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Nutritional Property of Endosperm Starches from Maize Mutants: A Parabolic Relationship between Slowly Digestible Starch and Amylopectin Fine Structure

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The relationship between the slow digestion property of cooked maize starch and its molecular fine structure was investigated. Results of the in vitro Englyst assay showed a range of rapidly digestible starch (RDS) (70.1−98.9%), slowly digestible starch (SDS) (0.2−20.3%), and resistant starch (RS) (0.0−13.7%) among the tested maize mutant flour samples. Further analysis showed that amylose content was significantly correlated ($R = 0.763$, $P < 0.001$) with RS amount but not with that of SDS, indicating that amylopectin is the starch molecule associated with SDS. Total starch debranching analysis revealed a parabolic relationship between SDS content and the weight ratio of amylopectin short chains (DP < 13, named SF) to long chains (DP ≥ 13, named LF), which means amylopectin with a higher amount of either short chains or long chains can produce relatively high amounts of SDS. Furthermore, debranching analysis of the SDS materials from samples with the highest and lowest weight ratios of SF/LF (both had a high amount SDS) showed significantly different profiles, indicating there is not a uniform molecular structure for SDS. Thus, genetic mutants of maize samples have a good potential to provide raw starch materials of high nutritional quality. An additional finding showed that a simple and comparably high-throughput technique of Rapid Visco-Analyzer (RVA) can be used to screen genetic mutants on the basis of their RVA profiles.

KEYWORDS: Slowly digestible starch (SDS); resistant starch (RS); starch gelatinization; nutrition; starch; amylopectin fine structure; maize; corn; mutant; Rapid Visco-Analyzer (RVA)

INTRODUCTION

Starch is the main energy-providing material in cereal- and tuber-based food products. Proper rate of glucose release and absorption from digesting starch may play important roles in human health by helping to maintain proper blood glucose levels. On the basis of the rate of digestion, starch has been classified as rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (*1*) to specify its nutritional quality related to physiological effects. RDS usually leads to a rapid increase of plasma glucose and insulin levels, whereas SDS is digested slowly with a moderate glycemic and insulinemic response. Chronic consumption of foods with high contents of RDS will cause a substantial fluctuation of glucose homeostasis regulatory hormones and high stress to the regulatory system, which might be associated with a series of health complications such as diabetes, cardiovascular disease, and obesity (*2*). Therefore, improving food quality with higher amounts of SDS and RS is of increasing interest to academic researchers, crop breeders, the food industry, and health professionals. Although there have been several reports on SDS preparation (*3*–*7*) using different techniques, the amount of SDS in regular food products is very low, and a fundamental understanding of the slow digestion property of starch is necessary to increase SDS content in processed foods.

In food processing, starch gelatinization, which is a process of disrupting starch crystalline structure with heat and moisture, causes a decrease or loss of the slow digestion property of native cereal starches (*8*). Gelatinized starch may disperse in a liquid suspension, form a thick paste, or exist in a completely amorphous state depending on the moisture content of the final food product. For these gelatinized starch forms, their granular or supramolecular structures are almost completely lost, and the molecular structure of starch likely becomes a major determinant of its functionality and nutritional property. Starch is composed of essentially linear amylose molecules and highly branched amylopectin molecules. Its properties mainly depend on the ratio of amylose to amylopectin and the fine structure of amylopectin, namely, the chain length distribution and its branching pattern. It is known that amylose content (1−30%)

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Table 1. Starch, Amylose, Rapidly Digestible Starch (RDS), Slowly Digestible Starch (SDS), and Resistant Starch (RS) Contents on the Basis of the Englyst Assay of Selected 18 Maize Flour Samples

no.	starch[a]	SD[b]	amylose[c]	SD	RDS (%)	SD	SDS (%)	SD	RS (%)	SD
6	40.93	0.01	11.08	3.8	80.78	2.96	20.28	3.96	0.00	2.34
11	51.80	1.46	5.66	0.04	76.77	3.26	20.35	4.59	2.88	3.21
12	58.63	0.52	26.36	0.05	77.08	2.03	16.33	2.61	6.60	1.63
13	56.98	0.96	41.43	2.98	80.67	2.19	12.38	3.73	6.95	3.01
14	59.11	0.66	32.74	2.15	78.47	1.12	16.88	2.18	4.66	1.86
15	57.28	0.81	12.46	0.54	91.96	1.35	11.58	2.89	0.00	2.55
16	59.11	1.05	5.64	0.01	89.86	3.19	10.69	3.97	0.00	2.36
17	51.68	0.17	75.4	2.56	70.09	1.05	16.20	1.31	13.71	0.79
18	48.10	0.47	8.69	0.92	97.41	1.81	4.79	2.16	0.00	1.16
19	56.99	0.06	31.38	1.98	95.14	2.88	6.71	2.89	0.00	0.23
20	54.35	0.80	66.74	4.09	84.17	2.86	12.18	3.57	3.65	2.13
23	60.25	2.52	49.56	3.21	79.92	1.13	7.17	2.52	12.92	2.25
26	54.98	1.51	71.9	3.61	72.57	0.74	14.49	4.02	12.94	3.95
33	58.22	0.77	36.17	2.87	98.92	1.22	0.17	2.52	0.91	2.25
36	67.27	0.87	33.51	5.90	82.79	2.95	9.38	4.02	7.83	3.95
45	60.56	0.22	63.94	0.63	84.43	0.69	9.69	3.42	5.87	2.74
46	58.56	0.99	58.30	1.58	80.80	0.95	7.52	4.02	11.68	3.95
47	60.02	0.82	48.73	3.90	79.32	0.69	11.08	2.70	9.60	2.62

[a] Percentage on the basis of wet flour weight. [b] Standard deviation [c] Percentage based on the dry weight of starch.

is negatively associated with the onset and peak minimum temperature of starch pasting (*9*) and that amylopectin is associated with temperature and enthalpy of starch gelatinization (*10*) in which amylopectin with more branches has a lower gelatinization temperature, whereas that with more long chains has a higher gelatinization temperature (*11*). The relationship between the molecular structure of starch and its nutritional property (digestion rate and extent) after starch gelatinization, however, is not well understood.

