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Slow glucose release property of enzyme-synthesized highly branched maltodextrins differs among starch sources

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ABSTRACT

Seven types of starch (waxy corn, normal corn, waxy rice, normal rice, waxy potato, normal potato, and tapioca) were selected to produce slowly digestible maltodextrins by enzymatic modification using a previously developed procedure. Branching enzyme (BE) alone and in combination with β-amylase (BA) were used to increase the amount of α-1,6 branching points, which are slowly hydrolyzed by mucosal α-glucosidases in the small intestine. The enzymatic treatments of all starches resulted in a reduction of the debranched linear chain length distribution and *weight-average* molecular weight. After α-amylolysis of the enzymatically synthesized-maltodextrins, the proportion of branched α-limit dextrins increased, and consequently a reduction in rate of glucose release by rat intestinal α-glucosidases *in vitro*. Among the samples, enzyme-modified waxy starches had a more pronounced effect on an increase in the slow digestion property than normal starches. These enzyme-modified maltodextrins show potential as novel functional foods by slowing digestion rate to attain extended glucose release.

1. Introduction

Plant starches are the main energy source in the human diet. For nutritional purpose, starch is generally classified into three types depending on its digestion rate: rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst & Hudson, 1996). RDS and SDS offer the advantage of a manipulation of postprandial blood glucose levels. A high proportion of SDS relative to RDS indicates a slow and prolonged release of glucose in the gastrointestinal tract. Such attenuated glycemic response profiles have been linked to reduced risk of type 2 diabetes and obesity (Jenkins et al., 2002). Chronic diseases related to unregulated blood glucose levels potentially can be ameliorated by changing the structure of starch to delay its digestion in the small intestine (Ao, Quezada-Calvillo, et al., 2007; Lee et al., 2013).

After food consumption, the starch component is first broken down by salivary and pancreatic α-amylases to linear maltooligosaccharides, mainly maltose and maltotriose, and α-limit dextrins (Englyst & Hudson, 1996), and then digested to glucose by the intestinal brush border α-glucosidases, maltase–glucoamylase and sucrase–isomaltase (Lin et al., 2010). The cleavage of α-1,6 linkages takes place at a slower rate than that of α-1,4 linkages (Zhang & Hamaker, 2009). Therefore, one of the strategies to produce SDS is to increase the amount of α-1,6 linkages (Ao, Simsek, et al., 2007; Lee et al., 2013). Any factors affecting enzyme activity and the susceptibility of substrate to hydrolytic enzymes will influence the rate of starch digestion (Tester, Karkalas, & Qi, 2004b), and as far as SDS is concerned, a main focus is on the substrate property.

Native starch is composed of essentially linear amylose and branched amylopectin, in which amorphous and crystalline elements are found in granule structure with some minor components (*e.g.* lipids, proteins, and phosphates) (Tester, Karkalas, & Qi, 2004a). It has a slow digestion property that depends on the amylose and amylopectin ratio, as well as the fine structure of amylopectin (*e.g.* the chain length distribution and its branching pattern) (Zhang & Hamaker, 2009). Differences in the compositional and structural properties are found among starches isolated from different botanical sources; and even in starches isolated from the same botanical

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source, but with different branching patterns in the amylopectin (Bello-Pérez, Paredes-López, Roger, & Colonna, 1996). Generally, most native starches are ideal SDS in which densely packed amorphous and crystalline regions in the starch granule simply cannot be readily accessed by digestive enzymes and hydrolysis is consequently delayed (Zhang, Ao, & Hamaker, 2006; Zhang, Ao, & Hamaker, 2008; Zhang, Venkatachalam, & Hamaker, 2006). However, the slow digestion property of granular starch is reduced or completely lost during food processing especially in the presence of excess water and high temperature which disrupts starch crystalline structure (Zhang, Venkatachalam, et al., 2006). Gelatinized starch is far more susceptible to digestive enzymes. The most desirable strategy for creating slowly digestible starch-based materials is to have the slow digestion property inherent in the starch molecular structure so that it is retained through processing.

The structural aspects of SDS related to mechanism and health implications have been reviewed (Lehmann & Robin, 2007; Zhang & Hamaker, 2009). The molecular structure of starch molecules is a major factor to achieve slow digestion property. In this respect, various methods have been reported to produce molecular structures that are slowly digestible; physical (Wongsagonsup, Varavinit, & BeMiller, 2008), chemical (Han & BeMiller, 2007), and enzymatic (Ao, Simsek, et al., 2007; Backer & Saniez, 2004; Cai & Shi, 2010; Casarrubias-Castillo, Hamaker, Rodriguez-Ambriz, & Bello-Pérez, 2012; Guraya, James, & Champagne, 2001; Le et al., 2009; Lee, Le, et al., 2008; Lee et al., 2013; Shin et al., 2004; Shin, Simsek, Reuhs, & Yao, 2008). Among these, enzymatic modification is perhaps the most desirable method offering high substrate selectivity and product specificity with low undesirable byproducts compared to physical and chemical modifications (Lee, Le, et al., 2008).

Enzymatic modification of starch to produce SDS usually involves hydrolysis and transglycosylation activities on starch molecules by α-glucosyltransferases (Van der Maarel & Leemhuis, 2013). Branching enzyme (BE, EC 2.4.1.18) is known to catalyze the hydrolysis of α-1,4 linked linear chains of amylose and amylopectin followed by transglycosyl activity to create new α-1,6 linked branch chains (Borovsky, Smith, & Whelan, 1976; Lee, Yoo, Ryu, Kim, & Yoo, 2008), thereby increasing the number of branched points. Also, these structures are often in a cyclized form (Takata, Takaha, Okada, Hizukuri, et al., 1996; Takata, Takaha, Okada, Takagi, & Imanaka, 1996). β-Amylase (BA, EC. 3.2.1.2) catalyzes the hydrolysis of α-1,4 linkages from non-reducing ends to remove maltose residues and partially shortens external branch chain length, as well as increases branch density (Ao, Simsek, et al., 2007; Lee et al., 2013). Using the activities of branching enzyme and β-amylase, new highly branched α-glucans have been produced. A proposed mechanism is shown in Fig. 1 (adapted from Le et al., 2009; Lee et al., 2013; Takata, Takaha, Okada, Takagi, et al., 1996).

