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Glucose Release of Water-Soluble Starch-Related α-Glucans by Pancreatin and Amyloglucosidase Is Affected by the Abundance of α-1,6-Glucosidic Linkages

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This study tested the hypothesis that an increased branch density (i.e., the percentage of α-1,6-glucosidic linkage) in water-soluble, starch-related α-glucans leads to reduced glucose release by pancreatin and amyloglucosidase. Malto-oligosaccharides and phytoglycogens were structurally analyzed and compared for their susceptibility to the enzymes. Malto-oligosaccharides were prepared by subjecting starch to α-amylase and β-amylase followed by ultrafiltration to enrich α-1,6-glucosidic linkages. The branch density of the oligosaccharide products reached up to 17%, determined by ^{1}H NMR. Phytoglycogens were extracted from six sweet corn lines, and analysis showed similar chain length distributions and a branch density range from 8.8 to 9.5%, as compared with 4.6% for normal corn starch and 5.7% for waxy corn starch. The digestion behavior of these α-glucans was correlated to branch density: Highly branched malto-oligosaccharides had much reduced glucose release as compared with starch, whereas the reduction of glucose release from phytoglycogen was relatively low. Particularly, the reduction of glucose release associated with enhanced branch density was caused by reduced hydrolysis by amyloglucosidase.

KEYWORDS: Soluble α-glucans; branch density; digestibility; malto-oligosaccharide; phytoglycogen; starch

INTRODUCTION

Overconsumption of carbohydrate is associated with obesity, diabetes, and cardiovascular disease (*1, 2*). Considering the high glycemic index of starchy foods, this work addresses the long-term goals to identify starch-related carbohydrates with reduced, slow, or extended glucose and energy release. Primary sources of carbohydrates are native starch, starch derivatives, starch hydrolysates, or plant-based starch analogues containing α-1,4 and α-1,6 linkages. The studies on the digestibility of starch-related α-glucans are of particular importance, not only because they constitute a major contribution to the calories in diet but also because of their broad availability from inexpensive agricultural resources.

Different factors contribute to the digestion properties of starch and starch-related glucans. The digestion of starch begins with the action of α-amylase in salivary fluid and continues in the duodenum by α-amylase released by the exocrine pancreas (*3*). Salivary and pancreatic α-amylases hydrolyze α-1,4-glucosidic bonds. The linear molecules are degraded into maltose and maltotriose, whereas the branched molecules are degraded into maltose, maltotriose, and small branched α-limit dextrins. The resulting luminal mixture of di-, tri-, and oligosaccharides is then hydrolyzed by the combined actions of maltase−glucoamylase (α-limit dextrinase) and sucrase−isomaltase complexes, each liberating free glucose.

It has been established that the mixture of pancreatin and amyloglucosidase can be used to measure starch digestibility in vitro (*4*). Fungal amyloglucosidase has been used to convert glucans to glucose, mimicking the activity of maltase−glucoamylase and sucrase−isomaltase in the small intestine.

Starch-related α-glucans can be swellable, soluble, or insoluble. Native starches are typical insoluble glucans. According to Englyst et al. (*4*), by its susceptibility to amylolytic enzymes, starch can be categorized into rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS). In addition, RS can be classified as RS type 1 (physically inaccessible), RS type 2 (granular), RS type 3 (retrograded), and RS type 4 (chemically modified). The strategy to produce different types of RS has been extensively discussed (*5*). This classification can also be employed to differentiate and prepare

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SDS, in which the susceptibility of insoluble α-glucans to amylases is manipulated.

In contrast, the knowledge on the digestibility of soluble starch-related α-glucans is not well-established; these glucans are very important in liquid, semiliquid, and solid food systems. One possibility to manipulate their digestibility is to enrich the content of α-1,6-glucosidic linkages. In theory, all of the α-1,6 branching linkages can be fully hydrolyzed; however, the hydrolysis rate of α-1,6 linkages is different from that of α-1,4 linkages (*6, 7*). Hitherto, very few studies have been reported to address the impact of α-1,6 linkage abundance, or branch density, on the digestibility of starch-related malto-oligosaccharides and higher molecular weight α-glucans. Recently, incubation of starch with transglucosidase to create α-1,6 linkages was reported to reduce enzyme digestibility (*8*), which was possibly due to the production of isomalto-oligosaccharides such as isomaltose.

In this study, the hypothesis is that, for water-soluble starch-related α-glucans, an enhanced branch density (i.e., the percentage of α-1,6-glucosidic linkage) leads to reduced enzymatic release of glucose. The objective of this work is to prepare highly branched starch-related soluble α-glucans with different branch densities and molecular weights and compare their enzyme digestibilities with that of starch. Two types of soluble α-glucan were used as model systems to establish the relationship between branch structure and enzyme digestibility. The first is highly branched starch hydrolysates enriched for α-1,6 linkages. This group of materials was studied due to their potential to replace regular starch hydrolysates in food systems. Regular starch hydrolysates, such as maltodextrin and corn syrup, are major commodities of the corn-refining industry. While these products have broad applications in food, their quick glucose release has been a health concern. In contrast, highly branched starch hydrolysates may provide the benefit of reduced glucose release. The second type of glucan is phytoglycogens extracted from sweet corn. The agricultural significance of phytoglycogen lies in its abundance and extractability from sweet corn containing the *su1* mutation, and approximately 60% of sweet corn acreage in the United States is planted with the *su1* and *se1* genotypes (*9*). Phytoglycogen is the primary starch analogue in the endosperms of corn (*10, 11*), rice (*12*), sorghum (*13*), and barley (*14*). It is also found in potato (*15*) and *Arabidopsis* (*16*).