The molecular structure of starch is determined by the enzymes involved in its biosynthesis (*12, 13*). For endosperm storage starch granules, each glucose unit is added in the form of ADP-glucose, which is converted from glucose-1-P through the action of ADP-glucose pyrophosphorylase (AGPase). The cytosolic ADP-glucose is transported into an endosperm amyloplast by the adenylate translocator (*bt1* gene) (*14*) for the synthesis of starch molecules and granules. The linear amylose is elongated by granule-bound starch synthase I (GBSSI), whereas amylopectin is synthesized by soluble starch synthases (SSII and SSIII), branching enzymes (SBEI, SBEIIa, and SBEIIb), and debranching enzymes (isoamylase type and pullulanase type). For maize endosperm starch, more than 20 genes involved in starch synthesis have been identified through genetic mutations (*15*). Maize mutants of waxy (*wx*, GBSSI defective), sugary-1 [*su1*, isoamylase defective (*16*)], sugary-2 [*su2*, SSIIa defective (*17*)], dull1 [*du1*, SSII defective (*18*)], and amylose-extender [*ae*, SBEIIb defective (*19*)] are commonly used crop lines in academic research and genetic breeding. Starch materials produced from single, double, or triple mutations of these genes have distinct phenotypes, and some are of high commercial value, for example, high amylose starch (*ae* mutation) for resistant starch production. These maize mutants are also invaluable tools to investigate the structure−nutritional property relationships of starches.

It has been known that starch gelatinization generally increases its digestibility, and most processed foods have a high amount RDS (*20*). However, the effect of the molecular structure of starch on its digestion rate and extent has yet to be systematically studied in great depth. In the current investigation, a series of maize mutants with different genotypes were used to examine the relationship between the nutritional properties of gelatinized starch and their molecular structures. More specifically, the effect of starch molecular structure on its slow digestion property was investigated to have a better understanding of SDS in processed food products, to develop a structural definition of SDS, and to provide the fundamental basis for modifying starch structures genetically or through physical or enzymatic means to increase the SDS content in regular processed food products.

MATERIALS AND METHODS

Materials. Sixty maize mutant flour samples were provided by Tate & Lyle (Decatur, IL); no detailed description of their genotypes and breeding history is available due to proprietary concerns. α-Amylase (EC 3.2.1.1) type VI-B from porcine pancreas (19.6 units/mg), amyloglucosidase (EC 3.2.1.3) from *Rhizopus* mold (21100 units/g), pepsin (EC 3.4.23.1) from porcine stomach mucosa (1:2500, 51 units/mg), pancreatin from porcine pancreas, invertase (EC 3.2.1.26) grade VII from baker's yeast (355 units/mg), and guar gum were purchased from Sigma Chemical Co. (St. Louis, MO). Glucose assay reagents and isoamylase (EC 3.2.1.68, 250 units/mL) were from Megazyme International Ireland Ltd. (Wicklow, Ireland).

Methods. *Starch Hydrolysis, Total Starch and Amylose Content Measurement, and Cooking Method.* The flour samples [10%, wet weight based (wb)] were cooked in a boiling water bath for 20 min, and then the cooked samples were maintained at 4 °C overnight before starch fractionation based on the Englyst test (*1*) with values expressed on a dry starch weight basis. Total starch content was measured on the basis of a heat-stable α-amylase and amyloglucosidase method using the Total Starch measurement kit from Megazyme International Ireland Ltd. Amylose content was measured using the Amylose/Amylopectin Assay that employs concanavalin-A to separate the two polymers (Megazyme).

Small-Scale Starch Isolation. Eighteen samples were chosen on the basis of their amount of RDS, SDS, and RS after the Englyst test. Starches from these selected maize flour samples were isolated according to a dimethyl sulfoxide (DMSO) method. Flour (100 mg, wb) was dissolved in 5 mL of DMSO in a boiling water bath with stirring for 1 h. After overnight stirring at room temperature to completely dissolve the sample, 25 mL of ethyl alcohol was added, and then centrifugation was performed at 5000*g* for 20 min to precipitate the dispersed starch molecules. The precipitates were further washed three times using ethyl alcohol to remove remaining nonstarch materials. After vacuum-drying overnight, the extracted starch samples were collected for further analysis.