In a previous study, we modified waxy corn starch using BE alone and in combination with BA to produce highly branched maltodextrins (Lee et al., 2013). The products showed slow digestion properties at the mucosal α-glucosidase level as well as slow glucose release *in vivo*. In view of the fact that starches from different sources differ in their structural features, seven starches from different botanical sources were selected for the present study to prepare slowly digestible maltodextrins by enzymatic modification using BE and BA. The goal was to make enzyme-modified SDS food starch products with desired slow glucose release property involving attenuation of glucose generation by the mucosal α-glucosidases. Molecular fine structures and digestibility of enzyme-treated starches from various sources were investigated *in vitro* using rat intestinal α-glucosidase-containing powder. This information provides additional information to better understand how sourcing of starch affects the ultimate structure and digestibility of enzyme synthesized highly branched maltodextrins.

2. Materials and methods

2.1. Materials

Waxy corn (WCS), normal corn (NCS), waxy rice (WRS), normal rice (NRS), waxy potato (WPS), normal potato (NPS), and tapioca starches (TS) were used as substrates for enzymatic modification. BE from *Rhodothermus obamensis* (Branchzyme®) was obtained from Novozymes North America, Inc. (Franklinton, NC) and β-amylase (BA) from barley extract (Optimalt® BBA) was obtained from Danisco US Inc., Genencor Division (Cedar Rapids, IA). In our previous work (Lee et al., 2013), we saw no confounding enzyme activities related to our experiments. The glucose assay kit was purchased from Megazyme (Wicklow, Ireland), and all other chemicals were purchased from Sigma–Aldrich Co. (St. Louis, MO).

2.2. Preparation of enzyme-modified starch products

Enzymatic modification of starch was performed using the previously reported method (Lee et al., 2013) with slight modification. Each starch was suspended in 50 mM sodium acetate buffer (pH 6.5, 5% (w/v), 300 mL) and was boiled to achieve pasting. The suspension was incubated with BE (500 U/g dry weight of starch, Novozymes unit) at 65 °C for 24 h with stirring to produce BE-treated starch. After 24 h, BE was inactivated by boiling for 10 min. The sample was dialyzed (MWCO: 3500) in de-ionized water, freeze-dried, and ground (Lab Grinder A10S1, Janke & Kunkel IKA Labortechnik, Germany) to pass through a 100-mesh sieve. To further reduce the proportion of the α-1,4 linkages, BE-treated starch powders were dissolved in 50 mM sodium acetate buffer (pH 5, 10% (w/v), 100 mL) and stirred for 10 min. The suspensions were incubated with BA (0.64% dry weight of starch) at 55 °C for 24 h to produce BEBA-treated starch. The BA was inactivated in a boiling water bath. Then, BEBA-treated starches were dialyzed (MWCO: 3500) in de-ionized water to remove the released oligosaccharides (mainly maltose) from the BA reaction and salt ions from the buffer solution. Samples were freeze-dried and passed through a 100-mesh sieve after grinding.

2.3. Determination of debranched linear chain length distribution using HPAEC

Individual enzyme-treated starches (1% (w/v), 900 μL) were combined with 100 mM sodium acetate buffer (pH 5.0, 100 μL) and then boiled for 20 min. The mixtures were incubated with pullulanase (0.72 U, Megazyme, Wicklow, Ireland) and isoamylase (0.1 U, Megazyme, Wicklow, Ireland) at 40 °C for 48 h to hydrolyze the α-1,6 linkages, using a modified method from MacGregor and Morgan (1984). The enzymes were inactivated by boiling for 10 min. Samples were filtered through a 0.45 μm nylon filter. Debranched linear chain length distributions were measured on a high-performance anion-exchange chromatography (HPAEC) system equipped with an electrochemical detector (ED40, Dionex, Sunnyvale, CA). The filtered sample (25 μL) was injected into a CarboPac PA-100 pellicular anion-exchange column (Dionex, Sunnyvale, CA) that was pre-equilibrated in eluent A (150 mM NaOH) at 1.0 mL/min. Chromatographic separation of the linear oligosaccharides from the samples was achieved by gradient elution from 100% eluent A to 100% eluent B (600 mM sodium acetate in 150 mM NaOH) (Lee, Le, et al., 2008). Chain length distribution with degree of polymerization (DP) up to 60 was characterized as percentage of the total peak area (Hanashiro, Abe, & Hizukuri, 1996). The *weight-average* degree of polymerization (DP_w) of the sample was calculated using the equipment software.

