has a positive relationship with crystallinity.[32] The DRC values for AS-modified normal and waxy rice starches

Table 1. Percentage of Different Fractions in Amylopectin Debranched Profiles of Amylosucrase Controls and Amylosucrase-Modified Starches[a]

	percent distribution (%)				
sample[b]	DP < 6	DP 6−12	DP 13−24	DP 25−36	DP ≥ 37
normal rice					
AS control	0.8 ± 0.0 b	29.4 ± 0.5 b	53.1 ± 1.2 a	11.4 ± 0.3 c	4.0 ± 0.2 b
AS-modified starch	2.8 ± 0.6 a	2.4 ± 0.0 c	49.5 ± 0.3 b	38.9 ± 1.1 b	6.4 ± 0.9 a
waxy rice					
AS control	0.7 ± 0.3 b	30.3 ± 0.2 a	52.4 ± 0.3 a	12.0 ± 0.5 c	4.5 ± 0.3 b
AS-modified starch	1.7 ± 0.3 b	2.9 ± 0.3 c	47.1 ± 0.3 c	41.4 ± 0.5 a	6.8 ± 0.8 a

[a]Values are mean ± standard deviation of duplicate analysis. Means carrying different letters within a column are significantly different ($p < 0.05$) by Duncan's multiple range test. [b]AS control, amylosucrase-control starch; AS-modified starch, amylosucrase-modified starch.

DOI: 10.1021/acs.jafc.6b01055
J. Agric. Food Chem. 2016, 64, 5045−5052

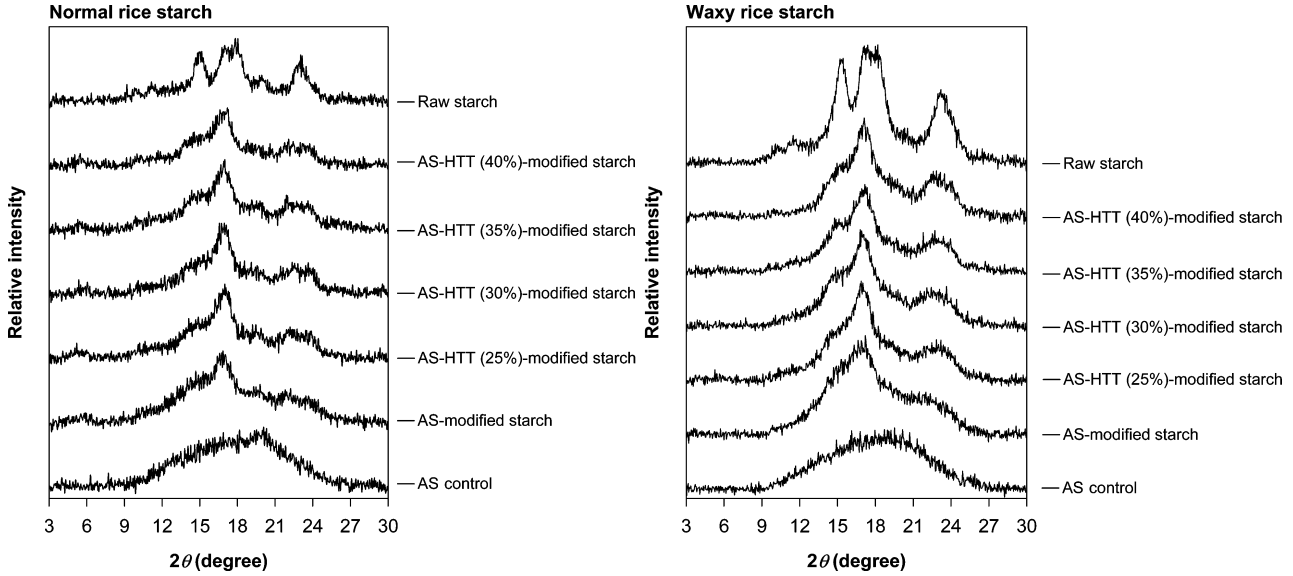

Figure 2. X-ray diffraction patterns of modified starches.