Material Preparation for Chain Length Distribution Analysis. Flour samples were digested first using the Englyst method, and the reaction was stopped at 20 and 120 min using ethyl alcohol with a final concentration of 80% (v/v). Precipitates were dried after centrifugation for 10 min at 5000*g*. Dried starch precipitates were first treated using the above DMSO method and dissolved in hot water, and deproteination (mostly enzymes added from the Englyst test) was performed by using 1:1 (v/v) phenol/chloroform. After the phenol/chloroform was added, the mixture was vortexed for 5 min and then centrifuged at 5000*g* for 10 min to separate the protein and organic layers from the aqueous layer, and then the middle layer of protein and the top layer of organic solvent were discarded. The procedure was repeated until the middle protein layer disappeared. The starch samples in the aqueous layer were precipitated by adding 90% ethyl alcohol, vacuum-dried, and bottled for debranching and chromatographic analysis.

Chromatographic Analysis of Debranched Amylopectin. Chain length distribution profiles of the debranched maize mutant starches and SDS and RS materials were analyzed using a high-performance size exclusion chromatography (HPSEC) system based on the method described by Zhang et al. (*8*).

Rapid Visco-Analysis (RVA). A Rapid Visco-Analyzer (Newport Scientific Inc.) was used to measure the pasting properties of starches isolated by the traditional method with toluene to remove the proteins. Starch (2.0 g) and double-distilled water were added to a final weight of 25.0 g, and the RVA was operated according to standard method 1 (short time procedure) from the RVA manual.

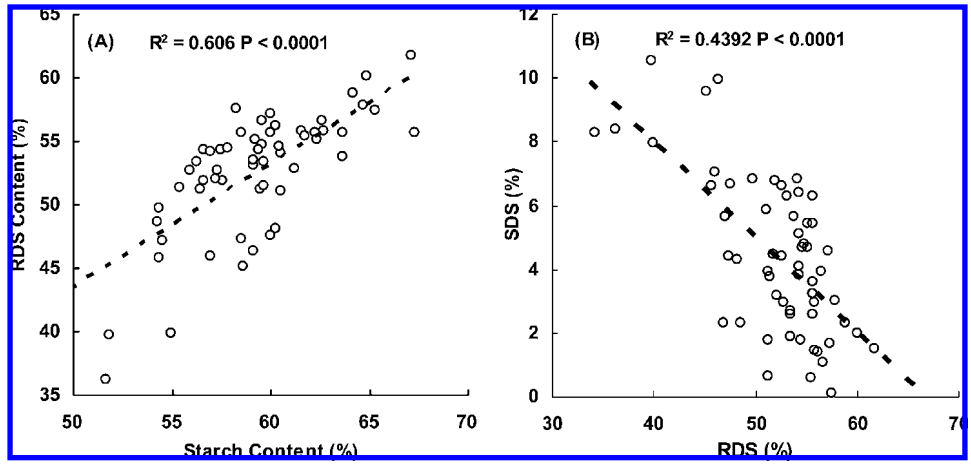

Figure 1. Correlations between (**A**) RDS content and starch content and between (**B**) RDS and SDS contents (same flour weight basis).

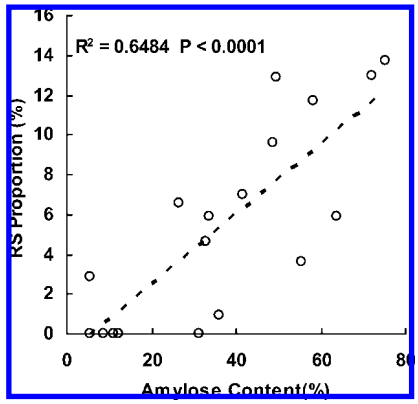

Figure 2. Relationship between RS content and amylose content of maize starches from the 18 selected samples from **Table 1**.

Statistical Analysis. Common statistical analysis was carried out using statistic tools from Microsoft Excel, and the *P* value was obtained by comparing the calculated *t* value

$$t = \frac{R \times \sqrt{\mathrm{df}}}{\sqrt{1 - R^2}}$$

where *R* is the correlation coefficient, df is the degree of freedom, with two tails probability from the table of percentage points of *t* distribution.

RESULTS AND DISCUSSION

Starch Digestion Property of Cooked Maize Flour Samples. The Englyst assay (*1*) is one of the commonly used methods to categorize starch fractions based on starch digestion rate. RDS, SDS, and RS are the three consecutive digestion fractions divided by reaction time and represent three different starch materials found in cooked starches and processed foods. RDS and glycemic index are highly correlated in processed foods (*21*). For SDS, slow and prolonged release of glucose of native maize starch was demonstrated in human subjects (*22*) and was concluded to be an ideal SDS material from the structural perspective (*23*). RS is resistant to digestion, and no glucose is available for glycemic response. However, the fermentation of RS in the large intestine generates short-chain fatty acids (SCFA) that are beneficial for colonic health (*24, 25*). Therefore, both SDS and RS, either alone or in combination, contribute to an improved nutritional quality of starch.

A range of RDS (70.1−98.9%), SDS (0.2−20.3%), and RS (0.0−13.7%) contents, on the basis of total starch, is shown for the screened maize mutant flour samples (**Table 1**, representing 18 samples selected from the 60 analyzed). RDS was the highest proportion for all the screened samples and is consistent with the finding that cooking leads to a great loss in SDS (*23*). Yet, different maize starch mutant genotypes showed variation in SDS and RS proportions, which indicates that genetic mutations have a high potential to change the nutritional quality of starch materials. Interestingly, a high correlation between RDS and starch contents (same flour weight basis) (**Figure 1A**) suggests a high-quality starch material with a high proportion of SDS, as well as RS, would be found in grains with lower yield of starch, and regular cereal-based food products with high content of RDS may be related to the utilization of crops with high yields. The most effective way to increase the content of SDS might be realized by substantially decreasing the amount of RDS (**Figure 1B**). Studies are needed to find ways to simultaneously maintain starch content and improve its nutritional quality.