MATERIALS AND METHODS

Materials. Starches used include normal corn starch (Tate & Lyle North America, Decatur, IL), waxy corn starch (National Starch Food Innovation, Bridgewater, NJ), maltodextrin Maltrin M100 (DE 11) (Grain Processing Corp., Muscatine, IA), and corn syrup Star-Dri-240 (DE 24) (Tate & Lyle). Enzymes used include α-amylase (SPEZYME ETHYL) and β-amylase (OPTIMALT BBA) (Genencor International, Rochester, NY). SPEZYME ETHYL is an α-amylase derived from *Geobacillus stearothermophilus* with a claimed activity of 6700−7300 unit/g, (where one unit is defined as the amount of enzyme required to hydrolyze 10 mg of starch per minute under specified conditions). OPTIMALT BBA is a barley β-amylase with a claimed activity of 1230 DP°/g, [where DP° (diastatic power) is the amount enzyme contained in 0.1 mL of a 5% enzyme solution that produces sufficient reducing sugars to reduce 5 mL of Fehling's solution when incubated with 100 mL of substrate for 1 h at 20 °C]. Sweet corn lines SU (containing the *su1* mutation) Early Sunglow, Silver Queen, Honey & Cream, and Peaches & Cream and SE (containing both *su1* and *se1* mutations) Kandy Korn H and Miracle were purchased from Burpee Co. (Warminster, PA). To obtain fresh kernels of Peaches & Cream, the seeds were planted in a greenhouse with controlled temperature and lighting and harvested at 20 days after pollination. The ears were placed in dry ice and transferred to a −70 °C freezer for storage.

Methods. *Preparation of Malto-Oligosaccharides.* Twenty-eight grams of each of normal corn starch and waxy corn starch was suspended in 400 mL of deionized water. The pH was adjusted to 5.5 by hydrochloric acid. The starch suspension was heated in a microwave to boiling. After the suspension was cooled, 28 μL of α-amylase (SPEZYME) and 56 μL of β-amylase (OPTIMALT) were added. The mixtures were incubated at 55 °C for 18 h before adjusting the pH to 2.7 and boiling for 10 min to denature the enzymes. The solution was centrifuged at 5000*g* for 10 min and filtered through a 2.0 μm Nalgene filter. The filtrate was termed hydrolysate-0 for each starch.

Ultrafiltration of hydrolsate-0 was performed using a membrane with molecular mass cutoff of 650 Da (650D Minimate TFF capsules, Pall Life Science, Ann Arbor, MI). The filtration was conducted under a tangential flow with constant cycling at room temperature, and the filtrate was discarded. When the retentate in the reservoir was reduced to 50 mL, deionized water was added to bring the volume to 400 mL. Hydrolysate-1 (25 mL) was sampled when the retentate was reduced to 50 mL after two additions of water. Hydrolysate-2 was collected after one more addition of water (375 mL) and volume reduction to 50 mL. Hydrolysates-0, -1, and -2 were freeze-dried.

Extraction of Phytoglycogen. To extract phytoglycogen from matured seeds, 10 g of dry kernels for each sweet corn line was ground, soaked overnight at 4 °C in 40 mL of deionized water, and homogenized using a mortar and pestle. To extract phytoglycogen from fresh kernels, the kernels of Peaches & Cream (50 g) were homogenized with 70 mL of distilled water using a mortar and pestle. The homogenates were centrifuged at 10000*g* for 20 min at room temperature (22 °C). The supernatants were collected, filtered, heated in a boiling water bath for 30 min, and centrifuged to remove insoluble materials. Soluble polysaccharides in the supernatant were precipitated by adding 3 volumes of ethanol. The precipitate was collected after 3−5 repetitions of dispersion−centrifugation in 80% ethanol and dehydrated by anhydrous ethanol and acetone.

^{1}H NMR Determination of α-1,6-Glucosidic Linkages in Malto-Oligosaccharides. ^{1}H NMR analyses were performed using a Varian Unity Inova 300 MHz NMR spectrometer (Varian Inc., Palo Alto, CA) as described by Gidley (*17*). Starch hydrolysate samples were dissolved in 1 mL of deuterium oxide (D_2O, Sigma), incubated for 2 h at room temperature, and lyophilized. The D_2O-treated samples were dissolved in 0.6 mL of D_2O, and ^{1}H NMR spectra were obtained at 80 °C. Maltose, isomaltose, and panose were used as standards to quantify the level of α-1,4- and α-1,6-glucosidic linkages. The ratio of α-1,4- to α-1,6-glucosidic linkages was determined from integration of the anomeric resonance at 5.4 and 5.0 ppm, respectively.

Determination of Molecular Weight Distribution of Malto-Oligosaccharides. Twenty microliters of 90% dimethyl sulfoxide (DMSO) solution of starch hydrolysates (approximately 0.2%) was subjected to high-performance size exclusion chromatography (HPSEC) analysis, using two connected Zorbax gel PSM 60-S columns (6.2 mm × 250 mm, Agilent Tech., Santa Clara, CA) at a flow rate of 0.5 mL/min, with DMSO as the mobile phase. The elution was monitored with a Waters 2414 refractive index (RI) detector (Waters, MA). Glucose, maltose, malto-pentaose (DP5), and pullulan with molecular masses of 5900, 11800, 22800, 47300, 112000, and 212000 Da (Polymer Laboratories, Amherst, MA) were used for column calibration.

Determination of Molecular Weight Distribution of Phytoglycogens. Samples were filtered (5 μm) and injected into a HPSEC system with a multiangle laser light scattering and a refractive index detector (HPSEC-MALLS-RI). The HPSEC system included an HR 16/50 column containing Sephacryl S-500 HR gel (Amersham Biosciences, Piscataway, NJ), a DAWN DSP-F laser photometer fitted with argon laser (λ= 488.0 nm) with a K-5-129 flow cell (Wyatt Technology, Santa Barbara, CA), and an Optilab 903 interferometric refractometer (Waytt Technology). The flow rate was set at 1.3 mL/min with a mobile phase of 0.02% NaN_3 (w/v) in deionized water. A d*n*/d*c* value of 0.146 was used in molecular weight calculation, and data processing was performed using ASTRA software (Version 4.9, Wyatt Technology).

