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Preparation of slowly digestible sweet potato Daeyumi starch by dual enzyme modification

A Ra Jo[a], Ha Ram Kim[a], Seung Jun Choi[b], Joon Seol Lee[c], Mi Nam Chung[c], Seon Kyeong Han[c], Cheon-Seok Park[d], Tae Wha Moon[a,e,*]

[a] Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea
[b] Department of Food Science and Technology, Seoul National University of Science and Technology, Seoul 01811, Republic of Korea
[c] Bioenergy Crop Research Institute, National Institute of Crop Science, Rural Development Administration, Muan-gun, Jeollanam-do 58545, Republic of Korea
[d] Department of Food Science and Biotechnology, Kyung Hee University, Yongin 17104, Republic of Korea
[e] Center for Food and Bioconvergence and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea

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ABSTRACT

Sweet potato Daeyumi starch was dually modified using glycogen branching enzyme (BE) from *Streptococcus mutans* and amylosucrase (AS) from *Neisseria polysaccharea* to prepare slowly digestible starch (SDS). Dually modified starches had higher SDS and resistant starch (RS) contents than control starch. The branched chain length distributions of the BE-modified starches indicated an increase in short side-chains [degree of polymerization (DP) ≤ 12] compared with native starch. AS treatment of the BE-modified starches decreased the proportion of short side-chains and increased the proportion of long side-chains (DP ≥ 25) and molecular mass. It also resulted in a B-type X-ray diffraction pattern and an increased relative crystallinity. Regarding thermal properties, the BE-modified starches showed no endothermic peak, whereas the BEAS-modified starches had a broader melting temperature range and lower melting enthalpy compared to native starch. The combined enzymatic treatment resulted in novel glucan polymers with slow digestion properties.

1. Introduction

Starch is the main carbohydrate in the human diet. According to the rate and extent of starch digestion *in vitro*, starch has been classified into three major fractions: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). RDS is rapidly digested and absorbed in the human small intestine, leading to a rapid elevation of blood glucose. SDS, as an intermediate starch fraction between RDS and RS, is digested slowly through the entire small intestine to provide sustained glucose release with a low initial glycemia and a subsequent slow and prolonged release of glucose (Lehmann & Robin, 2007). Another benefit of SDS-rich starch is its moderate impact on the glycemic index (GI). Clinical data have shown that a low GI diet is linked to a reduced risk of diabetes and cardiovascular disease (Jenkins et al., 2002). The glucose release property of SDS satisfies the fundamental basis for the beneficial effects of a true low-GI food with a slow and prolonged release of glucose (Zhang & Hamaker, 2009). RS refers to the starch fraction that cannot be digested in the small intestine, but is fermented in the large intestine into short-chain fatty acids (Englyst et al., 1992).

Recent studies on the production of SDS using physical, chemical, and enzymatic methods have been reported (He, Liu, & Zhang, 2008; Li et al., 2014; Miao et al., 2014; Shin, Kim, Ha, Lee, & Moon, 2005). Among them, enzymatic treatment has many advantages as it is safer for the environment as well as consumers and has more specific reactions and fewer by-products. The molecular structure of starch is thought to be the key determinant for its functionality in regard to food and nutritional properties (Zhang & Hamaker, 2009). Ao et al. (2007) employed various amylases and a transglucosidase to control the branch density and length to produce SDS using partial hydrolysis of normal maize starch. Miao et al. (2014) also tried dual enzyme treatment with β-amylase and transglucosidase to obtain maize starch of increased slow digestion property.

Glycogen branching enzyme (1,4-α-glucan branching enzyme, EC 2.4.1.18; BE) catalyzes the formation of α-1,6 branching points by transglycosylation in amylopectin and amylose, thereby

* Corresponding author at: Department of Agricultural Biotechnology, Seoul National University, Seoul 08826, Republic of Korea.
E-mail address: twmoon@snu.ac.kr (T.W. Moon).

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producing highly branched glucans. It cleaves α-1,4 glucans into linear chains and attaches a string of several glucose units to α-1,4 glucans, forming the α-1,6 glycosidic linkages. Therefore, BE could produce highly branched cyclic glucans by intramolecular α-1,6-branching activity (Takata, Takaha, Okada, Takagi, & Imanaka, 1996). Also, BE cleaves the α-1,4 glucosidic bond of the segment between clusters to produce amylopectin cluster from amylopectin (Le et al., 2009). Amylosucrase (AS; EC 2.4.1.4) is widely used to catalyze the transglycosylation reaction to produce an insoluble α-1,4 glucan using sucrose, releasing fructose (De Montalk, Remaud-Simeon, Willemot, Planchot, & Monsan, 1999). Rolland-Sabate, Colonna, Potocki-Veronese, Monsan, and Planchot (2004) suggested that AS accelerates the elongation of some external chains at their nonreducing ends in the presence of an acceptor such as a glucosyl unit.