In the screening process, an overnight cooling of cooked flour samples at 4 °C was performed before the Englyst assay in order to amplify the difference in digestion rates and extents among samples. A significant correlation ($R = 0.763$, $P < 0.0001$) between RS, although not SDS, and amylose content of the samples (**Figure 2**) indicates that amylose is the main starch molecule related to RS. It is well-known that starch is composed of essentially linear amylose and highly branched amylopectin molecules, and upon cooling, the leached amylose molecules during and after starch gelatinization reassociate rapidly to form crystalline structures (7−10 h), which are resistant to digestion. This is generally called short-term retrogradation (*26*), and an overnight cooling is long enough for amylose to complete the process of retrogradation to form RS. This result is consistent with literature reports, particularly for type 3 RS formed by amylose retrogradation (*27*). On the other hand, amylopectin, with its complex branched fine structure, is likely the major starch molecule related to the slow digestion property of starchy materials.

Fine Structure of Amylopectin and Relationship with SDS. The selected 18 maize flour samples shown in **Table 1**, with a range of contents of SDS and RS, were further analyzed to investigate the relationship between amylopectin fine structure and their slow digestion properties. Different chain length distribution profiles after total starch debranching were found, and some representatives are shown in **Figure 3**. It is clear that some starches are waxy mutants (e.g., samples 11, 6, and 15) with negligible amounts of amylose, some have a high amount of amylose (e.g., sample 17), and the remaining are intermediate in this respect (e.g., samples 14 and 33). Debranched amylopectin of sample 11 (**Figure 3**) was delineated into several

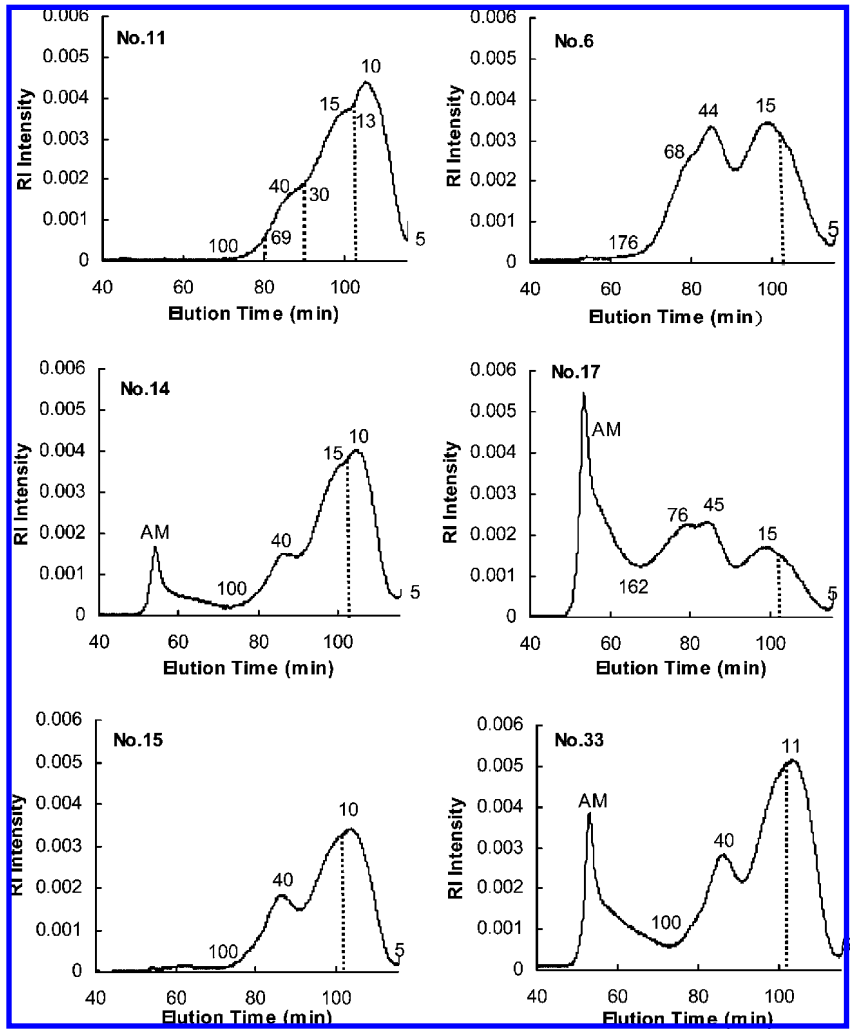

Figure 3. Representative size exclusion chromatograms of total starch debranching profiles of selected maize starch samples exhibiting a range of SDS and RS. Degrees of polymerization (DP) of 13, 30, and 69 are marked by the dotted line for sample 11 with DP 13 shown in all samples, which is found above the curve at the peak or shoulder.

fractions (DP < 13, DP 13−30, DP 31−69, and DP > 69), representing peaks of its chromatographic profile. There was a range in amounts of different fractions among the mutant samples (data not shown). On the other hand, most samples (amylopectin) showed a bimodal distribution similar to that of the debranched normal maize starch (*8*). On the basis of the study of Hizukuri (*28*) and the cluster model of Robin et al. (*29*), amylopectin molecules have A, B (B_1−B_4), and C chains in which A and B_1 chains are called short chains and others are collectively called long chains. The delineated fractions of (1) DP < 13 and (2) DP 13−30 together compose the short chains and likely correspond to A+B_1 chains, and the other remaining longer chain fractions compose the long chains and likely correspond to B_2−B_3 and other B chains of amylopectin with higher DPs. For the purpose of our discussion, the fraction of DP < 13 hereafter will be called the short-chain fraction (SF), and the other combined fractions will be called the long-chain fraction (LF). This delineation of the chains is consistent with results from the literature that short chains of DP 6−12, generally thought of as A chains in the cluster model, are an important marker for crystalline allomorphs (A, B, and C types crystalline structure) of amylopectin in the starch granules (*30*).