Determination of Chain Length Distribution and Branch Density of Phytoglycogen and Starch. Five milligrams of phytoglycogens, normal corn starch, and waxy corn starch was dispersed in 125 μL of 90% DMSO and heated in a boiling water bath for 10 min. Sodium acetate buffer (0.02 M, 875 μL, 50 °C, pH 4.75) was added to dispersions. The mixtures were heated again in a boiling water bath for 10 min and cooled to 37 °C in a shaking water bath. Isoamylase solution (Megazyme, 5 U/mL, 50 μL in acetate buffer) was added to each mixture. The mixtures were incubated for 24 h at 37 °C with shaking and then boiled for 10 min. The samples were cooled down, and pullulanase solution (Megazyme, 7.2 U/mL, 40 μL in acetate buffer) was added to each sample. The mixtures were incubated for another 24 h at 37 °C and then boiled for 10 min. Moisture was removed using a SpeedVac concentrator (Savant) with heating and vacuum, and the volume of each sample was adjusted to about 1 mL with DMSO. After the samples were vortexed and centrifuged to remove insolubles, a 20 μL aliquot was injected into to the HPSEC system. Using HPSEC chromatograms, the average chain length and branch density of each glucan and starch were determined as described by Yuan et al. (*18*). Briefly, the raw data were exported to an Excel spreadsheet. The DRI value (mass-based) at each sampling retention time was converted to a molar-based value using the calibration curve of molecular weight (MW, with unit of DP) and retention time. The average chain length was determined by the following equation: CL (chain length) = $\sum(NM)/\sum N$ from 10 to 20 min. N is the molar amount of glucans at molecular weight of M (with unit of DP). The branch density was determined as the inverse of CL.

Enzymatic Digestion by Pancreatin and Amyloglucosidase. The glucan solids in powder form (200 mg; dry base) were dispersed in 2 mL of distilled water and heated in a boiling water bath for 20 min. After they were cooled to 37 °C, sodium acetate buffer (4 mL, 0.25 M, pH 5.2), distilled water (3.2 mL), and solution (0.8 mL) with pancreatin, amyloglucosidase, or both enzymes were added sequentially. A 0.51 mg amount of pancreatin (Sigma P-7545, from porcine pancreas) and 0.05 units of amyloglucosidase (Sigma A-7095, from Aspergillus *niger*) were added for each mg of glucan. Enzymatic digestion was conducted in a water bath at 37 °C with constant shaking (120 rpm). One hundred microliter aliquots were taken at 20, 40, 60, and 120 min. The reaction was stopped with 900 μL of ethanol, and the samples were centrifuged for 10 min at 10000*g*. For digestions involving amyloglucosidase, the supernatant was analyzed by the glucose oxidase/peroxidase (GOPOD) procedure (Megazyme, Wicklow, Ireland). The degree of digestion was calculated as: glucose amount after digestion/amount of initial glucan) × 0.9 × 100. For digestion using pancreatin only, the extent of hydrolysis was determined using 3,5-dinitrosalicylic acid (DNS) assay (*19*). Hydrolysis for each sample was performed in duplicate followed by duplicate analysis for each replicate. The data were plotted using the average value of four measurements.

RESULTS AND DISCUSSION

Ultrafiltration Effectively Removes Maltose and Maltotriose from Starch Hydrolysates Produced from Coordinated Action of α-Amylase and β-Amylase. The coordinated action of α-amylase and β-amylase partially hydrolyzed starch and yielded a mixture of malto-oligosaccharides (hydrolysate-0) containing maltose, maltotriose, and a negligible amount of glucose (**Figure 1**). The molecular weight distribution of hydrolysate-0 was related to the origin of starch. For normal corn starch, the size distribution reached up to DP100, whereas for waxy corn starch the distribution was mostly below DP50. Hydrolysate-0 from both normal corn starch and waxy corn starch formed a stable clear solution, which implies an absence of linear chains long enough to haze via retrogradation.

As shown in **Figure 1**, ultrafiltration (MWCO of 650 Da) was effective in reducing the content of maltose (DP2) and maltotriose (DP3). For normal corn starch, the total amount of maltose and maltotriose was reduced from 74% in hydrolysate-0 to 44 and 18% in hydrolysate-1 and -2, respectively. Similarly,

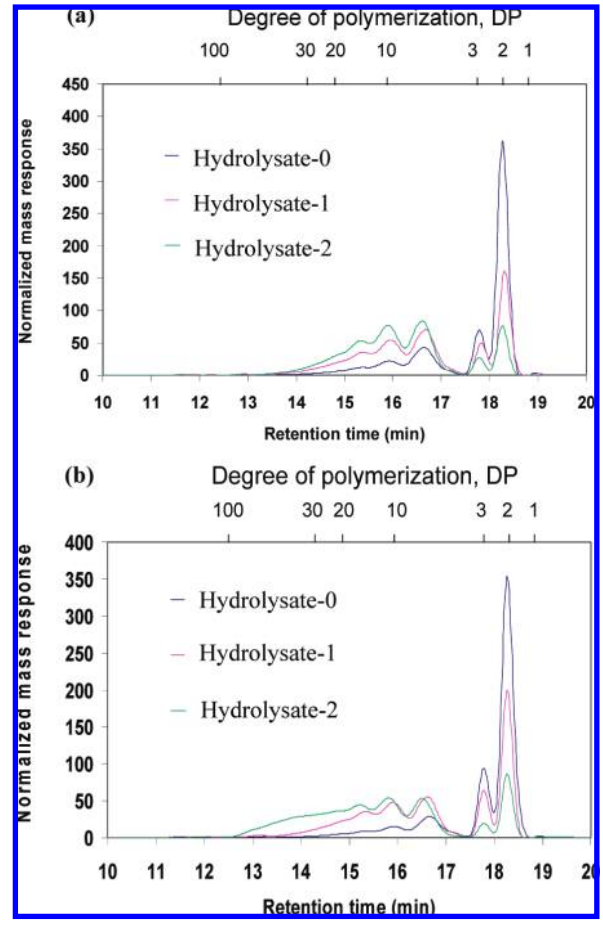

Figure 1. HPSEC chromatogram of waxy corn starch (**a**) and normal corn starch (**b**) hydrolysates after a combined α- and β-amylolysis (hydrolysate-0) and additional treatment by ultrafiltration (hydrolysate-1 and hydrolysate-2).