Sweet potato is one of the most important starch-producing crops worldwide. Although the trend in utilization of sweet potato is shifting away from a staple food to a processed food (Zhang, Wheatley, & Corke, 2002), very few studies have enhanced the nutritional quality of sweet potato starch to create value-added products. Enzymatic modification of starch can be applied to improve sweet potato starch and facilitate the development of new dietary applications using the slow digestion properties. The objectives of this study were to enhance the SDS content of sweet potato Daeyumi starch by changing its branch density and chain length using dual enzymatic modification and to elucidate the relationship between the changes in physicochemical characteristics during enzyme treatment and the digestibility of enzyme-treated starch.

2. Materials and methods

2.1. Materials

Daeyumi, a newly developed sweet potato variety, was obtained from Bioenergy Crop Research Institute, Rural Development Administration, Korea. Starch was isolated from sweet potato by alkaline extraction method (Yamamoto, 1981). Its apparent amylose content was 18.8%, determined following the method of Jayakody and Hoover (2002). Glycogen branching enzyme (BE) from *Streptococcus mutans* was obtained from the Food Enzyme Biotechnology and Functional Carbohydrate Laboratory of Yonsei University (Seoul, Korea). Dimethylsulfoxide (DMSO) and dinitrosalicylic acid (DNS) were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). All other reagents were of analytical grade.

2.2. Enzyme assay of branching enzyme and amylosucrase

BE activity was determined according to the procedure of Takata et al. (1994) with a slight modification. The substrate solution (50 μL, 0.1% (w/v) potato amylose type III (A0512, Sigma–Aldrich Co.) in 90% (v/v) DMSO was mixed with a diluted enzyme solution (50 μL) in 50 mM Tris–HCl buffer (pH 7.5) and the mixture was incubated at 30 °C for 20 min. The reaction was stopped by the addition of 1 mL of iodine reagent which was prepared by mixing iodine solution (0.026 g of I_2 and 0.26 g of KI in 260 mL distilled water) with 1 M HCl in 1:1 volume ratio, and the absorbance at 660 nm was measured. One unit (U) of BE activity corresponds to the amount of enzyme that degrades 1 μg/mL of amylose per min under the conditions described above.

The gene of AS from *Neisseria polysaccharea* was cloned and expressed in *Escherichia coli* BL21 (DE3). The enzyme was purified by affinity chromatography using Ni–NTA (nickel–nitrilotriacetic acid) resin (Qiagen, Hombrechtikon, Switzerland). AS activity was determined following the method of van der Veen et al. (2004) with a slight modification. A mixture of 100 μL of 4% (w/v) sucrose, 100 μL of 1% (w/v) glycogen, 250 μL of 50 mM Tris–HCl buffer (pH 7.0) and 50 μL of diluted enzyme in that buffer was prepared and reacted for 10 min in a shaking water bath at 30 °C and 80 rpm. The released fructose was quantified using DNS method (Miller, 1959). One unit (U) of AS activity was defined as the amount of enzyme that catalyzes the consumption of 1 μmol of sucrose per min in the assay conditions.

2.3. Preparation of dual enzyme-modified starch

Sweet potato starch (2%, w/v) was suspended in 50 mM Tris–HCl buffer (pH 7.5) to the final volume of 30 mL. The suspension was boiled with stirring for 30 min. After the solution was cooled to 30 °C, an appropriate amount of BE was added (0–48 U per mL of starch suspension) and incubated at 30 °C for 1 h. Enzyme reaction was terminated by placing the reaction mixture in boiling water for 30 min, and the pH was adjusted to 7.0 by adding 1 M HCl. Sucrose was added to be 200 mM, and the solution was cooled to 30 °C. Then, AS (1,333 U per mL of starch suspension) was added and incubated at 30 °C for 24 h. Three volumes of ethanol were added to stop the AS reaction, and it was washed three times with distilled water by centrifugation (10,000 × *g*, 10 min). The dual enzyme-modified starch samples were recovered, freeze-dried, ground, and put through a 100-mesh sieve. The same procedure was performed without AS addition for comparison (BE-modified starches). The BEAS-control was prepared following the same process for enzymatically-modified starch preparation without enzyme addition. All the experimental data were expressed as mean ± standard deviation of triplicates.

2.4. Determination of degree of branching

To examine the degree of branching, the changes in the amount of reducing sugars were determined by the DNS method before and after isoamylolysis of the BE-modified starch. Reducing power of the starch debranched by isoamylase (Megazyme, Bray, Ireland) was measured. Starch (10 mg) was dissolved in a mixture of 100 μL of 1 M NaOH and 200 μL of distilled water and boiled for 30 min. After the adjustment of pH to 7.0 using 1 M HCl, distilled water was added to a final volume of 1 mL. Sample solution (0.5 mL) was transferred into a microtube, and 0.49 mL of 50 mM sodium acetate buffer (pH 4.3) and isoamylase (10 μL, 1,000 U/mL, Megazyme) were added. This mixture was incubated at 45 °C for 2 h and boiled for 10 min to stop the reaction. The resultant mixture (0.5 mL) was transferred into a microtube, and DNS solution (0.5 mL) was added. The solution was boiled for 5 min and cooled immediately under iced water for 10 min. The absorbance was measured at 575 nm.