A *parabolic relationship* between SDS content and the weight ratio of SF (DP < 13) to LF (DP ≥ 13) of amylopectin was revealed (**Figure 4**), showing that starch samples with amylopectin having either a high proportion of short chains or a high proportion of long chains had a higher content of SDS. A middle value (~0.5) of SF/LF weight ratio had the lowest content of SDS, represented by a wild type normal maize starch. Thus, starch samples can be divided into two groups: group I with low values of SF/LF weight ratio (0.166−0.525) and group

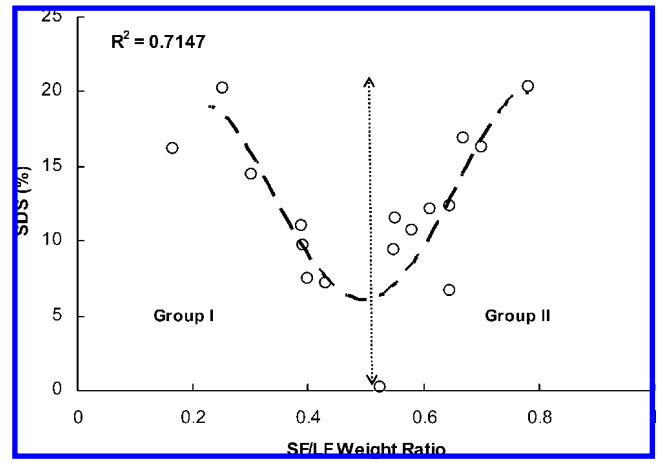

Figure 4. Parabolic relationship between proportion of SDS and the weight ratio of the short-chain fraction (SF, DP < 13) to the long-chain fraction (LF, DP ≥ 13) of debranched amylopectin. The two groups of starch samples were divided at the dotted line with the lowest percent SDS.

Table 2. Weight Ratio of SF/LF for Group I Samples

	sample							
	6	17	23	26	33	45	46	47
SF/L	0.251	0.166	0.431	0.303	0.525	0.392	0.400	0.387

Table 3. Weight Ratio of SF/LF for Group II Samples

	sample								
	11	12	13	14	15	16	19	20	36
SF/LF	0.782	0.700	0.647	0.669	0.552	0.580	0.645	0.612	0.548

II with high values of SF/LF weight ratio (0.525−0.782). The range in weight ratios of SF/LF for both groups demonstrates their large variations in chain length distribution (**Tables 2** and **3**).

It should be noted that the SF and LF fractions are not exactly the same as A chains and B chains in the classic cluster model of amylopectin showing a range of ratio of 1.0:1 to 1.5:1 (weight ratio of A to B) (*31*). However, the ratio SF/LF does indirectly represent the branch density of amylopectin (a high weight ratio of SF/LF indicates a high branch density), and both the high and low weight ratios of SF/LF indicate a large deviation from the normal type starch with a value of ∼0.5. The large variation of SF/LS among the selected samples is likely the result of variable activity, due to gene mutations, of the enzymes involved in starch synthesis. From available information in the maize genetics literature, a high or low branching of amylopectin is due to the interplay of genes encoded for branching and debranching enzymes. The *sugary-1* mutation, caused by inactivation of the debranching enzyme (*32*), accumulates phytoglycogen with a significant increase in branch density (*31*), compared to normal type amylopectin of ∼5% branches. On the contrary, the mutation in the amylose extender gene (*ae*) that encodes for the branching enzyme SBEIIb (*33*) severely decreases the branch density of amylopectin and significantly increases chain length (*34*). Although most of the literature reports are on the effects of gene mutations (not enzyme activity), increasing or decreasing the activity of branching or debranching enzymes is a possible way to change the molecular structure of amylopectin for desired nutritional properties. The parabolic relationship of SF/LF of amylopectin fine structure to SDS content provides direction for genetic manipulation to generate high nutritional quality raw starch materials.

This elucidated structural requirement of starch molecules for high nutritional quality can also be used as the basis for structural modification of regular starches to either increase the chain length or modify the branch pattern (including the length and arrangement of the branched chain and branch density) of amylopectin using a variety of methods. Amylopectin molecules with a combination of high branch density and longer chains (likely resulting in a substantial improvement of SDS) could possibly be produced using starch synthase to elongate the chains of a highly branched amylopectin substrate. A recent report by Putaux et al. (*35*) on enzymatic chain extension of glycogen might be a good example of a glucan possibly with a high amount of SDS on the basis of our current findings.