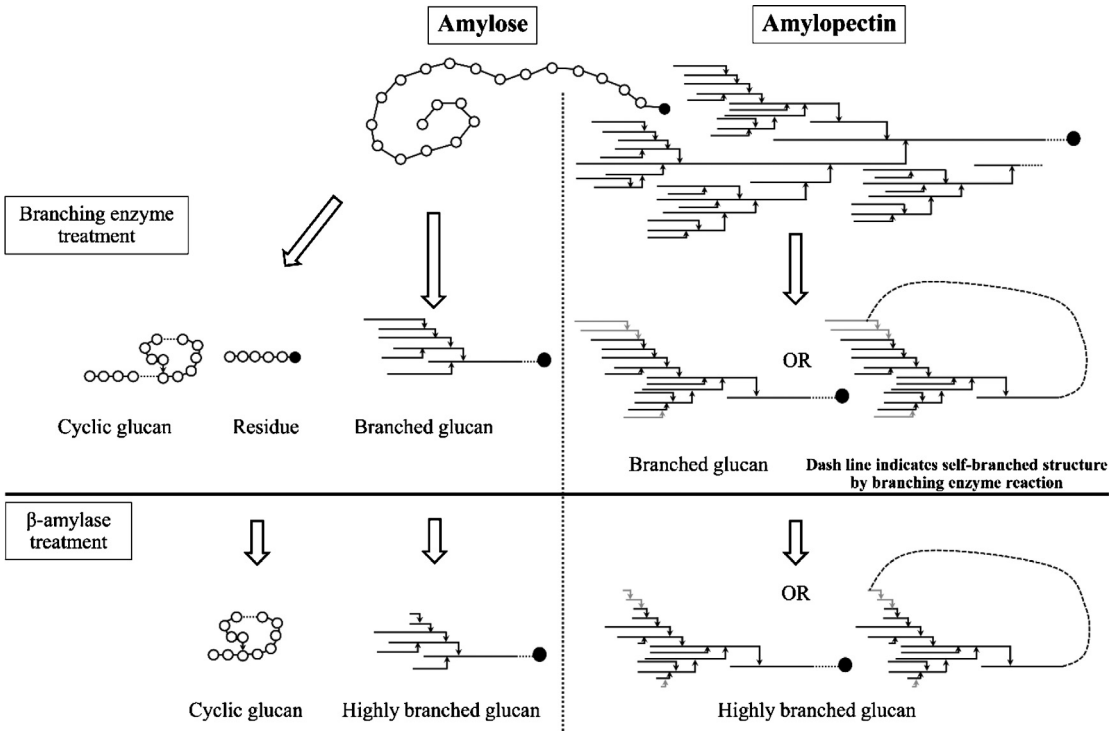

Fig. 1. Schematic diagram of enzymatic modification of starch using branching enzyme and β-amylase. Arrows are α-1,6 glucosidic linkages. The reducing end of a glucan chain is shown by a closed circle and gray line represents a newly generated branched structure ( ).

2.4. Determination of apparent weight-average molecular weight (M_w) by high-performance size-exclusion chromatography (HPSEC)

Apparent *weight-average* molecular weight (M_w) of each enzyme-modified starch was determined by high-performance size-exclusion chromatography (HPSEC) with a refractive index (RI) detector. Starch samples (1%, w/v) were dissolved in de-ionized water with 0.02% sodium azide, boiled for 20 min with stirring, and then filtered through a 0.45 μm nylon filter. The filtered samples (500 μL) were injected into a HPSEC-RI system equipped with a Varian 9010 pump (Varian Associates Inc., Sugar Land, TX), a Varian Star 9040 refractive index (RI) detector, two series HR 10/30 tandem columns, with the first containing Superdex 200 prep grade gel (fractionation range: M_w 1×10^3–1×10^5, GE Healthcare, Piscataway, NJ) and the second containing Superdex 30 prep grade gel (fractionation range: $M_w \leq 1 \times 10^4$, GE Healthcare, Piscataway, NJ). Mobile phase was de-ionized water with 0.02% sodium azide at a flow rate of 0.4 mL/min (Zhang, Ao, et al., 2006). Since no highly branched dextrins standards are commercially available, the columns were calibrated with the straight-chain pullulan standards. Although, the absolute M_w and molecular size distributions cannot be determined by this HPSEC method, the information of the relative or apparent M_w and molecular size distributions of each α-amylase hydrolyzed sample can be provided.

For the native starches, M_w was determined using HPSEC coupled with multi-angle laser light scattering (MALS, Dawn Heleos-II, Wyatt Tech. Corp., Santa Barbara, CA) and refractive index (RI, Optilab rEX, Wyatt Tech. Corp., Santa Barbara, CA) detectors at 35 °C. Starch suspensions (1%, w/v) were boiled for 20 min and then filtered through a 5 μm nylon filter. Injected samples (200 μL) were separated on a Sephacryl™ S-500 HR gel filtration media (GE Healthcare, Piscataway, NJ) using purified water (18.2 MΩ) with 0.02% sodium azide as mobile phase. Flow rate of the mobile phase was set at 1.3 mL/min (Ao, Quezada-Calvillo, et al., 2007). A *dn/dc* value of 0.146 mL/g was used in the molecular weight calculation, and data processing was performed using Astra software version V (Wyatt Tech. Corp., Santa Barbara, CA) (Yoo & Jane, 2002; Yu & Rollings, 1987).

2.5. Hydrolysis properties by human pancreatic α-amylase

Hydrolysis property of each enzyme-treated starch by human pancreatic α-amylase was investigated. Starch samples were solubilized in 10 mM PBS buffer (pH 6.9) at 1% (w/v) concentration and boiled for 20 min. After cooling, an aliquot of the sample (1 mL) was reacted with human pancreatic α-amylase (500 U, Meridian Life Science, Inc., Saco, Maine) at 37 °C for 24 h to produce α-limit dextrins (fully α-amylase hydrolyzed starch). The reaction was then stopped by boiling for 20 min to inactivate the enzyme. The hydrolyzed samples were analyzed for their relative molecular size by HPSEC-RI (Zhang, Ao, et al., 2006) using pullulan standards (Polymer Laboratories Inc. Amherst, MA).

2.6. Determination of glucose release property of starch samples by rat intestinal α-glucosidases

An aliquot of the α-limit dextrins (200 μL) was further incubated at 37 °C with the rat intestinal α-glucosidases (500 U, one unit (U) enzyme activity arbitrarily defined as 1 μg of glucose released from 1% maltose per 10 min at 37 °C). The amount of released glucose at different time intervals (0, 5, 10, 20, 60, 120, and 240 min) was determined by the glucose oxidase/peroxidase (GOPOD) method (Vasanthan, 2001, Chap. E2).