2.5. Determination of branched chain length distribution

The distribution of branched chain length of starches was determined after debranching the starch. Debranching method followed the procedure described above. Debranched sample was filtered through a 0.45-μm membrane filter and analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on CarboPac PA1 anion-exchange column (4 × 250 mm, Dionex, Sunnyvale, CA, USA). DP values from 1 to 7 were designated using a mixture of maltooligosaccharides (Sigma–Aldrich Co.).

2.6. Determination of molecular mass distribution

Starch (25 mg) was dispersed in 90% (v/v) DMSO (5 mL) and boiled for 15 min with vortexing at intervals. Ethanol (25 mL) was

added to this dispersion to precipitate starch. The pellet was collected by centrifugation (10,000 × g, 10 min), dissolved into 5 mL of 100 mM $NaNO_3$ solution, and boiled for 15 min. The starch sample was filtered through a 5.0-μm filter and injected into a high-performance size-exclusion chromatography-multi-angle laser-light scattering-refractive index (HPSEC-MALLS-RI) system, consisted of a pump (Jasco, Tokyo, Japan), with a 200 μL injector loop, a degasser (NO-OX Vacuum Station, Alltech, Deerfield, IL, USA), a multi-angle laser light scattering detector (DAWN DSP, Wyatt Technology, Santa Barbara, CA, USA), a differential refractive index detector (Opti-Lab, Wyatt Technology), and Shodex OH-Pak 804 and 806 columns (Showa Denko, Tokyo, Japan). The mobile phase used was aqueous 100 mM $NaNO_3$ solution containing 0.02% (w/v) sodium azide, which was filtered through a 0.22-μm filter and degassed before use. The flow rate was 0.4 mL/min, and the experimental data collected from the DAWN DSP/OptiLab system were processed with ASTRA software (Version 4.09.07., Wyatt Technology).

2.7. X-ray diffraction pattern and the degree of relative crystallinity

X-ray diffraction analysis was performed with an X-ray diffractometer (Model D5005, Bruker, Karlsruhe, Germany) (analysis parameters: 40 kV and 40 mA, CuK radiation λ = 0.15406 nm, time constant = 4 s, nickel filter). Starch sample was scanned through 2θ range from 3 to 30°. The degree of crystallinity was calculated according to the method of Nara and Komiya (1983) using Origin 7.5 software (MicroCal, Northampton, MA, USA).

2.8. Analysis of thermal properties

Thermal properties of starches were investigated using a DSC (Diamond DSC, Perkin-Elmer, Waltham, MA, USA). Indium was used as a calibration standard substance. An empty pan was used as a reference. Starch (10 mg) was placed in a high-pressure stainless steel pan, and distilled water (40 μL) was added. The sample pan was sealed, kept at room temperature overnight, and heated from 30 to 130 °C at 5 °C/min.

2.9. Starch digestibility

Starch digestibility was measured based on the method of Brumovsky and Thompson (2001) with a slight modification. Pancreatin (2 g, P7545, activity 8 × USP specification, Sigma–Aldrich Co.) from porcine pancreas was added to distilled water (24 mL) and stirred well for 10 min. This pancreatin dispersion was centrifuged (1500 × g, 10 min). The supernatant (20 mL) was moved into a beaker and then mixed with amyloglucosidase (0.4 mL) and distilled water (3.6 mL). For the determination of starch fraction, starch sample (30 mg) was placed into a 2 mL-microtube containing sodium acetate buffer (0.75 mL, 0.1 M, pH 5.2) with a glass bead. The microtube was equilibrated in a shaking incubator (240 rpm at 37 °C) for 10 min. Then, the enzyme solution (0.75 mL) was added, and the starch sample was incubated in a shaking incubator (240 rpm at 37 °C) for 10 or 240 min. Each microtube was collected at 10 or 240 min and immediately put in a boiling cooker for 10 min to deactivate the enzyme. The glucose content of the hydrolyzates after starch digestion was measured using a GOD-POD kit (BCS Co., Anyang, Korea) (Shin, Choi, Park, & Moon, 2010).

The RDS fraction was defined as the amount of glucose released after 10 min of digestion. The SDS fraction was measured as digested amount between 10 min and 240 min hydrolysis. The unhydrolyzed fraction after 240 min of digestion was defined as RS fraction.