Chain Length Distribution of Amylopectin and RDS, SDS, and RS. Although the chain length distribution of amylopectin generally showed a bimodal distribution, the fractions of the debranched linear chains can be further delineated to provide a more detailed analysis of the amylopectin fine structure related to RDS, SDS, and RS. On the basis of the cluster model (*28*), DP 5−9 represents the shortest A chains that cannot form double helices (*36*), DP 6−12 is generally typical A chains, DP 13−30 belongs to the range of B_1 chains (*37*), DP 31−69 belongs to the range of B_2 chains, and DP > 69 belongs to B_3 and other long B chains based on a repeating distance of ∼25 glucose units between clusters (*38*). Correlation matrices between starch digestibility values (RDS, SDS, and RS) and the amounts of each fraction of debranched amylopectin of groups I and II are shown in **Tables 4** and **5**. For group I samples with low SF/LF ratios (**Table 4**), a negative correlation was found between the short linear chain fraction (DP 5−30) and SDS, whereas a positive correlation was shown between SDS and the long linear chain fraction (DP 31−69), indicating that SDS is mainly determined, in this group, by the long chains of the amylopectin. A similar trend of relationship between different fractions of linear chains was also shown for RS, although it was not statistically significant. For RDS, the fraction of DP 5−30 was positively correlated, whereas the fraction DP > 30 was negatively correlated, indicating that high amounts of short chains are the main determinant of RDS in group I starches. For group II samples with high SF/LF ratios (**Table 5**), an opposite relationship appears with high and significant correlation between SDS and the broad short linear chain fraction of DP 5−30, although particularly those shortest DP linear chains of 5−9, suggesting that they are the major determinant of slow digestion as well as RS properties. The long linear chains of group II samples, specifically DP 30−69, appear to be the determinant of RDS. A significant positive relationship was shown between amylose content and RS content in the group I samples, whereas no significant relationship was found between amylose and RS in the group II samples. This might be related to the fact that samples with a high amount of amylose also have amylopectin with a low SF/LF ratio and that samples with a high ratio of SF/LF were mostly low in amylose content.

SDS and RS showed similar correlative trends to amylopectin fine structure (SF/LF weight ratio) within either group I or II

Table 4. Correlation Matrix of Amylopectin Structure with Digestibility for Group I Maize Genotypes with Low SF/LF Weight Ratios

fraction (AP)	SDS	*P* value	RS	*P* value	RDS	*P* value
DP5−9	−0.779	0.0133	−0.546	X[a]	0.832	0.0053
DP9−13	−0.855	0.0033	−0.455	X	0.807	0.0086
total SF	−0.83	0.0056	−0.495	X	0.823	0.0064
DP13−30	−0.636	X	−0.398	X	0.648	X
DP30−69	0.752	0.0193	0.502	X	−0.786	0.0119
DP > 69	0.721	0.0284	0.413	X	−0.703	0.0344
total LF	0.829	0.0057	0.497	X	−0.824	0.0063
SF/LF	−0.795	0.0104	−0.525	X	0.827	0.0059
amylose	0.157	X	0.852	0.0035	−0.732	0.0249

[a] Not statistically significant.

Table 5. Correlation Matrix of Amylopectin Structure with Digestibility for Group II Genotypes with High SF/LF Weight Ratios

fraction (AP)	SDS	*P* value	RS	*P* value	RDS	*P* value
DP5−9	0.689	0.0275	0.298	X[a]	−0.639	0.0467
DP9−13	0.279	X	0.447	X	−0.345	X
total SF	0.803	0.0051	0.538	X	−0.820	0.0037
DP13−30	0.123	X	0.237	X	−0.149	X
DP30−69	−0.715	0.0202	−0.751	0.0122	0.843	0.0022
DP > 69	−0.563	X	−0.144	X	0.430	X
total LF	−0.803	0.0051	−0.538	X	0.820	0.0037
SF/LF	0.801	0.0054	0.519	X	−0.810	0.0045
amylose	−0.264	X	0.394	X	−0.010	X

[a] Not statistically significant.