Table 2. Degree of Relative Crystallinities and Thermal Transition Properties of Raw Starches and Modified Starches[a]

sample[b]	deg of rel crystallinity (%)	thermal properties[c]				
		T_o (°C)	T_p (°C)	T_c (°C)	T_r (°C)	ΔH (J/g)
normal rice						
raw starch	45.9 ± 0.2 b	58.2 ± 0.1 i	66.1 ± 0.3 e	80.3 ± 0.3 g	22.1 ± 0.3 i	11.9 ± 0.4 b
AS control	12.8 ± 0.0 m	nd[d]	nd	nd	nd	nd
AS-modified starch	19.5 ± 0.1 k	76.2 ± 0.2 d	90.7 ± 0.4 c	102.6 ± 0.4 f	26.5 ± 0.6 f	10.5 ± 0.2 cd
AS-HTT 25%	24.4 ± 0.0 i	75.3 ± 0.3 e	91.5 ± 0.1 c	103.5 ± 0.6 e	28.3 ± 0.9 de	10.9 ± 1.1 bcd
AS-HTT 30%	26.7 ± 0.2 g	77.8 ± 0.1 c	92.8 ± 0.5 b	105.3 ± 0.2 bc	27.6 ± 0.1 e	10.3 ± 1.2 d
AS-HTT 35%	26.7 ± 0.0 g	79.7 ± 0.1 b	93.7 ± 0.0 b	105.1 ± 0.1 bc	25.4 ± 0.2 g	11.6 ± 0.1 bcd
AS-HTT 40%	26.3 ± 0.1 h	82.9 ± 0.1 a	95.3 ± 0.6 a	105.6 ± 0.1 b	22.7 ± 0.0 hi	11.6 ± 0.3 bcd
waxy rice						
raw starch	49.8 ± 0.5 a	58.0 ± 0.3 i	66.9 ± 0.1 e	74.6 ± 0.3 h	16.6 ± 0.6 j	14.3 ± 0.2 a
AS control	13.6 ± 0.3 l	nd	nd	nd	nd	nd
AS-modified starch	20.2 ± 0.2 j	71.7 ± 0.1 g	89.0 ± 0.1 d	103.2 ± 0.0 ef	31.5 ± 0.1 c	11.7 ± 0.3 bc
AS-HTT 25%	27.1 ± 0.2 f	69.7 ± 0.1 h	88.5 ± 0.2 d	104.2 ± 0.0 d	34.5 ± 0.1 a	15.3 ± 0.1 a
AS-HTT 30%	33.4 ± 0.1 e	73.7 ± 0.0 f	89.3 ± 0.6 d	106.6 ± 0.0 a	32.9 ± 0.0 b	14.1 ± 0.2 a
AS-HTT 35%	35.4 ± 0.2 d	76.3 ± 0.6 d	90.9 ± 1.0 c	104.9 ± 0.4 c	28.6 ± 0.3 d	15.4 ± 0.7 a
AS-HTT 40%	38.0 ± 0.3 c	80.0 ± 0.5 b	91.7 ± 0.1 c	103.5 ± 0.1 e	23.5 ± 0.6 h	15.5 ± 0.7 a

[a]Values are mean ± standard deviation of duplicate analysis. Means carrying different letters within a column are significantly different ($p < 0.05$) by Duncan's multiple range test. [b]AS control, amylosucrase-control starch; AS-modified starch, amylosucrase-modified starch; AS-HTT x%, amylosucrase-modified starch followed by hydrothermal treatment at x% moisture content. [c]T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; T_r, melting temperature range; ΔH, melting enthalpy. [d]Not detected.

were 19.5% and 20.2%, respectively. This is approximately 7% higher than the values for their corresponding AS controls, which lost the crystalline structure of raw starches. The branches of amylopectin in AS-modified starches, which contain an amylose-like long linear structure, might reassociate and organize into a crystalline structure more easily than those of control starches. AS-HTT-modified rice starches had notably higher DRC values than AS-modified starches, and they were dependent upon the moisture level, especially in the waxy starch. When HTT was carried out at the same moisture content, the DRC values of AS-HTT-modified waxy rice starches were higher than those of modified normal rice starches.