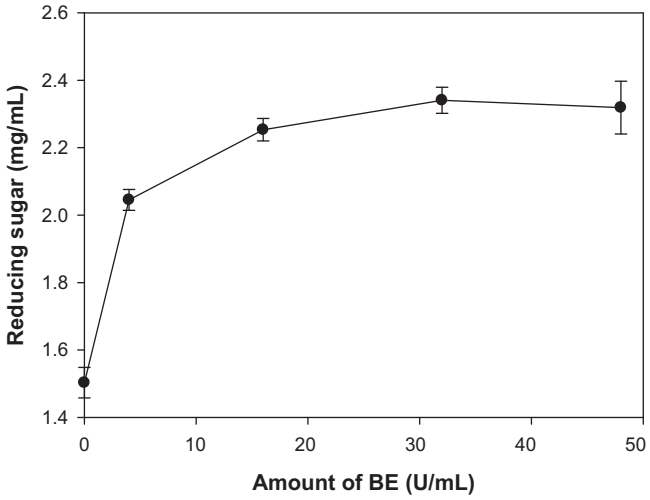


Fig. 1. Reducing power of BE-modified sweet potato starches.

3. Results and discussion

3.1. Reaction of branching enzyme on sweet potato starch

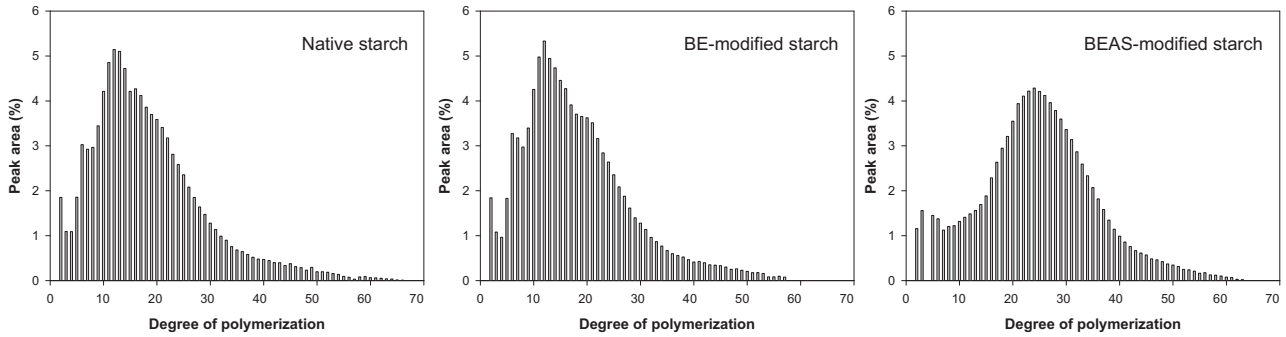
The BE action on sweet potato starch was investigated by determining the amount of reducing sugar after isoamylolysis. As the amount of BE increased, a slight increase in the amount of reducing sugar was observed (Fig. 1). The amount of reducing sugar for the starch modified with 48 U/mL for 1 h was 2.32 mg/mL, 1.5-times higher than the control starch (1.50 mg/mL). The increment in reducing power of the BE-modified starch after isoamylolysis suggested that novel branching points were introduced into sweet potato starch (Kim, Ryu, Bae, Huong, & Lee, 2008).

3.2. Molecular mass and branch chain length distributions of enzymatically modified starch

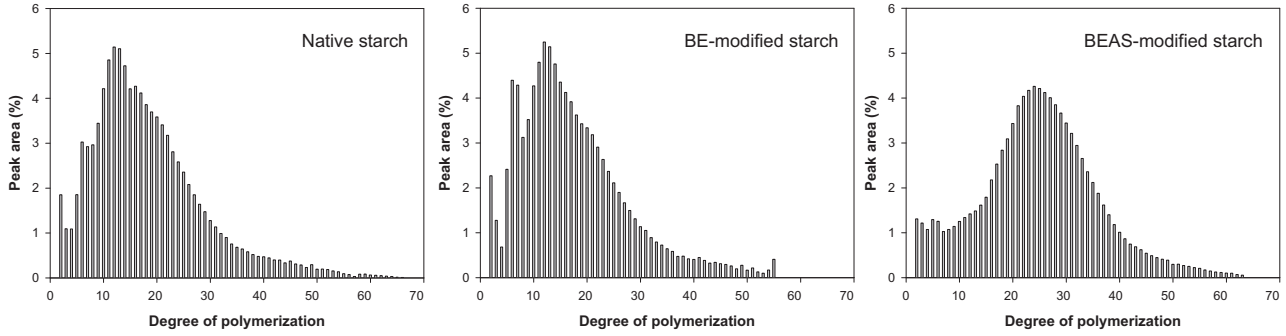
Weight-average molecular masses of enzymatically modified starches determined using a HPSEC-MALLS-RI system are presented in Table 1. The large reductions in molecular masses were observed after BE treatment as reported previously (Le et al., 2009). With increasing amounts of BE, the molecular mass steadily decreased and the reduction level proportionally increased. These results suggested that amylopectin and amylose in sweet potato starch with fairly large molecular masses were extensively modified into highly branched amylopectin clusters by the degrading and branching actions of BE. The molecular mass increased from 1.2- to 2-fold according to the level of BE, as the result of additional AS reaction.