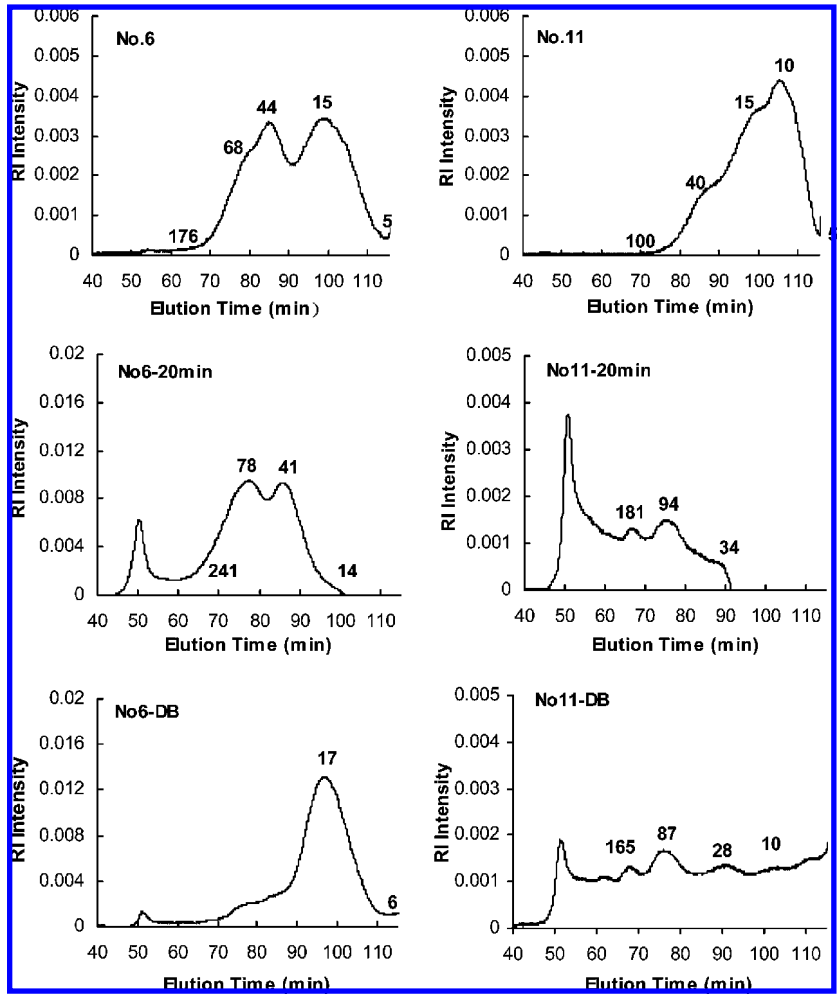

Figure 5. HPSEC profiles of samples 6 and 11, which represent group I and II starches with the highest content of SDS. "20 min" means digested for 20 min using the Englyst assay, and DB means the digested sample is further debranched with isoamylase. The top chromatograms are controls of debranched starch from samples 6 and 11.

samples, whereas RDS showed an opposite correlation. Thus, the property of SDS is closer to that of RS than RDS. RS in gelatinized starch is a result of retrogradation and, in this way, is a physical entity (*39*), and SDS generated after starch gelatinization, at least for group I samples, is likely also a physical entity (dependent on retrogradation). The opposite relationship between SDS-RS/RDS and fractions of debranched amylopectin in group I and II starches indicates that the mechanisms of the slow digestion property for the two groups are different. For group I starches with low SF/LF weight ratios, amylopectin molecules are longer, with comparably fewer branches, whereas amylopectin molecules of group II starches have more short chains, particularly of DP 5−9, and a higher degree of branching. SDS in group I samples is likely a physical entity with long B chains facilitating association among molecules, whereas SDS in the group II samples is likely a chemical entity that is inherent in the special chemical structure (high branching density and short chains) of its amylopectin molecules. The localized difference in arrangement of amylopectin branched chains and their length are apparently the bases for the parabolic relationship between SDS and amylopectin fine structures.

Chain Length Distribution Profiles of SDS Materials. As SDS is the starch fraction digested between 20 and 120 min (*1*), SDS material was prepared by stopping the reaction at 20 min of samples 6 and 11, representing the highest SDS contents from group I and II starches, respectively. Because both samples had little RS and are waxy starches (from their chromatographic profiles showing no amylose), the prepared samples (stopped at 20 min) were considered to be pure SDS materials for further analytical work.

For sample 6 of the group I starches with a high proportion of long chains, HPSEC profiles of the SDS material showed two peaks of DP 41 and 78 in the digestion products. The first peak (at the void volume) was likely undigested or partially digested amylopectin. After debranching with isoamylase, a single peak with DP of 17 was formed (**Figure 5**, No.6-DB), which was different from the original structure (**Figure 5**, No.6). For sample 11 of the group II starches with a high proportion of short chains (more branches), the chain length profile of the SDS material included undigested or partially digested amylopectin molecules (**Figure 5**, No.11−20min) and several small peaks with DPs of 34, 94, and 181. Multiple peaks were found after debranching (**Figure 5**, No.11-DB). The peak area of the fractions with a DP < 34 was ~39% of the total area, indicating a substantial proportion of small molecules in the debranched material. This result is consistent with results shown above (**Table 2**) that very short chains are the main determinant of SDS in group II samples with a high ratio of SF/LF.

In the Englyst assay, both α-amylase and amyloglucosidase are present in the reaction system. It is known that α-amylase cleaves only α-1,4 glycosidic linkages, and for amyloglucosidase, the rate of cleaving α-1,6 linkages is much slower than

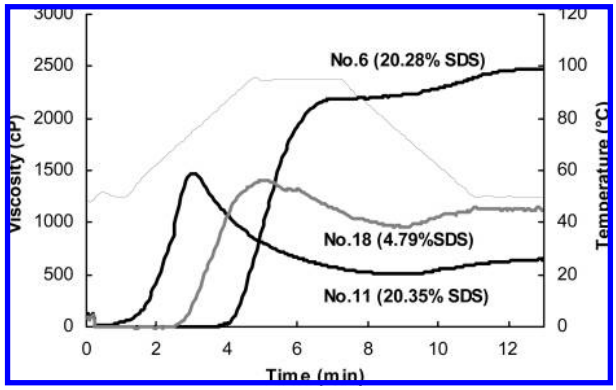

Figure 6. RVA profiles of three maize starches. The label indicates the proportion of SDS.

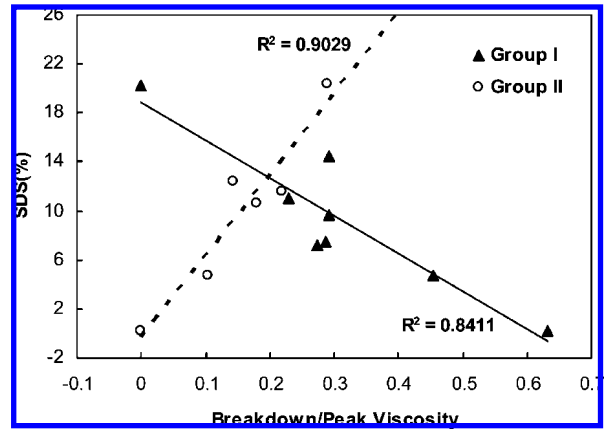

Figure 7. Relationship between SDS content and the ratio of breakdown to peak viscosity. Group I is samples with a high proportion of long chains, and group II is samples with a high proportion of short chains.