During the incubation to produce the modified starches, the double helices are presumably formed between elongated branch chains of amylopectin that were adjacent. As aforementioned, the longer linear chains could easily form the double helical structure and their aggregation could also be accelerated. In general, retrogradation, also referred to as recrystallization or reassociation, goes through the three steps of nucleation, propagation, and maturation and is dependent upon temperature.[33] Although the temperature for AS reaction (30 °C) was not perfect for the nucleation, it was quite far from melting temperatures of AS-modified starches (71.7 and 76.2 °C for AS-modified waxy and normal starches, respectively), and the creation of nucleation seeds by newly formed double helices could be promoted during AS reaction. The storage temperature employed in the HTT in this study is not conducive to nucleation, but might allow propagation and/or maturation of crystals to a certain extent. The temperature of 100 °C is slightly higher than the melting temperature of AS-modified starches (Table 2). Therefore, some parts of the

DOI: 10.1021/acs.jafc.6b01055
J. Agric. Food Chem. 2016, 64, 5045−5052

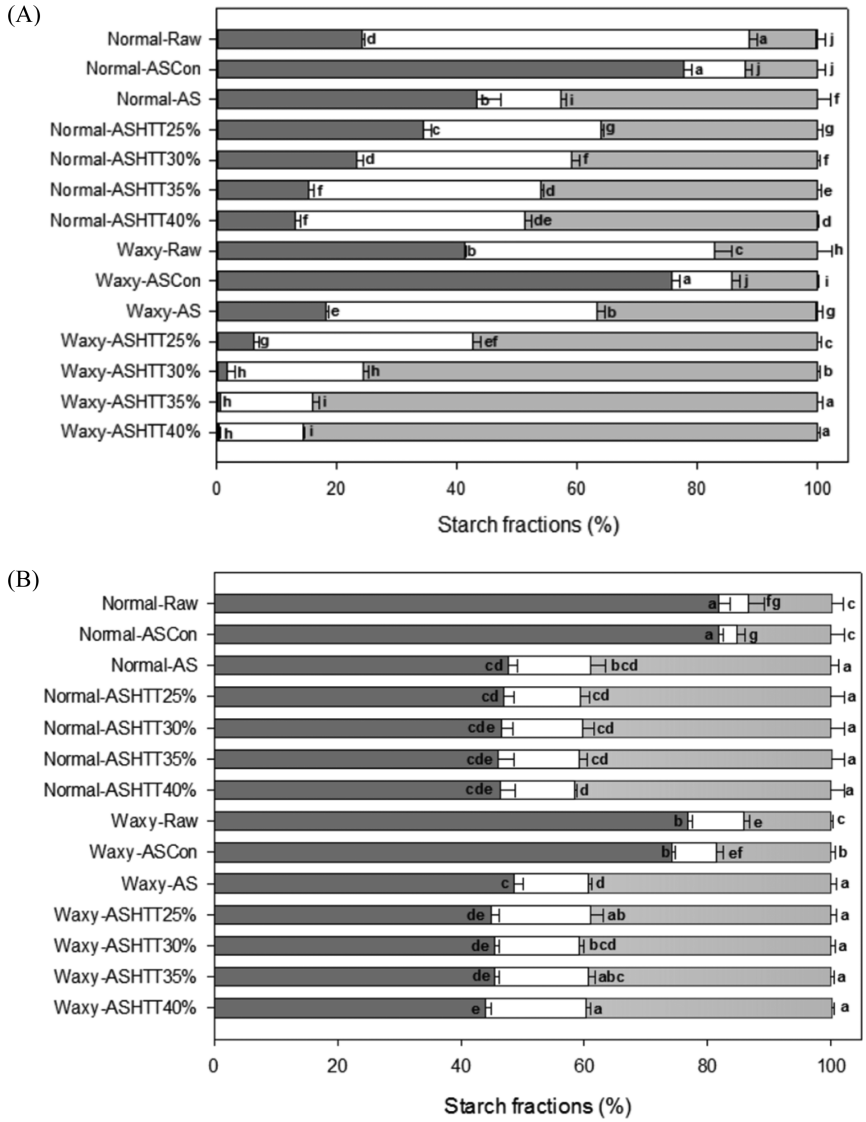

Figure 3. Digestibility of modified normal and waxy rice starches before (A) and after (B) cooking. Dark gray bars = RDS; white bars = SDS; lilght gray bars = RS. Different letters within the same fractions indicate significant difference ($p < 0.05$) by Duncan's multiple range test.

crystallites in AS-modified starches could melt during HTT, and the temperature may favor propagation and/or maturation of crystals. At this point, the unmelted portions of the crystalline structure of AS-modified starches can function as nuclei for propagation and maturation steps, leading to the growth of crystallites. Hence, crystalline structures that developed during AS modification could become more perfect during HTT, increasing the DRC of AS-HTT starches to a greater extent than that of AS-modified starches.