The amylopectin branched chain length distributions of enzymatically modified starches were examined after complete debranching by isoamylase. Grouping of the degree of polymerization (DP) was performed as described by Hanashiro, Abe, and Hizukuri (1996), in which chain length distributions are typically classified into four groups (A, B_1, B_2, and B_3), corresponding to chain lengths of DP 6–12, 13–24, 25–36, and ≥37, respectively. However, since the amylopectin chains were separated into four fractions based on their length, location in amylopectin, and whether they carried other chains, this fractionation is no longer valid for enzymatically modified starches. As shown in Fig. 2, the chain length distribution for unmodified sweet potato starch was characterized by the maximum DP 13–24. After BE treatment, the branched chain length distribution showed a little shift to short chains. Also, the chains longer than DP 60 disappeared. An increased amount of BE resulted in a pronounced maximum at low DP (DP 6–7).

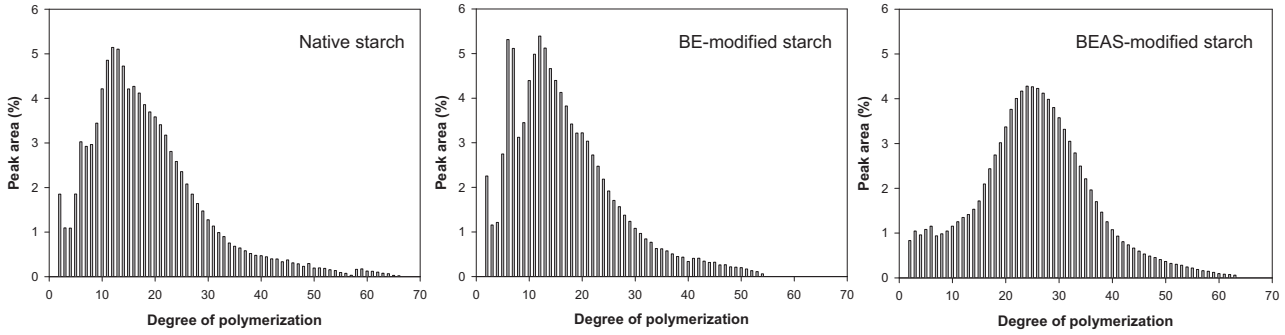
A: starch modified with 4 U of BE



B: starch modified with 16 U of BE



C: starch modified with 32 U of BE



D: starch modified with 48 U of BE

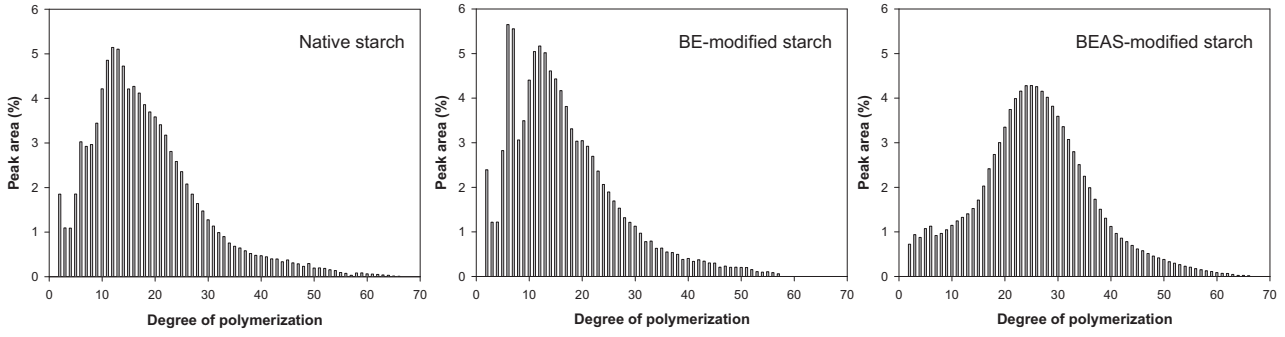


Fig. 2. Branched chain length distributions of BE- and BEAS-modified starches.

Table 1
Molecular mass and relative crystallinity of BE- and BEAS-modified starches.

Native	Weight-average molecular mass ($\times 10^6$ g/mol)		Relative crystallinity (%)	
	64.3 ± 5.1		25.4 ± 0.6	
	BE-modified starch	BEAS-modified starch	BE-modified starch	BEAS-modified starch
Amount of BE				
4 U/mL	6.0 ± 2.7	7.3 ± 2.2	16.4 ± 0.0	18.6 ± 0.0
16 U/mL	2.0 ± 1.6	3.5 ± 1.3	16.3 ± 0.0	19.9 ± 0.0
32 U/mL	1.3 ± 1.0	2.7 ± 1.1	15.3 ± 0.0	22.3 ± 0.0
48 U/mL	1.4 ± 1.1	2.0 ± 1.3	12.5 ± 0.0	25.3 ± 0.0