2.7. Statistical analysis

Results were expressed as mean ± standard deviation values. Data were analyzed using one-way analysis of variance (ANOVA) procedure; where analysis showed significant differences

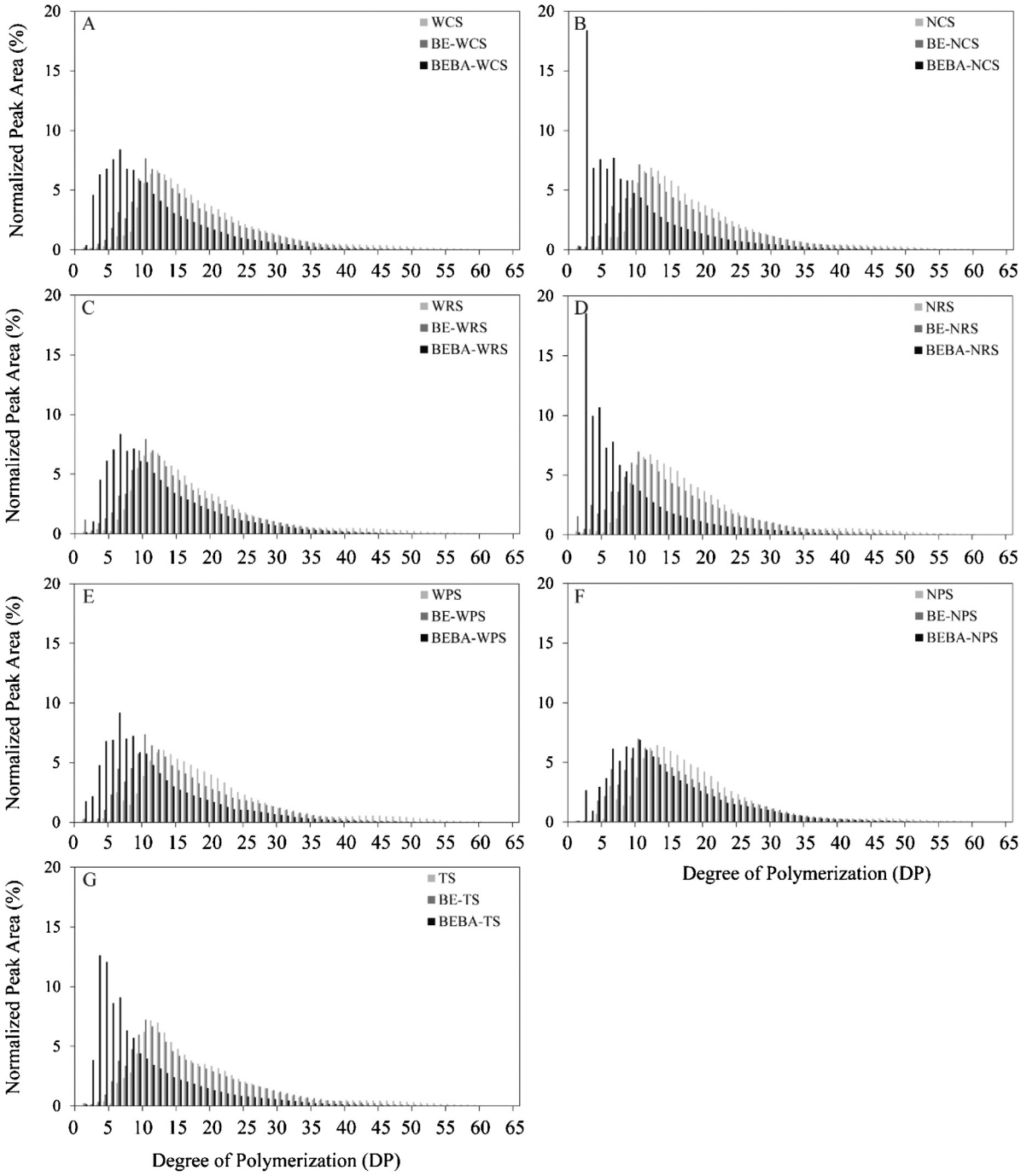

Fig. 2. Debranched linear chain length distribution of native and enzyme-modified starches determined by HPAEC.

($p \leq 0.05$), means were compared using Duncan's multiple range test. Statistical analysis was performed using PASW Statistics 18 software (SPSS Inc., Chicago, IL).

3. Results and discussion

3.1. Debranched linear chain length distribution of native and enzyme-modified starches

Normalized HPAEC chromatograms of debranched linear chain length distributions from native, BE-, and BEBA-treated starches are shown in Fig. 2 and the computed results are summarized in Table 1. Chain length distributions were classified into different ranges of degree of polymerization (DP) from short to long chains as follows: DP < 6, 6–12, 13–24, 25–36 and DP ≥ 37 chains (Hanashiro et al., 1996). The relative molar distribution profiles were different based on starch botanical sources. Generally, Fig. 2 shows that the chromatograms of A-type starches (*e.g.* WCS, NCS, WRS, NRS, and TS) had a peak position at shorter chain lengths (DP 11–12) than those of B-type starches, *i.e.* WPS and NPS (DP 13). Table 1 shows that all native starches had a comparatively low amount of very short chains (DP < 6) (0.5–1.4%) and high amount of DP 13–24 chains (45.8–55.9%). The A-type starches contained larger proportions of short (DP 6–12) chains (26.0–32.5%), than the B-type starches (23.1–23.8%).

BE-treatment resulted in a decrease in the proportion of long (DP ≥ 13) chains, and an increase in short (DP ≤ 12) chains, leading to a reduction of the *weight-average* degree of polymerization

Table 1
Chain length distributions (%) and *weight-average* DP of debranched native and enzyme-treated starches from different botanical origins.