When the same moisture content was applied, DRC was higher in AS-HTT-waxy starches than in AS-HTT-normal starches, which was consistent with the melting enthalpy (ΔH) measured by DSC. Amylose may have acted as a diluent that intrudes on the interaction of amylopectin double helices, preventing their development into perfect crystals. This is consistent with a previous report[34] proposing that amylose disrupts the crystalline packing of amylopectin.

Thermal Transition Properties. Before AS modification, all starches underwent gelatinization by boiling. Therefore, DSC thermograms of modified starches displayed the retrogradation endotherm caused by the melting of reassociated amylose and amylopectin. Onset (T_o), peak (T_p), and conclusion (T_c) temperatures, the melting temperature range ($T_r = T_c - T_o$), and melting enthalpy (ΔH) comprise the gelatinization thermal transition properties for raw starches and retrogradation thermal transition properties for modified starches.

Raw rice starches showed endothermic thermograms ranging from about 58 °C to above 70 °C. For raw normal rice starch, there was an additional peak at 95 °C, representing amylose–lipid complexes[35] (data not shown). Endothermic peaks were not observed for the AS-control starches. These results are consistent with X-ray diffraction patterns, showing considerable amorphous regions. The appearance of endothermic peaks in the AS-modified starches suggested that elongated chains were related to the degree of retrogradation. As shown in Table 1, AS modification decreased the proportion of relatively short chains and increased the proportion of longer chains. This higher proportion of long chains could be more effective than short chains for recrystallization and formation of denser crystalline structures. Yuan et al.[36] also previously proposed that the

DOI: 10.1021/acs.jafc.6b01055
J. Agric. Food Chem. 2016, 64, 5045−5052

retrogradation process is influenced by the chain length distribution of amylopectins.

In AS-HTT-modified starches, T_o and T_p gradually increased with moisture content, while T_r decreased. This reflects the fact that HTT of AS-modified starches caused further rearrangement, leading to a more stable, dense, and rigid crystalline structure. T_o and T_c are related to the melting of the weakest and strongest crystallites, respectively.[37,38] Increased T_o suggests that HTT made the weakest crystallites of AS-modified starch stronger. Some AS-HTT-modified starches had a narrower T_r than AS-modified starches, indicating that some crystallites with high homogeneity or uniform sizes and perfection level were developed by HTT.

According to a previous study,[39] enthalpy is due to the melting of imperfect amylopectin-based crystals, with potential contributions from both crystal packing and helix melting enthalpies. Thus, HTT could cause higher crystalline perfection and formation of larger crystallites.[31,40] These results were in agreement with the DRC results. AS-HTT-modified waxy rice had a greater ΔH than AS-modified starch, whereas the ΔH for normal starches remained constant. Since it has been proposed that amylose disrupts the crystalline packing of amylopectin,[34] this difference suggests that the crystalline structure of dually modified starch could be influenced by the presence of amylose, as discussed earlier.

In Vitro Digestibility. The RDS, SDS, and RS content of starch samples are presented in Figure 3A. Raw normal and waxy rice starches showed a typical digestibility pattern of A-type cereal starches with considerable SDS content. The AS-control starches showed very high RDS content in both normal and waxy rice starches. When raw starch was gelatinized for easy access of AS, both crystalline and semicrystalline structures were destroyed. As a result, the AS-control starches contained amorphous regions to a considerable extent that were easily hydrolyzed by α-amylase.

AS modification improved the slow digestion properties of starches. The RS content in normal-AS-control starch considerably increased from 12.0 to 42.6% through AS modification, but there was only a small increase in SDS content. The effect of AS modification on RS content of waxy starch was very similar to that of normal starch. However, the change in SDS content of waxy starch after AS modification was significantly different ($p < 0.05$) from that of normal starch. Both SDS and RS contents of AS-modified waxy rice starch increased more than 4-fold and 3-fold, respectively, compared with the AS-control. Although a similar rise in RS content was observed in normal and waxy starches, the effect of AS modification on SDS content was greater in waxy starch than in normal starch. This was in agreement with previous results.[12] Generally, tolerance to hydrolysis by digestive enzymes increases with retrogradation.[33] The degree of retrogradation tends to be higher as the amount of long branched chains increases,[41] indicating that the elongation of branched chains by AS could be a major cause of the increase of SDS and/or RS. Increments in SDS and RS fractions by AS modification were verified in vitro and also in vivo, which showed lowered maximum glucose levels and delayed peak times after consumption in mice.[42]