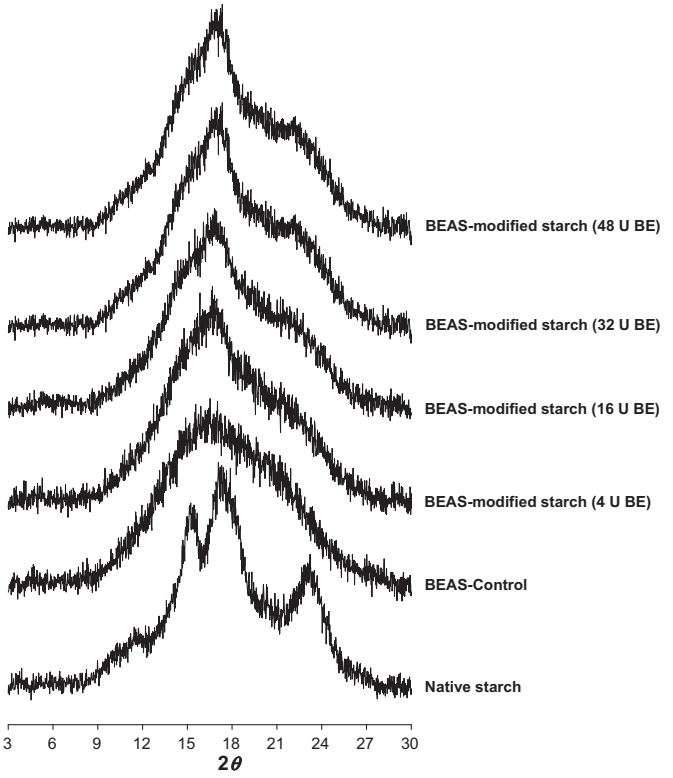


Fig. 3. X-ray diffractograms of BEAS-modified sweet potato starches.

The larger proportion of short chains (DP 6–7) could have been due to the introduction of short-branched chains onto the external amylopectin molecule by BE. These results corresponded to those of a previous study (Kim et al., 2008), suggesting that maltohexaose and maltoheptose were introduced into the branches by BE. The maximum DP in branched chain length distribution for the BEAS-modified starches was revealed over the range of DP 25–36. Elongation by the AS reaction shifted the largest proportion of the DP group from DP 13–24 to 25–36, which was similar to the results of Shin et al. (2010). These changes in branch density and chain length distributions of amylopectin by the dual enzymatic modification could enhance the slow digestion property of the modified starch, due to the formation of more perfect crystallites and reduced accessibility of digestive enzymes (Shin et al., 2010).

3.3. X-ray diffraction pattern and relative crystallinity of enzymatically modified starch

The X-ray diffractograms are displayed in Fig. 3, and Table 1 shows the degrees of relative crystallinities of native and enzymatically modified starches. Native sweet potato starch showed a C_A-type pattern, which has main peaks at 15, 17, 18, and 23°. This pattern corresponded to a C- or C_A-type pattern of sweet potato starch reported by Hizukuri, Kaneko, and Takeda (1983). No peak was detected in the BEAS-control starch, indicating that the crystalline structure disappeared and relative crystallinity decreased because of gelatinization prior to the enzyme reaction. Also, no peak was detected in BE-modified starch, regardless of the amount of BE used (data not shown). In general, starch retrogradation easily occurs in the presence of amylose and amylopectin long chains. However, the unique structure of the BE-modified starch (short and highly branched amylopectin clusters) may not be ideal for retrogradation. After AS treatment of the BE-modified starch, the X-ray diffraction pattern changed from amorphous to B-type with a distinct peak at 22° as described in a previous study (Kim, Kim, Moon, & Choi, 2014). Kalichevsky, Orford, and Ring (1990) also reported that longer chains were related to retrogradation, and Pohu, Planchot, Putaux, Colonna, and Buleon (2004) reported that B-type structures resulted from the aggregation of longer linear chains. Therefore, the development of B-type crystalline structure of the BEAS-modified starches could have been attributable to the elongation of branched chains by AS treatment.

Regarding the relative crystallinity of the BE-modified starches, the intensity of the crystalline pattern decreased with increasing BE (Table 1). In contrast, a marked increase in relative crystallinity was observed for the BEAS-modified starches compared with only BE-modified starch, suggestive of an increase in crystalline regions (Cooke & Gidley, 1992). These results were in accordance with the differential scanning calorimetry (DSC) parameters, which showed increased ΔH after the additional AS treatment.

3.4. Thermal properties of enzymatically modified starch

The stability of the crystal structure of native and enzymatically modified starches has been extensively investigated using DSC. The thermal parameters (T_o, T_p, T_c, and T_r) and the enthalpy change (ΔH) are summarized in Table 2. Native sweet potato starch showed a typical gelatinization pattern with a large gelatinization endotherm appearing at approximately 70 °C, corresponding to the melting of crystallites in native granules (Cruz-Orea, Pitsi, Jamee, & Thoen, 2002). The gelatinization peak was absent in all BE-modified starches, indicating the loss of original crystalline structure (Han et al., 2006).