Sample	Control[a]					BE-treated[b]					BEBA-treated[c]				
	Degree of polymerization (DP)				DP_w	Degree of polymerization (DP)				DP_w	Degree of polymerization (DP)				DP_w
	(<6)	(6–12)	(13–24)	(25–36)	(≥37)		(<6)	(6–12)	(13–24)	(25–36)	(≥37)		(<6)	(6–12)	(13–24)	(25–36)	(≥37)	
WCS	0.9	26.0	51.0	15.1	6.9	19.1	3.5	36.6	43.3	13.4	3.2	16.4	25.7	42.1	25.0	6.2	1.0	11.1
NCS	0.5	26.2	52.4	14.3	6.6	18.9	5.1	36.6	41.3	13.0	4.0	16.4	39.9	35.4	18.6	5.0	1.1	9.3
WRS	1.2	32.5	47.6	11.8	6.8	18.0	5.5	40.4	40.4	11.3	2.4	15.3	18.9	44.2	27.9	7.5	1.5	12.2
NRS	1.4	28.3	50.8	12.1	7.4	18.6	8.4	37.3	39.4	11.6	3.2	15.4	46.7	32.6	15.4	4.4	0.8	8.5
WPS	0.9	23.1	52.3	14.8	8.9	19.9	4.1	38.2	40.5	13.9	3.3	16.3	22.5	44.1	24.7	7.3	1.5	11.6
NPS	1.2	23.8	55.9	13.6	5.4	18.6	4.3	36.5	42.5	13.2	3.5	16.4	10.3	42.2	34.6	10.4	2.5	14.3
TS	1.1	31.9	45.8	13.6	7.7	18.6	3.8	38.0	40.4	14.0	3.9	16.6	37.3	36.1	19.7	5.8	1.1	9.9

[a] Control: untreated native starches.
[b] BE-treated: branching enzyme-treated starches (BE-starches).
[c] BEBA-treated: β-amylase-treated BE-starches (BEBA-starches).

(DP_w) from 18.0 to 19.9 for native starches to 15.3–16.6 for BE-treated starches (Table 1). All BE-treated starches had peak at DP 10 which was lower than the peak for the native starches (Fig. 2). These results indicate that BE mainly reacts on longer chains, and transferred moieties of glycosyl residues to either the same or different amylose and amylopectin molecules to produce new α-1,6 linked branch chains, resulting in overall shorter linear chain lengths (Le et al., 2009; Lee, Yoo, et al., 2008; Takata, Takaha, Okada, Hizukuri, et al., 1996).

The BE-treated starches were further modified using BA to produce more highly branched maltodextrins by decreasing external α-1,4 linkages. As shown in Fig. 2, all BEBA-treated starches had peaks at shorter chain lengths than the native or BE-treated starches, except for the BEBA-NPS (peak at DP 10) which was not much affected by the BA reaction. The BEBA-treated normal starches (NCS, NRS, and TS) had peaks at shorter chain lengths (DP 2–3) than the BEBA-treated waxy starches (WCS, WRS, and WPS) (DP 6), the former which is similar to a β-limit dextrin. Table 1 shows a substantial increase in the proportion of very short chains (DP < 6) from 3.5% to 8.4% for the BE-treated starches to 10.3–46.7% for the BEBA-treated starches and a substantial decrease in the longer (DP ≥ 13) chains. With the exception of BEBA-NPS, the BEBA-treated normal starches had a higher proportion of very short chains (DP < 6) (37.3–46.7%) than the BEBA-treated waxy starches (18.9–25.7%), but lower proportion of DP 6–12 (32.6–36.1% *vs.* 42.1–44.1%) and DP ≥ 13 chains, leading to the lower DP_w for the BEBA-normal starches (8.5–9.9) compared with the BEBA-waxy starches (11.1–12.2). In the case of BEBA-normal starches, the BE reaction may induce addition of α-1,6 linkages on the amylose molecule to produce an amylopectin-like structure with less densely branched structures compared to amylopectin BEBA-modified maltodextrins.

It should be noted that the HPAEC chain length distribution in this study only considers linear chains up to about DP 60–100 which are chains that comprise amylopectin. It is possible that linear chains of DP greater than this amount in the native starch (*i.e.* amylose or super-long chains of amylopectin) could also have participated in the enzyme modification reaction. Casarrubias-Castillo et al. (2012) reported a significant reduction of the percentage of long chains (DP > 205), which represent amylose, by enzymatic (β-amylase and β-amylase-transglucosidase) treatments for plantain and mango starches.

3.2. *Weight-average molecular weight (M_w) of native and enzyme-treated starches*

A substantial reduction of the M_w of about four orders of magnitude was observed for all BE-treated starches as compared with their native counterparts (Table 2). It is generally known that amylopectin consists of multiple clusters connected by long linear chains (Hizukuri, 1986). Based on enzymatic properties of BE, our results indicate that the α-1,4 linked segments between amylopectin clusters were hydrolyzed to release cluster units. Similar findings were observed by others (Le et al., 2009; Lee et al., 2013). In addition, the reduction of M_w could also be attributed to the action of BE on a linear chain of amylose to form a branched linkage (Borovsky et al., 1976) and a cyclic amylose (Takata, Takaha, Okada, Takagi, et al., 1996).

As expected, BA treatment of the BE-treated products led to a further decrease in M_w due to hydrolysis of the external linear branch chains of amylopectin. A reduction of M_w of about 20.6–24.8%, 10.5–11.3%, and 37.8% was observed for BEBA-treated cereal starches (WCS, NCS, WRS, and NRS), BEBA-treated potato starches (WPS and NPS), and BEBA-TS, respectively, as compared with their BE-treated counterparts. This decrease in the M_w increased the number of short chains (Table 1) and the

Table 2
weight-average Molecular weight (M_w) of native and enzyme-treated starches.

Sample	Weight-average M_w (Da)		
	Control[a]	BE-treated[b]	BEBA-treated[c]
WCS	17.78×10^8	9.59×10^4	7.43×10^4
NCS	8.72×10^8	8.22×10^4	6.18×10^4
WRS	5.13×10^8	8.47×10^4	6.57×10^4
NRS	8.37×10^8	8.54×10^4	6.78×10^4
WPS	11.90×10^8	12.90×10^4	11.54×10^4
NPS	4.26×10^8	12.97×10^4	11.50×10^4
TS	9.91×10^8	9.81×10^4	6.10×10^4

[a] Control: untreated native starches.
[b] BE-treated: branching enzyme-treated starches (BE-starches).
[c] BEBA-treated: β-amylase-treated BE-starches (BEBA-starches).

amount of branched α-limit dextrins (Table 3) of the BEBA-treated products.

