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Research Note

Low Digestion Property of Amylosucrase-modified Waxy Adlay Starch

Eun Jeong Kim[1], Ha Ram Kim[1], Seung Jun Choi[2], Cheon-Seok Park[3], and Tae Wha Moon[1,4,*]

[1]*Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Korea*
[2]*Department of Food Science and Technology, Seoul National University of Science and Technology, Seoul 01811, Korea*
[3]*Department of Food Science and Biotechnology, Kyung Hee University, Yongin, Gyeonggi 17104, Korea*
[4]*Center for Food and Bioconvergence, and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea*

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*Corresponding Author
Tel: +82-2-880-4854
Fax: +82-2-873-5095
E-mail: twmoon@snu.ac.kr

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Abstract Structural and digestion properties of amylosucrase-modified waxy adlay starch were investigated. The unique reaction of amylosucrase caused a decrease and an increase in the proportion of short chains and long chains, respectively, via attachment of glucosyl units to the non-reducing ends of branch chains. The *in vitro* digestion profile of amylosucrase-modified starch revealed that elongated branch chains were the main reason for high contents of slowly digestible and resistant starches due to formation of a more perfect crystalline structure via easy association between elongated branch chains. The glucose response in mice after consumption of amylosucrase-modified starch was similar to the response for commercial resistant starch with a gradual increase followed by a gradual decrease in blood glucose concentrations over a prolonged time. Both *in vitro* and *in vivo* tests were used to verify increased resistance to digestive enzymes caused by amylosucrase modification.

Keywords: amylosucrase, adlay starch, digestibility, blood glucose level

Introduction

Starch is an important dietary energy source for humans. For nutritional purposes, starch is generally classified into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) depending on the rate of digestion (1). RDS is digested rapidly and, thus, directly affects the blood glucose level. SDS is defined as a starch fraction that is digested slowly, but completely. RS is starch that is not digested in the small intestine but is fermented by microbial flora in the large intestine to produce short chain fatty acids. Therefore, SDS and RS are drawing attention as ingredients for low glycemic index foods to lower the risk of developing diabetes and cardiovascular diseases (2,3).

Recently, production of starch with high SDS and RS contents via enzymatic modification has been preferred rather than using other methods due to safety and health issues for both humans and the environment (4,5). In this study, amylosucrase (E.C. 2.4.1.4) from *Neisseria polysaccharea* was used to produce a starch with low digestibility. Amylosucrase catalyzes a transglycosylation reaction to produce (1→4)-α-glucans using sucrose as a substrate while releasing fructose (6). In the presence of an acceptor containing glucosyl units, amylosucrase catalyzes elongation of external chains at non-reducing ends (7,8). Previous studies reported that amylosucrase effectively increased the *in vitro* digestion resistance of starches (9-12). However, there are apparently no reports that verify the low glycemic effect of amylosucrase-modified starch *in vivo*.

In this study, waxy adlay starch was used as an acceptor of amylosucrase. Adlay (*Coixlachryma-jobi* var. *mayuen* stapf., called soft shelled Job's tears) is a grass crop widely grown in East Asia that has long been used for traditional medicine and food products (13). However, attempts for efficient use of adlay starch are lacking although grains are abundant in starch (14-17). Therefore, in this study, adlay starch was modified using amylosucrase to increase the digestion resistance, and the glycemic effect was examined both *in vitro* and *in vivo*.

Materials and Methods

Materials Raw waxy adlay was obtained from an experimental field at the National Crop Experiment Station located in Yeoncheon-gun, Gyeonggi-do, Korea. Adlay starch was isolated following an alkaline steeping method with slight modification, as previously described (14). The gene of *Neisseria polysaccharea* amylosucrase (AS) was cloned and expressed in *Escherichia coli*, and AS was purified following the method of Jung *et al.* (18). One unit of AS

corresponded to the amount of enzyme that catalyzed production of 1 μmol of fructose per min in the presence of 8 g/L sucrose and 2 g/L glycogen. All reagents were of at least analytical grade.