for waxy corn starch, the total amount of maltose and maltotriose was reduced from 80% in hydrolysate-0 to 34 and 18% in hydrolysate-1 and -2, respectively.

Size Distribution of Hydrolysates Demonstrates Stepwise Degradation of Amylopectin Clusters. The modal sizes of the major malto-oligosaccharides were ca. DP6, 11, and 17, and this distribution was not affected by the origin of starch. These populations consist of amylopectin cluster residues with defined size ranges. **Figure 2** depicts the formation of these populations. **Figure 2a** shows two connected clusters in an amylopectin molecule, with an average number of branches per cluster of approximately 7, which is specific for the *wx* genotype of maize (*20*). Each cluster is composed of a branched region and a linear region, which are responsible for the amorphous and crystalline lamellaes of starch granules, respectively (*21*). **Figure 2b** shows the β-limit dextrin of two clusters, in which the branched region is retained and the external linear chains are hydrolyzed to DP2−3 for A type chains and DP1−2 for B type chains (*22*). The trimmed clusters are connected by linear sections not accessible to β-amylase.

In the presence of α-amylase and β-amylase, amylose and amylopectin external chains are converted to maltose and maltotriose by exo- and endohydrolytic actions. In addition, the hydrolysis products of amylopectin also include a range of trimmed clusters and their branching zones with nonreducing end stubs shorter than DP3. As shown in **Figure 2c**, populations of DP40, DP17, DP11, and DP6 can be formed. This result is related to the structural work of Bertoft's group (*23*). Conceiv-

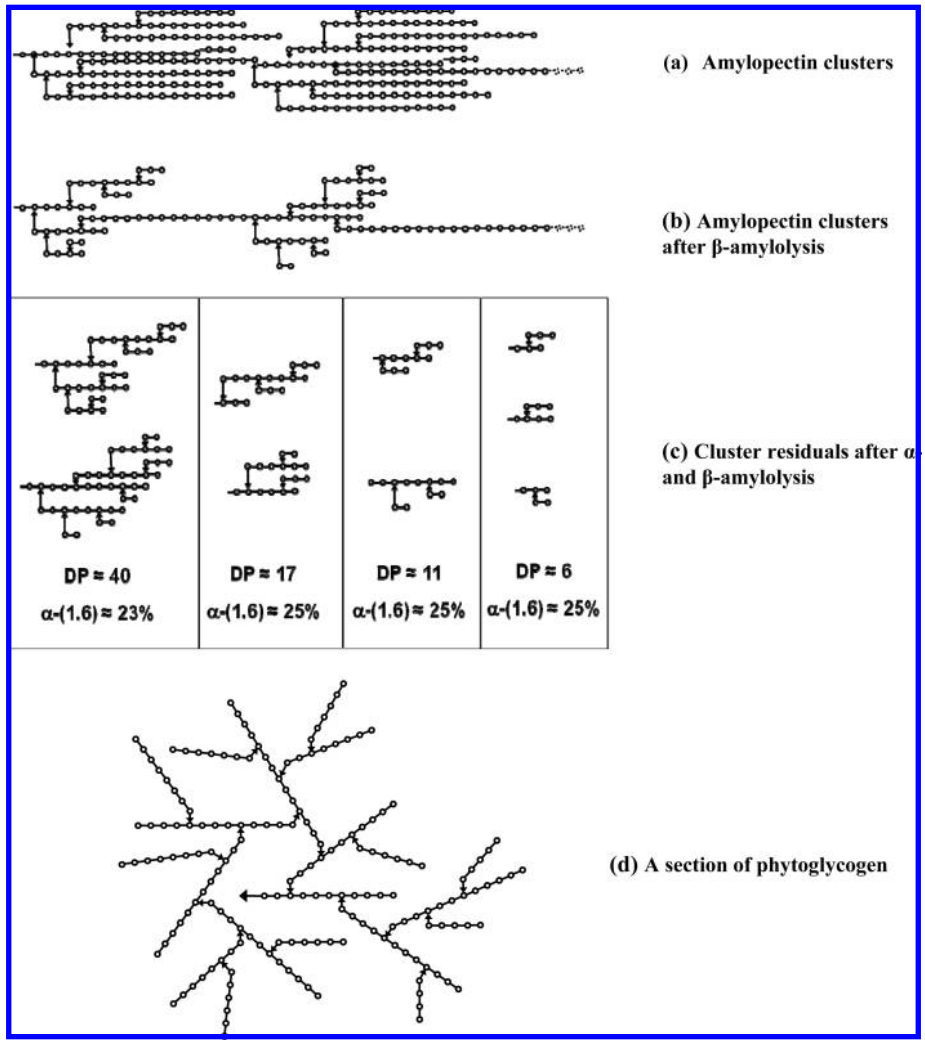

Figure 2. Schematic description of two amylopectin clusters before (**a**) and after (**b**) β-amylolysis. (**c**) Structures of cluster residues after both α- and β-amylolysis. The reducing end is on the left for molecules and molecular moieties. (**d**) Schematic of phytoglycogen.

ably, the α-amylolysis results in size reduction of branching zones by cleaving the intracluster α-1,4 linkages. Such hydrolysis occurs between adjacent branch points and should be restricted by the distance between branch points. A previous study (*20*) showed that the modal value of branch distance is mostly in the range of DP3−4. Branch distances at the higher end of this distribution (e.g., DP ≥ 4) are preferentially attacked by α-amylase, whereas a lower branch distance (e.g., DP ≤ 2) may retard α-amylolysis. Extensive α-amylolysis of α-1,4 linkages among branch points may cause the dissembling of high MW populations.