3.3. Quantification of α-limit dextrin content

An amount of branched α-limit dextrins of native starches and enzyme-modified starches was evaluated after complete hydrolysis by using human pancreatic α-amylase. Size-exclusion chromatographic tracings with two different regions, linear and branched α-limit dextrins (Jones et al., 1983), are illustrated in Fig. 3, and the calculated results are summarized in Table 3. The identity of the two different regions of the chromatogram corresponding to the linear maltooligosaccharide and branched α-limit dextrin fractions were established by HPAEC and HPSEC-DMSO systems (Lee et al., 2012, 2013; Lin et. al 2012). In general, the native normal starches displayed lower proportion of branched α-limit dextrins (12.6–13.9%) than the waxy starches (14.5–16.3%) due to the presence of amylose with very low amount of α-1,6 linkages. As expected, BE treatment resulted in an increase in the proportion of branched α-limit dextrins of all starch samples and the BE-treated waxy starches had higher proportion of branched α-limit dextrins (22.0–23.7%) than the BE-treated normal starches (17.3–19.8%). A further increase in the proportion of branched α-limit dextrins was obtained by the BEBA treatment. With the exception of the BEBA-TS sample, the BEBA-treated waxy starches exhibited higher proportion of branched α-limit dextrins (31.7–35.8%) than the BEBA-treated normal starches (23.5–29.3%). Among the BEBA-treated products, the highest proportion of branched α-limit dextrins (35.8%) was found for the BEBA-WRS and BEBA-TS samples, whereas the lowest value (23.5%) was observed for the BEBA-NPS sample. These BEBA-treated products were used to show that highly branched structures reduce hydrolysis rate and to test the hypothesis that starch sources with higher proportion of BEBA branched fraction had slower digestion.

3.4. Digestion properties of native and enzyme-modified starches

To evaluate the property of glucose release from native and enzyme-treated starches, gelatinized starch samples were incubated with pancreatic α-amylase followed by rat intestinal α-glucosidases. The digestion profiles, as measured by glucose release, are shown in Fig. 4. For all starch types, the gelatinized, untreated starches had the highest digestion rate and extent. In general, enzymatic treatments with BE alone and in the combination of BA resulted in a decrease in digestibility of the enzyme-treated products as compared with their control counterparts. As expected, the BEBA-treated products had lower digestibility than their native and BE-treated counterparts due to their higher amounts of branched α-limit dextrins (Table 3) which are slowly digested by rat intestinal α-glucosidases. Percentage reduction in digestibility was calculated by subtracting the amount of glucose released from BEBA-treated starches by α-glucosidase reaction at 240 min from the control untreated counterparts and dividing by the latter. BEBA-treated waxy starches exhibited higher reduction in digestibility (13.2–22.6%) than the BEBA-treated normal starches (4.7–16.2%), except for the BEBA-TS sample. Unlike the other BEBA-treated normal starches, the BEBA-TS sample showed a high reduction of digestibility of 22.1%. These results are consistent and correlate with the data on the amount of branched α-limit dextrins presented in Table 3. There were significant positive correlations of the amount of reduction in glucose released at 240 min, compared to the untreated starch, and the amount of branched α-limit dextrins; BE-treated ($R^2 = 0.75$, $p \leq 0.05$) (Fig. 5A) and the BEBA-treated ($R^2 = 0.86$, $p \leq 0.05$) starches (Fig. 5B). This provides clear evidence that hydrolysis rate of the α-glucosidases is limited by the amount of branched structures, and that starch source is a significant factor relating to proportion of branched α-limit dextrins in enzyme-treated starches.

It is worth noting that the digestion rate and extent observed during the first 60 min of digestion for all starch types were not

Table 3
Relative HPSEC peak area (%) of branched and linear α-limit dextrins[a].

Sample	Area for branched (α-1,6 linked) oligosaccharides			Area for linear (α-1,4 linked) maltooligosaccharides		
	Control[b]	BE-treated[c]	BEBA-treated[d]	Control[b]	BE-treated[c]	BEBA-treated[d]
WCS	15.5 ± 1.8	23.7 ± 0.2	31.7 ± 1.2	84.5 ± 1.8	76.3 ± 0.2	68.3 ± 1.2
NCS	12.6 ± 0.4	17.3 ± 1.1	25.7 ± 0.4	87.4 ± 0.4	82.7 ± 1.1	74.3 ± 0.4
WRS	16.3 ± 2.7	23.2 ± 0.4	35.8 ± 1.6	83.7 ± 2.7	76.8 ± 0.4	64.2 ± 1.6
NRS	13.9 ± 1.1	19.8 ± 0.2	29.3 ± 1.2	86.1 ± 1.1	80.2 ± 0.2	70.7 ± 1.2
WPS	14.5 ± 1. 0	22.0 ± 0. 7	31.7 ± 1.3	85.5 ± 0.9	78.0 ± 0.7	68.3 ± 1.3
NPS	13. 8 ± 0.5	19.1 ± 0.3	23.5 ± 0.3	86.2 ± 0.5	80.9 ± 0.3	76.5 ± 0.3
TS	13.4 ± 1.4	18.4 ± 0.2	35.8 ± 1.3	86.6 ± 1.4	81.6 ± 0.2	64.2 ± 1.3

[a] Mean ± standard deviation values of triplicate determinations.
[b] Control: untreated native starches.
[c] BE-treated: branching enzyme-treated starches (BE-starches).
[d] BEBA-treated: β-amylase-treated BE-starches (BEBA-starches).