Regardless of starch composition, HTT of AS-modified starches commonly reduced RDS content gradually with increasing moisture level. However, the effect of HTT on SDS and RS contents of AS-modified starches was dependent on the starch composition. In the case of AS-modified starch, HTT decreased the content of RDS by converting it to SDS and RS, indicating the concurrent increases in SDS and RS through HTT. The yield of conversion to SDS and RS was higher after HTT under high moisture contents. However, the result of the digestibility test for AS-HTT waxy starches was beyond expectations. The RDS and SDS of AS-modified waxy starch were concurrently converted to RS, making it less accessible to digestive enzymes. HTT at 40% moisture level resulted in an almost complete conversion of RDS in AS-modified waxy starch to RS. Many researchers have claimed that HTT of granular starch changes its digestion pattern, increasing or decreasing the content of SDS and RS, depending on the starch origin and treatment conditions.[9,31,43] When the starch is subjected to HTT, changes in granule stability, crystalline orientation, and starch chain interactions can alter the digestibility of starch.[44] The current study applied HTT to retrograded starches, in contrast to previous studies on native granular starches. Because of the different starch states, the explanation for the effect of HTT on granular starch could not be directly applied to trace the phenomenon that happened in this study.

HTT could promote the development of crystalline structures by rearranging starch molecules in amorphous regions.[9] The decrease in RDS content in AS-HTT-modified starches reflected the formation of a more compact crystalline structure due to the interaction between elongated long chains. The dramatic increase in the SDS as opposed to the RS content of AS-HTT-normal rice starches indicated that the crystalline structure that developed during HTT was not perfect, compact, or rigid enough to form RS. In contrast, RS formation was favored in AS-HTT-waxy rice starch, which showed a 20−50% increase with addition of 25−40% of water. The HTT of AS-modified waxy starch could create a more ordered semicrystalline structure, resulting in highly ordered structured RS. The results also suggested that dual modification of starch from different origins caused different crystalline orders, thereby affecting starch digestibility.

We also examined the effects of cooking on changes in the digestion properties of modified starches (Figure 3B). Cooking did not significantly change the digestive properties of AS-modified normal starch ($p > 0.05$, Figure 3). Cooking markedly increased the RDS contents of AS-HTT-modified normal starches in concurrence with a decrease in their SDS contents, resulting in values similar to those of AS-modified rice starch. However, their RS contents rarely changed after cooking. The AS-modified waxy rice starch showed little change in RS content, but it lost a significant amount of SDS ($p < 0.05$) after cooking. The cooked AS-HTT-modified waxy rice starches also showed a large increase in RDS content and large decreases in SDS and RS contents. Therefore, the comparison of in vitro digestibility in uncooked and cooked AS-HTT-modified starches suggested that HTT strengthened the crystalline structures and increased their perfection levels in AS-modified starches. However, those crystalline structures strengthened by HTT did not have enough heat stability to maintain their rigid structures under cooking conditions.

In conclusion, dual modification could remarkably alter the structural physicochemical characteristics of starch, leading to tolerance to enzymatic hydrolysis and increases in SDS and RS content. Through the elucidation of the effect of elongated chains and re-formation of crystal structures, this study helps explain how dual modification affects the physicochemical and digestion properties of rice starches dependent on the presence

DOI: 10.1021/acs.jafc.6b01055
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of amylose. The changes in physicochemical properties induced by dual modification could be used for development of food products with high SDS and RS content.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.6b01055.

Level combinations in central composite response surface design and digestibilities of AS-modified waxy rice starches (PDF)

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Funding

This work was supported by a National Research Foundation of Korea (NRF) grant funded by the Korean government (Ministry of Science, ICT & Future Planning, No. NRF-2014R1A2A1A11050918).

Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

AS, amylosucrase; HTT, hydrothermal treatment; DP, degree of polymerization; XRD, X-ray diffraction; DRC, degree of relative crystallinity; RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch; T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; T_r, melting temperature range ($T_r = T_c - T_o$); ΔH, melting enthalpy

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