After AS treatment of BE-modified starches, the dually modified starches showed a higher melting temperature than the native starch. AS could promote the formation of the double helices which contribute to crystalline structure in BEAS-modified starches through interchain association. Because the branched chains elongated by AS reaction bestowed the ordered and crystalline structures on the amorphous region of BE-modified starches, the increased melting temperature could have been the result of a crystalline structure formed with amylopectin long chains (Han et al., 2006).

After AS treatment, the T_r of the BEAS-modified starches increased compared with that of the native and BEAS-control starches. The difference in T_r of starch may be due to the presence of crystallites composed of numerous small crystallites, each possessing slightly different crystal strengths (Vasanthan & Bhatty,

Table 2
Thermal properties of BE- and BEAS-modified starches.

Amount of BE		T_o (°C)	T_p (°C)	T_c (°C)	T_r (°C)	ΔH (J/g)
	Native	61.7 ± 0.6	70.0 ± 0.2	82.3 ± 0.4	20.7 ± 0.9	16.6 ± 1.6
0 U/mL	BE-modified starch	46.7 ± 0.3	54.1 ± 0.3	65.3 ± 0.1	18.6 ± 0.2	2.9 ± 0.2
	BEAS-modified starch	72.1 ± 1.0	91.1 ± 0.1	106.1 ± 0.3	34.0 ± 1.2	8.5 ± 0.4
4 U/mL	BE-modified starch			N.D.		
	BEAS-modified starch	70.5 ± 0.8	89.8 ± 0.5	108.3 ± 2.3	37.8 ± 1.5	8.3 ± 0.5
16 U/mL	BE-modified starch			N.D.		
	BEAS-modified starch	72.6 ± 0.4	90.6 ± 0.4	109.1 ± 1.1	36.4 ± 1.1	8.5 ± 0.6
32 U/mL	BE-modified starch			N.D.		
	BEAS-modified starch	71.7 ± 0.6	89.2 ± 0.6	108.3 ± 2.8	37.6 ± 2.2	9.2 ± 0.7
48 U/mL	BE-modified starch			N.D.		
	BEAS-modified starch	70.7 ± 1.3	91.5 ± 1.8	110.4 ± 1.0	39.6 ± 1.3	12.2 ± 1.9

T_o, T_p, and T_c indicate the onset, peak, and conclusion temperatures of melting, respectively.
$T_r(=T_c - T_o)$ indicates the melting temperature range.
ΔH indicates the melting enthalpy.

1996). Additionally, Lehmann and Robin (2007) suggested that the different percent distribution of chain length could be associated with different sizes and amounts of double helix as well as the crystalline structure of starch. Thus, the broad T_r implies that BEAS-modified starches have various chains composing the crystallites and presumably produced several different crystallites during the enzyme reaction. This diversity of crystallites likely induced the formation of more perfect crystallites and elevated crystallinity values. It could also be a good evidence for that the elongated branched chains by AS modification were in favor to form stronger and more stable crystallites with more homogenous composition (Lopez-Rubio, Flanagan, Gilbert, & Gidley, 2008).

The ΔH ranged from 8.3 to 12.2 J/g for the BEAS-modified starches depending on the amount of BE used, which was lower than native starch but higher than BEAS-control starch. The enthalpy of gelatinization is due to melting of imperfect amylopectin-based crystals, with potential contributions from both crystal packing and helix melting enthalpies (Lopez-Rubio et al., 2008). Because the high proportion of long chains and low proportion of short chains could easily form the relatively more perfect and strong crystallites, the observed increase in ΔH of the BEAS-modified starches could have been caused by longer and more branched amylopectin chains.

3.5. In vitro *digestion pattern of enzymatically modified starch*

The digestion profiles of enzymatically modified starches are given in Table 3. The BE-modified starches showed very similar digestion patterns to the BEAS-control starch, with high RDS content and fairly low SDS and RS contents (the digestion pattern of BEAS-control starch is not shown). However, after the AS reaction of BE-modified starches, the RDS content decreased significantly, whereas the SDS and RS contents were greater compared with no AS-treated starches, regardless of the amount of BE. In the BEAS-modified starches, with increasing amount of BE used, the RDS content gradually decreased with a concomitant increase in SDS and RS contents. The greater extent of branching points produced by higher amount of BE resulted in more accessible reducing ends for elongation reaction of AS. This result showed consistency with the study of Shin et al. (2010) which reported that waxy starches revealed a greater decrease in digestibility than normal starches did after AS treatment.