Preparation of AS-modified starch Enzyme modification was performed as described in a previous study (10). Briefly, 2% (w/v) starch suspension in a 100 mM sodium citrate buffer at pH 6.0 containing 100 mM sucrose was prepared to reach a final volume of 30 mL. The starch suspension was boiled for 10 min, then cooled to 30°C. AS was added at 40,000 U followed by incubation in a waterbath at 30°C and 80 rpm for 40 h for reaction. The enzyme reaction was stopped using addition of 3 volumes of ethanol, and AS-modified starch was collected by centrifugation at 10,000×*g* for 10 min. Control sample was prepared following the same procedure, except for enzyme addition.

Characterization of structural properties The branch chain distribution of starch samples was determined after debranching using a Dionex-300 high-performance anion exchange chromatography system (Dionex, Sunnyvale, CA, USA) with a pulsed amperometric detector following a previous method (12). Crystalline properties were characterized using a D5005 X-ray diffractometer (Bruker, Karlsruhe, Germany) and a Diamond differential scanning calorimeter (DSC) (Perkin-Elmer, Waltham, MA, USA), as previously described (12). Mean values and standard deviations were obtained from triplicate experiments.

Determination of starch fractions Following the method of Englyst *et al.* (1) with modification described by Shin *et al.* (19), starch was categorized as RDS, SDS, and RS, which were hydrolyzed into glucose within 10 min, 10-240 min, and not hydrolyzed within 240 min, respectively. This procedure produced RDS, SDS, and RS contents that were similar to contents produced using the method of Englyst *et al*. (1).

Glucose tolerance testing in mice Eight female ICR mice were used for each treatment group and individually housed in an approved laboratory animal facility for a 7-day adaptation period. Mice were given a 500 μL of 7.5% (w/v) starch suspension or a 7.5% (w/v) glucose solution via an oral zonde needle after 16 h of fasting. Blood samples were taken from the tail artery at 0, 30, 60, 90, 120, 150, 180, and 240 min after consumption of the starch suspension or glucose solution, and blood serum glucose levels were measured using an Accu-Chek Active Glucose System (Roche Ltd., Basel, Switzerland). All institutional and national guidelines for the care and use of laboratory animals were followed.

Results and Discussion

Characterization of structural properties Structural properties of

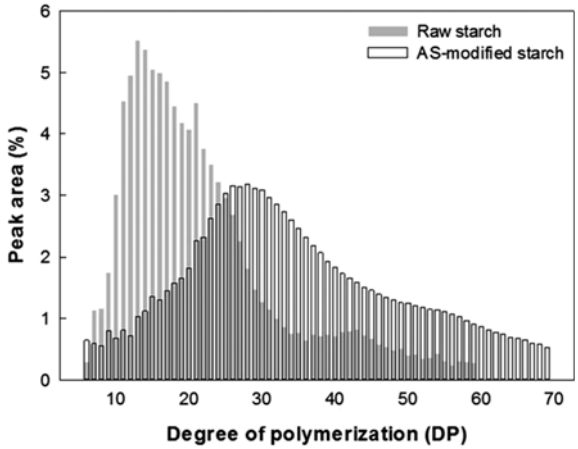

Fig. 1. Branch chain length distributions of raw and AS-modified starches.

AS-modified adlay starch exhibited consistency with previous reports (9-11). AS-modified adlay starch had a broader branch chain length distribution range than unmodified starch, showing a decrease in the proportion of short chains and the opposite for long chains (Fig. 1). The degree of polymerization (DP) of the most abundant chain and the longest detectable chain were shifted from DP 13 to 28, and from DP 59 to 69, respectively. The proportion of long chains (DP 25) showed an increase of approximately 44.0%, indicating that intense attachment of glucosyl units at non-reducing ends occurred via an AS reaction.

Native waxy adlay starch displayed an A-type crystalline structure with 43.9±0.8% relative crystallinity. The crystalline structure of control starch disappeared due to a gelatinization process, resulting in a low degree of crystallinity of 18.2±1.7%. The relative crystallinity of AS-modified starch was 29.1±1.1% with a B-type crystalline pattern that is generally observed in retrograded starch (20), indicating development of a more densely organized crystalline structure than for control starch (10,12,21). Association among elongated chains during an AS reaction can be understood as a phenomenon similar to retrogradation, which is accelerated by amylopectin with a longer average chain length (22,23). DSC results for high melting temperatures of AS-modified starch indicated development of crystalline structure. While control starch showed no endothermic peak, the melting enthalpy of AS-modified starch was 10.8±0.1 J/g, with a range of 72.3-112.3°C with a peak temperature of 93.9±0.1°C. The relatively broad melting range indicated that AS-modified starch had a heterogeneous structure (24) adopted via elongation, which corresponded to the polydispersity value (data not shown).