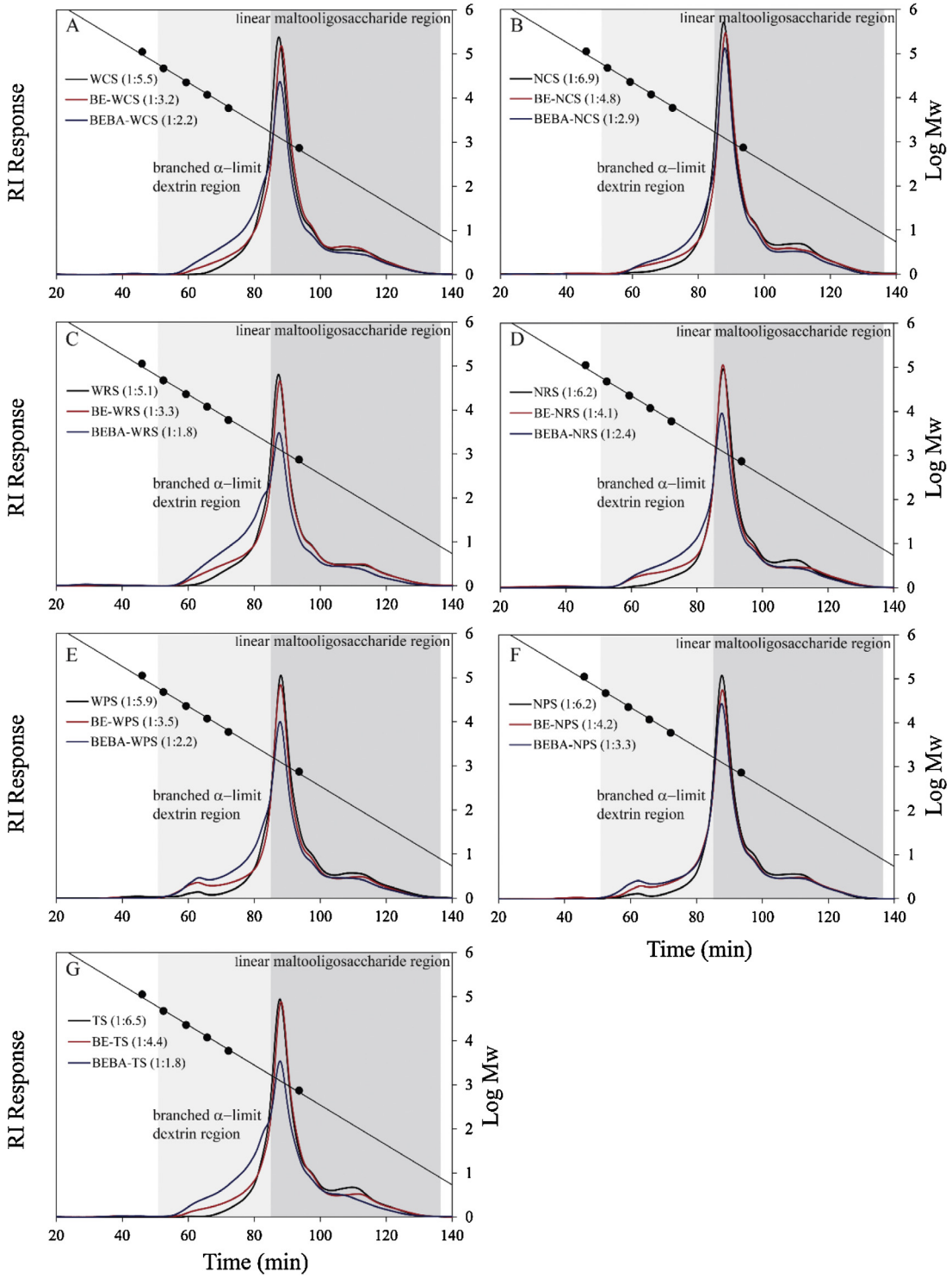

Fig. 3. Molecular weight distributions of native and enzyme-treated starches by HPSEC-RI after human pancreatic α-amylase treatment. The two different regions stand for branched and linear maltooligosaccharides, respectively. The ratios in abbreviated sample names of figures (A)–(G) represent the area ratio of branched α-limit dextrins region to linear maltooligosaccharide region.

significantly different between the enzyme-treated and untreated (control) samples (Fig. 4). This is contrary to our recent report where four different recombinant mucosal α-glucosidases (ctMGAM, ctSI, ntMGAM, and ntSI) were used to evaluate hydrolysis properties of BE- and BEBA-treated WCS (Lee et al., 2013). An explanation could be that the rat intestinal α-glucosidases used in this study are multi-complex enzymes with high impurity and low activity when compared with the recombinant enzymes. The rat intestinal α-glucosidases were chosen on the basis that they are very similar to the human intestinal α-glucosidases to simulate the digestibility in the human intestinal tract (Oku, Tanabe, Ogawa, Sadamori, & Nakamura, 2011).