The previous studies revealed that the fine structure of amylopectin is related to the slow digestion property in some retrograded starches (Ao et al., 2007; Zhang, Ao, & Hamaker, 2008; Zhang, Sofyan, & Hamaker, 2008). Amylopectin with a high proportion of either short chains or long chains tends to produce a high amount of SDS, indicating that there is a parabolic relationship between the SDS content and the weight ratio of amylopectin short chains to long chains. If amylopectin has a high amount of branches and short chains, its inherent molecular structure is not favorable for rapid enzyme digestion. On the contrary, if amylopectin has a high weight ratio of long chains to short chains, these long chains could form the slowly digestible structure. Taking into account the above hypothesis, the BE-modified starch would show the slow digestion property due to its high branching point and weight ratio of short chains to long chains. However, our result of *in vitro* digestion of the BE-modified starches did not agree with the previous findings (Ao et al., 2007; Li et al., 2014; Zhang, Ao, et al., 2008; Zhang, Sofyan, et al., 2008). It has been reported that the digestibility of starch is negatively correlated with the molecular weight of starch polymers (Sandhu & Lim, 2008). Therefore, presumably the higher digestion rate of BE-modified starches than had been expected could be due to the great reduction of molecular weight induced by BE reaction, although BE modification could cause an increase in the number of branching points. It implies that the negative effect originated from the BE-induced reduction of molecular weight on the digestibility of BE-modified starches was superior to the positive effect caused by the increased branching points.

Structural changes in the BE-modified starches by the consecutive AS modification seemed to affect the formation of SDS and RS (Table 3). The main reason for the increment in SDS and RS contents could be easy retrogradation of elongated chains after AS treatment (Kim et al., 2014). The large portion of long chains, resulting in a high weight ratio of long chains to short chains in the BEAS-modified starch, tended to form more perfect crystalline structures having the resistance to starch-digestive enzymes. Probably, the double helical structures formed with the long chains, represented in B_2 and B_3 fractions, would be the core structure with slow digestion property, and the chains shorter than B_2 and B_3 may act as anchor points to slow the digestion of branched chain fractions of B_2 and B_3 (Zhang, Sofyan, et al., 2008). Since the BE-modified starch produced under a greater amount of BE had a large ratio of 1,6 linkages to 1,4 linkages, it should have a higher number of anchor points. Therefore, the BEAS-modified starches could show a high SDS content, especially when the BE treatment level was high.

Crystalline parameters showed consistency. As noted earlier, the BE-modified starches showed no endothermic peak, regardless of applied BE unit and their relative crystallinity decreased with increasing BE unit. This reflected the loss of double helical order and crystallinity in amylopectin. However, the BEAS-modified starches showed a marked increase in enthalpy value and relative crystallinity compared with those of their respective BE-modified starches. This indicated that the dual enzymatic modification

Table 3
Contents of RDS, SDS, and RS in BE- and BEAS-modified starches.

Amount of BE		Starch fraction (%)		
		RDS	SDS	RS
	Native	17.4 ± 0.8	40.2 ± 0.8	42.4 ± 1.4
0 U/mL	BE-modified starch	80.8 ± 0.8	6.3 ± 0.2	12.9 ± 0.7
	BEAS-modified starch	40.9 ± 0.8	18.4 ± 1.0	40.6 ± 0.4
4 U/mL	BE-modified starch	76.1 ± 1.7	8.5 ± 2.0	15.4 ± 3.5
	BEAS-modified starch	40.4 ± 0.7	25.0 ± 1.8	34.6 ± 1.0
16 U/mL	BE-modified starch	79.7 ± 1.4	4.8 ± 2.5	15.5 ± 2.6
	BEAS-modified starch	33.1 ± 0.6	28.3 ± 0.5	38.6 ± 1.1
32 U/mL	BE-modified starch	77.6 ± 1.0	5.7 ± 1.3	16.7 ± 1.4
	BEAS-modified starch	35.2 ± 0.9	28.2 ± 1.3	36.7 ± 0.4
48 U/mL	BE-modified starch	75.7 ± 0.6	6.4 ± 1.8	17.8 ± 1.2
	BEAS-modified starch	23.9 ± 1.3	34.8 ± 3.1	41.3 ± 3.1

utilized in this study caused increase in the crystalline regions and the opposite in the armophous regions. Zhang, Venkatachalam, and Hamaker (2006) proposed that SDS plays an important role in the interplay between crystalline lamellae and amorphous lamellae within the crystalline regions. The crystalline regions are more resistant to enzyme hydrolysis, while the amorphous regions are more susceptible.

In sum, both the structural change of amylopectin and retrogradation in the BEAS-modified starches likely contributed to their slow digestion property.

4. Conclusions

Combined enzymatic modification of sweet potato Daeyumi starch with BE and AS resulted in a marked increase in SDS and RS fractions. The SDS and RS contents of dual-enzymatically modified starches increased from 6.3% to 25.0–34.8% and from 12.9% to 34.6–41.3%, respectively, depending on the amount of BE. Accelerated retrogradation of amylopectin influenced by increased number of branch points and elongated chain length brought slow digestion property to the starch. The BEAS-modified sweet potato starch could be utilized as blood glucose level controlling ingredient for food products requiring low glycemic response.

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