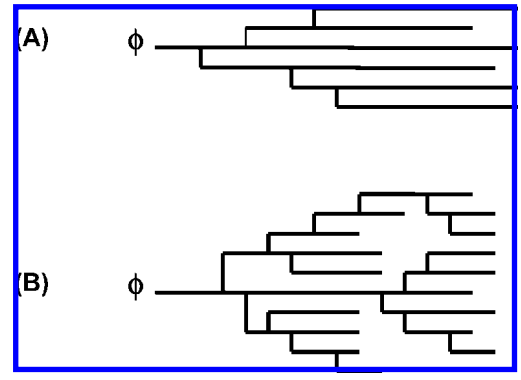

Figure 8. Diagram representing the amylopectin molecules with greater proportion of long chains (**A**) and greater proportion of short chains (**B**).

that of cleaving α-1,4 linkages (*40*). The residues after Englyst digestion at 20 min are generally small molecules with a high degree of branches (i.e., α-limit dextrins), and, apparently, their molecular weight distribution profiles are dependent on the molecular structure of amylopectin, particularly its branching pattern (*41*). Although it is difficult to determine specifically what part of the molecule was digested on the basis of the chromatogram of the SDS material, SDS material produced from samples 6 and 11 did show distinctly different molecular weight distribution profiles before and after debranching, reflecting the large difference in molecular structures between group I and II samples.

Pasting Property of Samples. In the current study, we have shown that there is an intimate relationship between the molecular structure of starch and its susceptibility to enzyme digestion. However, in vitro digestion procedures such as the Englyst test are labor intensive and costly to screen samples. The molecular structure of starch has been intensively studied in relation to starch functionality in food systems, and there are many available simple methods to test the functionality of starch, such as pasting properties using the RVA. Simple procedures to screen the nutritional property of starches from a large number of crop lines would save time and expense.

The pasting properties of starches (or flours) have been shown to be related to the structure of the starch molecules after starch is gelatinized. Using the RVA, amylose content was found to be correlated to paste setback and final viscosity (*42*), whereas the ratio of DP 6−12 to DP 6−24 linear chains of amylopectin was related to gelatinization temperature and peak viscosity; the peak viscosity increased as the ratio decreased, whereas a high ratio was correlated to a reduced gelatinization temperature (*43*). Han and Hamaker (*44*) found that the proportion of long chains of amylopectin was negatively correlated to paste breakdown, whereas the proportion of short chains was positively correlated. These data suggested that a simple RVA pasting profile could reflect the starch molecular structure of the tested sample and, thus, its nutritional property.

A typical RVA pasting profile is described using parameters of gelatinization time, peak time, peak viscosity, trough viscosity, final viscosity, and calculated breakdown (peak − trough), setback (final − trough), and consistency (final viscosity − peak viscosity). Substantial differences of pasting profiles among three different waxy-based starches are shown in **Figure 6**. These data are consistent with those found in the literature (*45*). Sample 11 starch with more short chains had lower gelatinization temperature and higher breakdown, whereas sample 6 starch, with more long chains, had a higher gelatinization temperature and less breakdown. Sample 18, as a typical waxy mutant starch, showed a pasting curve between those of samples 11 and 6 and had a lower SDS amount than that of either sample 11 or sample 6. Consistently, a highly significant positive correlation between SDS content and the ratio of breakdown to peak viscosity was found for group II samples with more short chains, and a highly significant negative correlation was found for group I samples with more long chains (**Figure 7**). Therefore, RVA, as a simple and quick analytical method, may be used to screen samples for SDS on the basis of reference samples with known fine structures and nutritional properties.

Conclusions. A parabolic relationship between starch structure and the slow digestion property of cooked starch was revealed for the first time, showing starch with either a high amount of short chains (DP < 13) or a high amount of long chains (DP ≥ 13) has a higher content of SDS. Thus, there are at least two types of amylopectin molecular structures that are characteristic of SDS materials. A schematic representation of their molecular fine structures is shown in **Figure 8**. This relationship between amylopectin fine structure and SDS suggests that mutations of starch-synthesizing enzymes, or in vitro modification of starches by enzymes, to introduce these long or short chains with high branching are necessary to obtain raw materials with comparably high amounts of SDS. Normal untreated starches used in most food products are less likely to yield an optimal nutritional property. A simple viscosity-based screening method was also introduced to assess the SDS property. Because starch gelatinization and the following retrogradation are two important processes in regular food preparations, the effect of starch gelatinization and retrogradation on starch

digestibility in the context of starch molecular fine structure was additionally examined to elucidate the mechanism of the slow digestion property of cooked starches and is presented in a companion paper.

ABBREVIATIONS USED

DP, degree of polymerization; SDS, slowly digestible starch; RDS, rapidly digestible starch; RS, resistant starch; DSC, differential scanning calorimetry; SF, short-chain fraction; LF, long-chain fraction; RVA, Rapid Visco-Analyzer; DMSO, dimethyl sulfoxide.

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