Digestion of AS-modified starch Raw starch showed a typical *in vitro* digestion profile for granular starch with an A-type crystalline structure, and control starch showed a high RDS content (Table 1). AS-modified starch showed a low RDS content that was half of the control starch value, but SDS and RS contents were increased,

Table 1. RDS, SDS, and RS contents of raw, control, and AS-modified starches

Starch fraction[1)]	Starch sample		
	Raw	Control	AS-modified
RDS (%)	31.3±1.1[b2)]	49.8±0.8[a]	24.5±1.2[c]
SDS (%)	58.3±1.0[a]	17.2±1.8[c]	24.0±0.1[b]
RS (%)	10.4±1.8[c]	33.0±2.3[b]	51.5±1.4[a]

[1)]RDS, rapidly digestible starch; SDS, slowly digestible starch; RS, resistant starch

[2)]Values with different superscripts within a row are significantly different (p<0.05) based on Duncan's multiple range test.

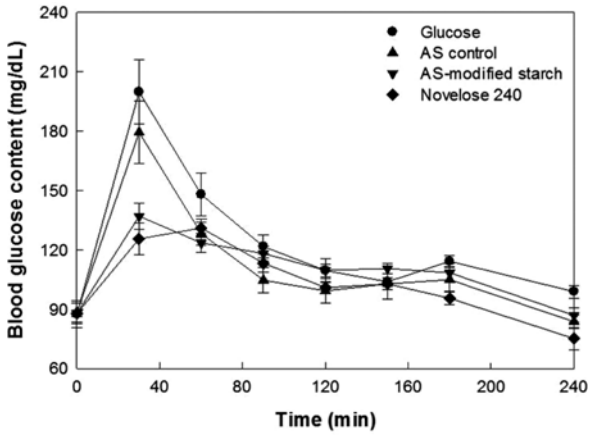

Fig. 2. Mean blood glucose concentrations in mice after intake of starch samples.

compared with control starch. Changes in digestibility, a decrease in the RDS content, and increases in SDS and RS contents were probably related to elongation of branch chains and elevation of relative crystallinity due to the AS reaction (10,12). The primary reason for an increase in SDS and RS contents in AS-modified starch was probably easy reassociation among elongated chains as abundant long chains tended to form more perfect crystalline structures with a reduced degree of susceptibility to enzymatic hydrolysis.

Postprandial blood glucose levels in mice after uptake of glucose, control starch, AS-modified starch, and Novelose 240 (Ingredion, Westchester, IL, USA) were measured (Fig. 2). The maximum glucose levels throughout the measurement range for glucose, control starch, AS-modified starch, and Novelose 240 were 199.8, 179.4, 137.0, and 131.0 mg/dL, respectively. A negative correlation between the RS content and the maximum glucose level was identified (r= −0.986, p<0.01), indicating that the high RS content caused a low glucose response. Glucose and control starch caused increases in postprandial blood glucose levels for the first 30 min after consumption, then steep decreases of approximately 50 mg/dL in the following 30 min. In contrast, AS-modified starch caused a gradual change with a steady blood glucose level over 30-90 min. This pattern was similar to the pattern for Novelose 240, which contained 54% RS, indicating the dominant RS characteristics of AS-modified starch. AS-modified starch allowed a sustained blood glucose level enabling a continuous supply of blood glucose throughout the digestive process, based on *in vivo* analysis.

In conclusion, reduction of the RDS content and increases in SDS and RS contents occurred after AS modification. Reduced *in vitro* digestibility caused by AS modification was indicated by lower maximum blood glucose levels and delayed peak times after consumption in mice. The reduced digestibility of AS-modified starch was verified both *in vitro* and *in vivo*, suggesting use for food applications requiring a low glycemic response.

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Disclosure The authors declare no conflict of interest.

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