As shown in **Figure 2c**, the theoretical branch density of different populations is ca. 25%, with the higher MW populations at the lower end of this range. It appears that 25% could be the upper limit of α-1,6 linkage enrichment by removing small linear oligosaccharides.

α-1,6-Glucosidic Linkages Were Enriched by Ultrafiltration. Figures 1 shows an effective enrichment of population DP6, DP11, DP17, and larger molecules by ultrafiltration. ^{1}H NMR confirmed the enrichment of α-1,6 linkages from 7.4 to 16% for the hydrolysate of normal corn starch and from 8.7 to 17% for waxy corn starch (**Table 1**). The complete removal of maltose and maltotriose may be accomplished by exhaustive ultrafiltration; however, the yield of DP6 population can be substantially reduced. In fact, a comparison among hydrolysates-0, -1, and -2 for both types of starch shows that the ratio of population DP6 to DP11 and DP17 declined with extended ultrafiltration. From the perspective of industrial application, an optimized operation should be identified to maximize the branch density without a substantial loss of low MW branched glucans.

Table 1. Percentage of α-1,6-Glucosidic Linkages of Starch Hydrolysates Determined by ^{1}H NMR

sample	percentage of α-1, 6-glucosidic linkages (%)
normal corn starch-based	
hydrolysate-0	7.4
hydrolysate-1	12
hydrolysate-2	16
waxy corn starch-based	
hydrolysate-0	8.7
hydrolysate-1	15
hydrolysate-2	17

Phytoglycogens from Different Sweet Corn Lines Show Similar Molecular Weight Distribution and Fine Structure. The molecular weight distribution of extracted phytoglycogen determined by HPSEC-MALLS-RI is shown in **Figure 3**. Two peaks in the chromatogram indicate two major glucan populations. **Table 2** compares the weight-average molecular weight (Mw) of both populations among phytoglycogens from different sources. Generally, peak 1 ranges from 1.2 to 2.2 × 10^8 (ca.

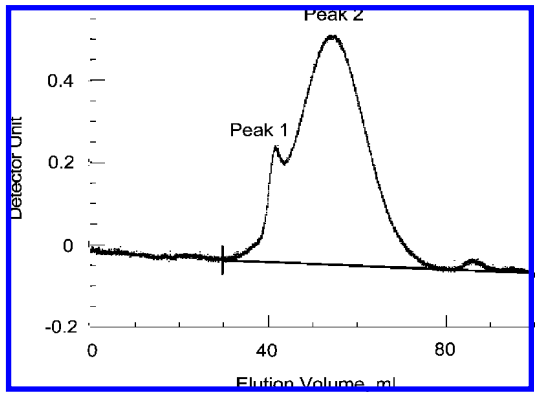

Figure 3. HPSEC-MALLS-RI chromatogram of phytoglycogen from sweet corn Silver Queen. Two major glucan populations are labeled as peak 1 and peak 2. The RI detector signal is shown here. The MALLS was used to determine the molecular weight of each peak (**Table 2**). The chromatograms of phytoglycogens from other sweet corn lines share a similar pattern.

Table 2. Weight Average Molecular Weight of Phytoglycogens from Different Sweet Corn Lines

	peak 1 (Da)	peak 2 (Da)
Early sunglow	1.2×10^8	1.5×10^7
Silver Queen	1.5×10^8	2.0×10^7
Honey & Cream	2.2×10^8	1.9×10^7
Peaches & Cream	1.5×10^8	2.2×10^7
Kandy Korn	1.4×10^8	2.0×10^7
Miracle	2.2×10^8	1.6×10^7

7.4 to 13.6 $\times$ 10^5 DP), and peak 2 ranges from 1.5 to 2.2 $\times$ 10^7 (ca. 9.3 to 13.6 $\times$ 10^4 DP). It appears that the molecular size of phytoglycogen is not substantially affected by the genetic background of maize. The chain length distributions of phytoglycogens are not significantly affected by the genetic background as well (**Figure 4**). Two populations of side chains can be identified, in which the lower and higher molecular weight populations have a modal length of around DP7 and DP15, respectively. No long chain population was detected, which implies that the branches are not clustered.

A schematic of the phytoglycogen molecule with randomly branched glucan chains is shown in **Figure 2d**. For model construction, the DP7 and DP15 populations as well as the average chain length of β-limit dextrin (CL_β) of DP8 (*22*) was considered. CL of B chains may contain 1, 2, or more internal sections between two adjacent branches, depending on the role of a specific B chain in the structure.

In contrast, debranched amylopectin from either normal corn starch or waxy corn starch shows a polymodal feature characteristic of amylopectin cluster structure (*24*). Three populations can be identified from debranched amylopectin: DP12, DP16, and DP40. The DP40 population is characteristic of the intercluster chains. The peak over DP100 for normal corn starch corresponds to amylose molecules. For waxy corn starch, there is no such peak due to the lack of amylose.