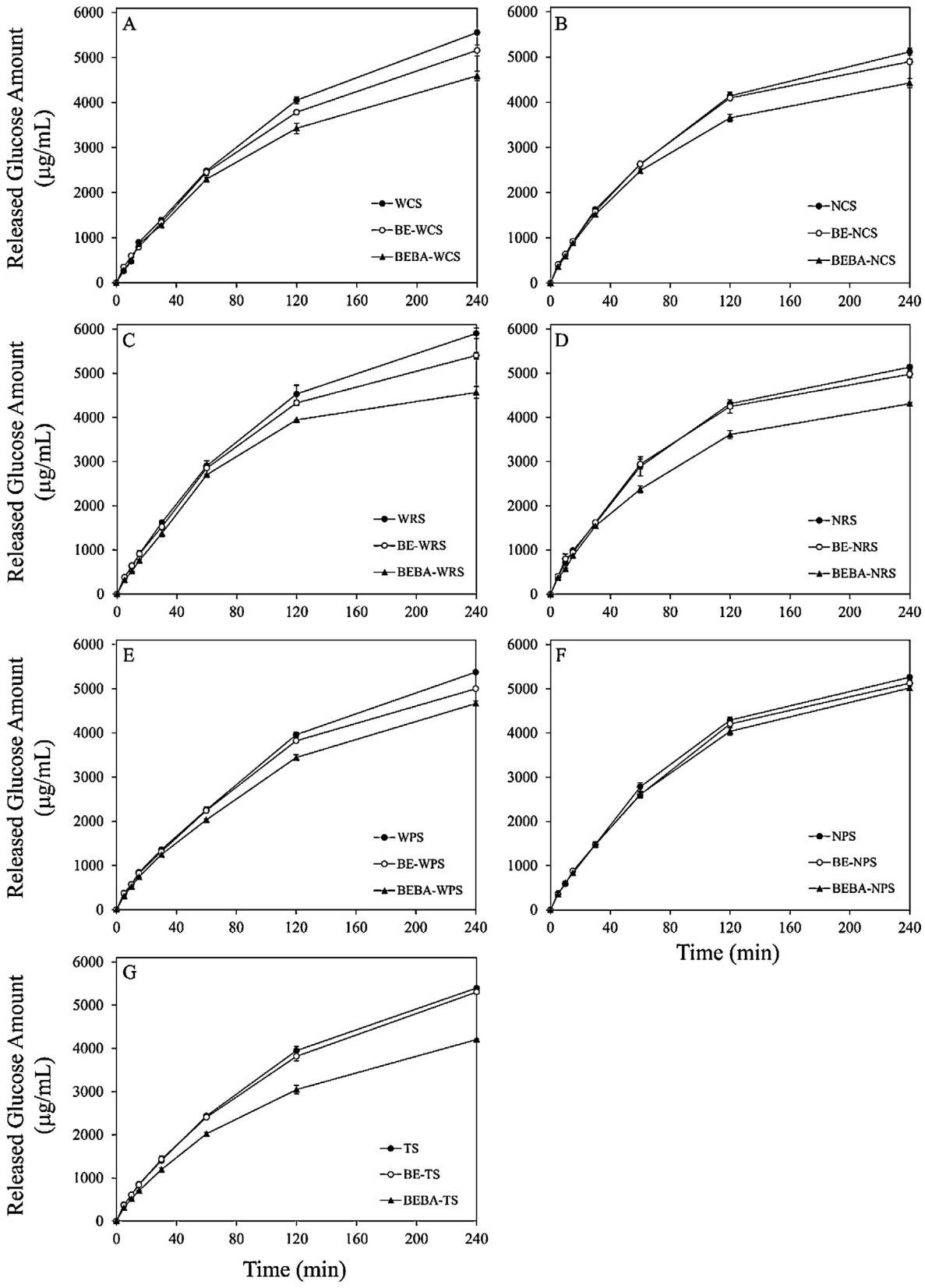

Fig. 4. Hydrolysis properties of native starches and enzyme-modified maltodextrins (1%, w/v) using the rat intestinal α-glucosidases (500 U). As the amount of α-1,6 linkages were increased, the released glucose amounts decreased by the rat intestinal α-glucosidase reactions.

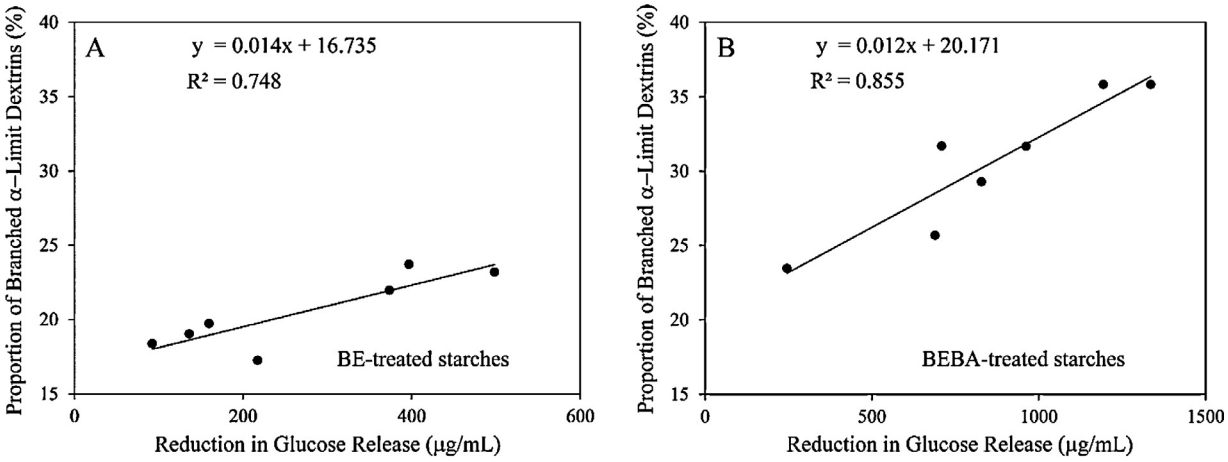

Fig. 5. Relationship between proportion of branched α-limit dextrins (%) and the amount of reduction in glucose released (μg/mL) from (A) BE-treated starches and (B) BEBA-treated starches at 240 min by the rat intestinal α-glucosidase reaction compared to their control counterparts.

4. Conclusions

The enzyme modifications, *i.e.* BE and a combination of BE and BA (BEBA), performed on seven starches, led to decrease in starch digestion rate as measured at the α-limit dextrin level using rat intestinal α-glucosidases *in vitro*. The BEBA-treated starches had significantly less glucose released than the BE-treated starches. α-Limit dextrins from different starch sources differed in α-glucosidase digestion to glucose and was determined to be structurally based. Amount of glucose generation at the α-glucosidase level correlated with amount of branched α-limit dextrins of the different starch sources (measured as proportion of branched α-limit dextrins of total branched plus linear dextrins). Starch sources with the slowest digestion to glucose were tapioca and waxy corn, potato, and rice starches. In the case of the waxy starches, absence of long-chain amylose and greater abundance of amylopectin favored BEBA modification to form highly branched molecular structures, and consequently a reduction in digestibility. Thus, slow digestion property of enzyme-modified maltodextrins may be optimized through selection of certain starch sources that would lead to a higher proportion of branched α-limit dextrins. Such BEBA-modified maltodextrins are potentially useful to be applied as functional food ingredients because of their slow glucose release property perhaps addressing diet-related metabolic conditions of type 2 diabetes and obesity, as well as for the athletic.

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