The formation of phytoglycogen is associated with the starch biosynthesis pathway in corn endosperm. Generally, starch branching enzyme (SBE) isoforms form branches by cleaving a section of linear glucan and attaching it to another chain by an α-1,6-glucosidic linkage (*25*). On the other hand, starch debranching enzyme (DBE) isoforms selectively cleave the branches to allow for the formation of ordered structure among linear chains (*26*). The *su1* mutation conditions the deficiency of DBE isoform SU1, which drastically reduces the debranching

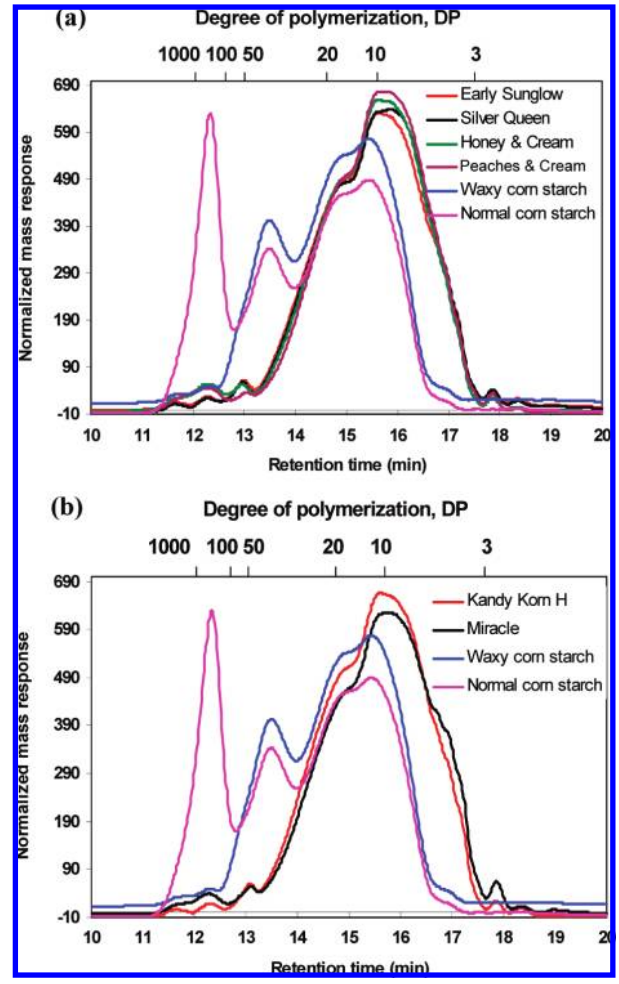

Figure 4. HPSEC chromatograms of debranched phytoglycogens from SU sweet corn lines (**a**) and SE sweet corn lines (**b**). Normal corn starch and waxy corn starch are used for comparisons.

efficiency and leads to randomly branched, water-soluble phytoglycogen (*10, 11*). In this type of mutant, a major reduction of starch biosynthesis is usually accompanied with the formation of phytoglycogen.

In addition to simple sugars and a minor amount of starch, phytoglycogen is a primary carbohydrate of sweet corns. It is interesting that the fine structure of phytoglycogen is essentially unaffected by genetic background, which implies an equivalent carbohydrate digestion property of sweet corns as far as phytoglycogen is concerned. From the perspective of plant carbohydrate metabolism, phytoglycogen can be used as a model α-glucan for studying in vivo functions of SBE.

Phytoglycogens Have Substantially Higher Branch Density than Starch. The number-average chain length (CL_N) of normal starch (DP21.9) is higher than that of waxy starch (DP17.4) due to the presence of amylose (**Table 3**). The CL_N of phytoglycogens ranges from DP10.5 to DP11.4. The data shown here are comparable with those reported by Yun and Matheson (*22*). In that work, the CL_N values of waxy starch and phytoglycogen were reported to be DP18 and DP12, respectively. The branch densities calculated were 5.6 and 8.3% for waxy starch and phytoglycogen, respectively. The branch density of phytoglycogens in our study ranges from 8.8 to 9.5% (**Table 3**), nearly twice that of starch from normal corn (4.6%) and waxy corn (5.7%).

Enhanced Branch Density Leads to Reduced Enzyme Digestibility for Malto-Oligosaccharides. Hydrolysate-2 of

Table 3. Number-Average Chain Length and Branch Density of Starches and Phytoglycogens

sample	number-average chain length, DP	branch density, %[a]
starch		
normal corn	21.9	4.6
waxy corn	17.4	5.7
phytoglycogen		
Early Sunglow	11.2	8.9
Silver Queen	11.0	9.1
Honey & Cream	11.4	8.8
Peaches & Cream	11.0	9.1
Kandy Korn	11.2	8.9
Miracle	10.5	9.5

[a] Branch density is calculated as the inverse of number-average chain length.

both normal corn starch and waxy corn starch has a significantly reduced degree of digestion by enzymes (**Table 4**). The degree of digestion of hydrolysates-2 of each type of starch remained at a lower level than that of starch through out the entire 120 min digestion process. In contrast, hydrolysate-1 showed a moderately reduced digestibility as compared with hydrolysate-0, and hydrolysate-0 showed a digestibility similar to that of the corresponding starch. At 20 min, the degree of digestion of all hydrolysates was lower than that of starch, suggesting a reduced initial digestion. At later stages, the degree of digestion of hydrolysates-0 increased to a level close to that of starch.

One plausible explanation is that the difference in digestibility among different hydrolysates is correlated with branch density. In hydrolysates-1 and -2, the highly branched populations (DP7, DP11, and DP17) were enriched by removing maltose and maltotriose. Therefore, an enrichment of highly branched glucan fraction causes reduced digestibility. Because the hydrolysates were products of substantial α-amylolysis, it is unlikely that the pancreatic α-amylase (in pancreatin) was a major factor of glucan degradation. Instead, the glucose released by amyloglucosidase was probably the rate-limiting step of digestion, and the branch density affects the digestion process by modulating glucan susceptibility to amyloglucosidase. In fact, past studies of branched oligosaccharides showed that α-1,6-glucosidic linkages are less susceptible to amyloglucosidase (from *Aspergillus niger*) than α-1,4 linkages (*6, 7*). Clearly, highly branched malto-oligosaccharides extracted from starch showed a reduced digestibility as compared with starch and regular maltodextrin and corn syrup, and this study shows that the highly branched low-digestibility α-glucan can be directly extracted from starch hydrolysates.

Enhanced Branch Density Correlates with Reduced Enzyme Digestibility for High Molecular Weight Glucans. The degree of digestion of phytoglycogens from both SU and SE sweet corn lines at 20, 40, and 60 min was lower than that of normal and waxy corn starches (**Table 5**). At 120 min, phytoglycogens of Early Sunglow and Honey & Cream showed a glucose release lower than that of starch. Generally, the reduction in digestibility of phytoglycogens is not as pronounced as that of hydrolysate-2 of both normal corn starch and waxy corn starch, which may be due to a lower branch density of phytoglycogens than that of hydrolysate-2.

To clarify the enzymatic factors affecting the digestion property of starch and phytoglycogen, separate treatments with pancreatin and amyloglucosidase were conducted. The pancreatin hydrolysis rates of starch and the phytoglycogen from the fresh and matured kernels of Peaches & Cream line are compared in **Table 6**. Both phytoglycogens and starches approached their degradation limits in only 20 min, suggesting that the conversion from large molecules to α-dextrins is a rapid step. The reduced degradation of phytoglycogen is evident throughout the entire enzymatic treatment, suggesting a greater abundance of α-dextrins resistant to further α-amylase degradation.

For both phytoglycogen and starch, glucose release by amyloglucosidase takes 60 min to reach approximate limits (**Table 7**). It is interesting that the digestion rates with amyloglucosidase are similar to the rates with both pancreatin and amyloglucosidase (**Table 5**). It is likely that, with the digestion conditions used in this study, the glucan degradation by pancreatic α-amylase is rapid enough to yield an efficient amount of α-dextrins as the substrate for amyloglucosidase.

It has been reported that the hydrolysis rate of the α-1, 4 linkage of maltose is 28-fold faster than that of α-1,6 linkage of isomaltose (*5, 6*). For soluble α-glucans, it is likely that the hydrolysis of α-1,6 linkage is the rate-limiting step of glucose release; therefore, the methods to increase the amount of α-1,6 linkage may have potential for the reduction of digestibility. There are a number of approaches to increase the amount of α-1,6 linkages; for example, Rydberg et al. (*27*) and Andersson et al. (*28*) reported using starch branching enzymes to increase glucan branch density. Recently, incubation with transglucosidase was reported to reduce enzyme digestibility (*8*), possibly by producing a number of isomalto-oligosaccharides. In this study, it was shown that ultrafiltration can effectively enhance branch density and modulate digestibility and that phytoglycogen, which is naturally produced by plants, has a lower digestibility than starch.

Factors of Designing Insoluble and Soluble Starch-Related Glucans for Modulated Digestibility. Different factors contribute to the digestion properties of insoluble and soluble starch-related glucans. The digestion begins with the action of

Table 4. Time−Course Glucose Release (% Based on Starch) by Pancreatin and amyloglucosidase from Starch Hydrolysates as Compared with That of Waxy Corn Starch, Normal Corn Starch, Maltodextrin, and Corn Syrup[a]

sample	0 min	20 min	40 min	60 min	120 min
hydrolysates of normal corn starch					
hydrolysate-0	0.76 ± 0.04 ab	58.2 ± 3.0 bcde	78.0 ± 5.8 abcd	78.3 ± 6.9 abc	85.5 ± 2.4 abc
hydrolysate-1	0.35 ± 0.01 b	56.3 ± 4.9 cde	79.9 ± 3.6 abcd	76.7 ± 4.1 abc	81.6 ± 3.7 abc
hydrolysate-2	0.81 ± 0 ab	54.1 ± 1.0 e	68.9 ± 7.0 bcd	66.3 ± 5.0 c	75.2 ± 2.9 c
hydrolysates of waxy corn starch					
hydrolysate-0	0.76 ± 0.03 ab	55.3 ± 1.9 de	79.2 ± 3.4 abcd	82.7 ± 3.0 ab	87.3 ± 1.9 ab
hydrolysate-1	0.93 ± 0.03 ab	55.3 ± 2.8 de	68.5 ± 3.3 cd	75.6 ± 2.0 abc	79.7 ± 5.5 abc
hydrolysate-2	0.83 ± 0 ab	52.3 ± 5.6 e	66.7 ± 1.2 d	69.9 ± 3.8 bc	76.9 ± 2.9 bc
waxy corn starch	0.17 ± 0.01 b	69.9 ± 7.4 ab	85.9 ± 3.4 a	88.0 ± 6.3 a	89.7 ± 4.5 a
normal corn starch	0.17 ± 0.02 b	71.9 ± 2.6 a	84.5 ± 4.2 abc	87.0 ± 2.0 a	86.9 ± 1.5 ab
maltodextrin	0.47 ± 0 b	69.5 ± 3.3 abc	85.3 ± 5.9 ab	81.9 ± 3.2 ab	89.0 ± 3.0 a
corn syrup	1.83 ± 0.05 a	67.9 ± 2.4 abcd	81.7 ± 3.9 abcd	88.1 ± 1.9 a	84.0 ± 1.0 abc

[a] Data are expressed using means ± standard deviations, $n = 4$. Significant differences in each column are denoted by different letters ($p < 0.05$).

Table 5. Time−Course Glucose Release (% Based on Dry Glucan) from Phytoglycogens and Starches by a Combined Hydrolysis of Pancreatin and Amyloglucosidase

sample	0 min[a]	20 min	40 min	60 min	120 min
Early Sunglow	N	67.6 ± 2.3 a[b]	79.8 ± 8.0 a	83.6 ± 2.0 abc	84.3 ± 4.2 b
Silver Queen	N	65.7 ± 3.2 a	76.2 ± 3.1 a	84.6 ± 3.3 abc	93.9 ± 5.0 ab
Honey & Cream	N	65.2 ± 2.6 a	76.8 ± 4.9 a	83.2 ± 2.1 abc	85.5 ± 1.3 b
Peaches & Cream	N	66.2 ± 5.5 a	78.1 ± 4.7 a	82.0 ± 1.1 bc	89.7 ± 4.2 ab
Kandy Korn H	N	65.0 ± 3.6 a	74.6 ± 6.0 a	80.4 ± 2.9 bc	89.1 ± 5.7 ab
Miracle	N	65.1 ± 2.1 a	74.6 ± 7.4 a	78.8 ± 1.2 c	87.9 ± 2.3 ab
waxy corn starch	N	68.4 ± 4.5 a	82.9 ± 6.2 a	93.0 ± 1.5 c	97.3 ± 0.8 a
normal corn starch	N	73.1 ± 2.6 a	85.0 ± 2.0 a	89.3 ± 1.8 ab	91.0 ± 4.3 ab

[a] Glucose release negligible for this group. [b] Means ± standard deviations, $n = 4$. Significant differences in each column are denoted by different letters ($p < 0.05$).

Table 6. Time−Course Reducing Sugar Release from Phytoglycogens and Starches with the Hydrolysis of Pancreatin (Maltose Equivalent mg/10 mg Starch)

sample	0 min	20 min	40 min	60 min	120 min
fresh Peaches & Cream	0.05 ± 0 ab[a]	8.38 ± 0.33 a	8.75 ± 0.4 ab	8.74 ± 0.13 b	9.05 ± 0.24 bc
matured Peaches & Cream	0.03 ± 0.005 ab	7.72 ± 0.39 a	7.66 ± 1.27 b	8.42 ± 0.26 b	8.60 ± 0.22 c
waxy corn starch	0.07 ± 0.002 a	9.25 ± 0.35 a	9.47 ± 0.36 ab	9.12 ± 0.58 ab	9.91 ± 0.28 ab
normal corn starch	0.02 ± 0.009 b	8.49 ± 0.93 a	9.85 ± 0.26 a	9.74 ± 0.26 a	10.10 ± 0.18 a

[a] Means ± standard deviations, $n = 4$. Significant differences in each column are denoted by different letters ($p < 0.05$).

Table 7. Time−Course Glucose Release (% Based on Dry Glucan) from Phytoglycogens and Starches by Amyloglucosidase

sample	0 min	20 min	40 min	60 min	120 min
fresh Peaches & Cream	0.1 ± 0.01 ab[a]	59.1 ± 1.8 b	67.2 ± 2.3 b	83.6 ± 3.9 a	83.6 ± 2.3 b
matured Peaches & Cream	0.14 ± 0 a	58.1 ± 5.3 b	76.7 ± 2.2 ab	83.3 ± 3.8 a	85.8 ± 2.2 b
waxy corn starch	0.14 ± 0 a	75.9 ± 3.6 a	88.5 ± 1.4 a	88.5 ± 6.8 a	95.2 ± 2.0 a
normal corn starch	0.02 ± 0 b	74.5 ± 0.98 a	81.9 ± 7.4 a	84.7 ± 1.7 a	97.9 ± 1.7 a

[a] Means ± standard deviations, $n = 4$. Significant differences in each column are denoted by different letters ($p < 0.05$).

α-amylase in salivary fluid and the duodenum (*3*). The linear molecules are hydrolyzed into maltose and maltotriose, and the branched molecules are degraded into maltose, maltotriose, and small branched α-limit dextrins. These di-, tri-, and oligosaccharides are hydrolyzed to glucose by the combined actions of maltase-glucoamylase (α-limit dextrinase) and sucrase-isomaltase complexes. For insoluble starch-related glucans, the degradation of particles by α-amylase can be essential to their conversion to glucose. The classification of RS is based on the mechanism affecting carbohydrate susceptibility to enzyme. For example, RS-1 provides physical barrier to α-amylase, and RS-2, RS-3, and RS-4 reduce α-amylase accessibility at granular, crystalline, and molecular levels. In contrast, the action of amyloglucosidase is primarily to convert α-dextrins to glucose and is unlikely to be rate-limiting.

For soluble α-glucans, the accessibility to α-amylase is unlikely to be a rate-limiting step of glucose release. The conversion from α-dextrin to glucose possibly governs the digestion rate. The primary factor is not the physical accessibility but the branch structure that affects the glucan susceptibility to amyloglucosidase (in vitro) or maltase−glucoamylase and sucrase−isomaltase (in vivo). Abdullah et al. (*29*) found that fungal amyloglucosidase releases glucose from panose, isomaltotriose, and isomaltotetraose much slower than from maltotriose and maltotetraose, and Taravel et al. (*30*) showed that glucose release by α-limit dextrinase was much lower from isomaltose and panose than from maltose, maltotriose, and maltotetraose. In this study, starch-based malto-oligosaccharides and high molecular weight α-glucans were analyzed, and it appears that for soluble glucans the branch density is a primary factor for in vitro enzyme digestibility. The information obtained may lead to effective production of soluble α-glucans with modified digestibility using both genetic and postharvest strategies. For example, the highly branched oligosaccharides can be produced in a corn-refining facility that is capable of enzymatic starch conversion or hydrolysis, with a possible addition of an ultrafiltration operation. Along with the production of oligosaccharides, the coproduct that contains small sugars (e.g., DP2 and DP3) can still be used or converted to glucose. For phytoglycogen, probably further enzymatic treatment is necessary to improve the branch density to make α-glucans with substantially reduced digestibility.

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