Project Title: Value-added Utilization of GEM Normal and High-amylose Line Starch

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Project Overview

This research project is aiming to characterize starches produced by the GEM projects and to develop value-added utilization of these starch lines. Two types of starch, high-amylose maize starch developed from GEM amylomaize project of Dr. Mark Campbell at Truman State, and normal maize starch extracted from GEM lines supplied by the GEM Coordinator, Dr. Mike Blanco were the main genotypes used in the study. The resistant starch (RS) contents (39.4%-43.2%) of three new GEM lines of amylomaize starches (GUAT209:S13//OH43*ae*/H99*ae* 1-2-1, GUAT209:S13//OH43*ae*/H99*ae* 4-4-2-1, GUAT209:S13//OH43*ae*/H99*ae* 4-4-2-1) were substantially higher than that of four other inbred lines, H99*ae*, OH43*ae*, B89*ae*, and B84*ae* (11.5%-19.1%). The three new GEM line amylomaize starches also consisted of larger amylose contents and higher conclusive gelatinization temperatures than the other inbred lines as reported previously. To understand the starch structures of these three new GEM line amylomaize starches, which were responsible for the large resistant starch contents, we analyzed the structures and contents of the intermediate components and the starch lipid complex, molecular weight of the resistant starch, and morphology of the resistant starch remaining in the granules after enzyme hydrolysis. A poster of this study presented at the American Association of Cereal Chemists International in September, 2006 received an Outstanding Poster Award. Selectively crossed normal cornstarch lines aiming to improve enzyme digestibility of cornstarch have been produced during the growing season of 2006. The starch samples are in the process of being isolated and analyzed for their enzyme digestibility, chemical structures, and physical properties.

Objectives

Objectives of this research project are to identify starch lines of desirable characteristics and to develop value-added utilization of GEM starch. Specific objectives of the study are:

1. To identify high-amylose maize starch lines for resistant starch production.
2. To produce easily digestible normal cornstarch to be used for feed of small-animal.

Progress Made in 2006

Objective 1. To identify high-amylose maize starch lines for resistant starch production

The progress made in 2005 showed that the resistant starch (RS) content and the amylose content of the new GEM lines of high-amylose maize starches, GUAT209:S13//OH43ae/H99ae 1-2-1-2, GUAT209:S13//OH43ae/H99ae 4-4-2-1-1, and GUAT209:S13//OH43ae/H99ae 4-4-2-1-2, were substantially larger than other high-amylose maize mutant starches of H99ae, OH43ae, B89ae, and B84ae. The amylopectin and the intermediate component contents of the high-amylose maize starches, determined using gel permeation chromatography, are shown in Table 1. The amylopectin contents of the new GEM line high-amylose maize starches (10.7 – 13.9%) were smaller than that of the other high-amylose inbred line starches (25.4 – 33.5%). The intermediate-component contents of these three new GEM line starches (36.1 - 45.0%), however, were larger than that of most other inbred lines (22.4 – 27.0%) except OH43ae starch (52.0%). (The intermediate components include starch molecules that have branched structures but lower molecular weight than amylopectin).

Table 1. Amylopectin and the intermediate component (IC) contents of high-amylose maize starches

Sample	Pedigree	% amylopectin	% IC
1	GUAT209:S13//OH43ae/H99ae 1-2-1-2	11.6±0.4[a]	36.1[c]
2	GUAT209:S13//OH43ae/H99ae 4-4-2-1-1	10.7±0.3	40.5
3	GUAT209:S13//OH43ae/H99ae 4-4-2-1-2	13.9±1.1	45.0
4	H99ae	33.5±0.9	27.0
5	OH43ae	25.4±0.6	52.0
6	B89ae	33.0±0.6	24.6
7	B84ae	32.3±0.7	22.4

Weight-average molecular weights (M_w) and z-average gyration radii (R_z) of amylopectin molecules of the high-amylose maize starches were determined using a high-performance size-exclusion chromatography equipped with multi-angle laser-light scattering and RI detectors. Molecular weights of the intermediate components were determined on the basis of a pullulan standard calibration curve. The results (Table 2) showed that the M_w of the new GEM line starch amylopectins and the intermediate components were smaller than that of the other lines, except the amylopectin of OH43ae starch. It is not known why OH43ae has such a high intermediate component content, although this may be related to the lower amylopectin content and absolute amylose content.

Table 2. Molecular weights and gyration radii of amylopectin and the intermediate components

		Amylopectin		IC
Sample	Pedigree	Mw $\times 10^7$ (g/mol)[a]	Rz (nm)[b]	Mw $\times 10^4$ (g/mol)[c]
1	GUAT209:S13//OH43ae/H99ae 1-2-1	4.8(0.1)[d]	158(6)	1.2±0.0
2	GUAT209:S13//OH43ae/H99ae 4-4-2-1	4.2(0.2)	151(7)	1.3±0.1
3	GUAT209:S13//OH43ae/H99ae 4-4-2-1	5.2(0.2)	173(11)	1.3±0.1
4	H99ae	7.7(0.0)	196(3)	1.5±0.0
5	OH43ae	2.5(0.1)	105(8)	1.6±0.1
6	B89ae	9.0(0.3)	213(24)	1.9±0.1
7	B84ae	6.0(0.3)	162(7)	1.9±0.1

[a] Weight-average molecular weight. [b] Z-average radius of gyration. [c] The molecular weight at the peak. [d] Standard deviation.

Branch chain lengths of the intermediate components (in general) were different from that of the amylopectin molecules. Amylopectin molecules consisted of more short branch-chains of DP<12 (7.7 – 13.3%) and B3 and B4 chains (detectable longest chains DP80-99) than the intermediate components (1.8 – 9.2% and DP74-80, respectively). The amylopectin molecules for all of the lines had average branch chain-lengths of DP32.0 – 37.6. The intermediate components for all of the lines of different molecular weights also displayed different branch chain-length distributions. The small molecular-weight intermediate components consisted of longer average chain length (DP38.7 – DP50.6) than the large molecular-weight intermediate components (DP31.6 – DP38.5). The average branch chain-lengths of the amylopectin, the large and small intermediate components are summarized

in Table 3. The new GEM line high-amylose maize starches consisted of amylopectin with shorter branch chains but the intermediate components with longer branch chains than other inbred high-amylose maize starches.

Table 3. Average branch chain-lengths of amylopectin and the large and small molecular-weight intermediate components of the high-amylose maize starches

			Intermediate Components	
Sample	Pedigree	Amylopectin DP	Large MW DP	Small MW DP
1	GUAT209:S13//OH43ae/H99ae 1-2-1	32.0	36.9	50.6
2	GUAT209:S13//OH43ae/H99ae 4-4-2-1	35.6	36.5	47.3
3	GUAT209:S13//OH43ae/H99ae 4-4-2-1	32.5	38.5	48.5
4	H99ae	36.8	35.3	45.2
5	OH43ae	37.6	31.6	43.0
6	B89ae	36.8	35.3	43.4
7	B84ae	36.2	34.8	38.7

Thermal properties of gelatinization of the high-amylose maize starches are shown in Table 4 and that of the melting amylose-lipid complex are shown in Table 5. All the starch samples displayed similar onset gelatinization temperature (64.5-67.2°C). But the conclusion temperatures of the new line starches were above the water boiling temperature (106.0 to 107.7°C) and were substantially higher than that of the other starch samples (88.0 –99.8°C), which were in agreement with the results reported previously. The results indicated that starch granules of the new line starches were not completely gelatinized after cooking at the boiling temperature. The new GEM line starches and the OH43ae starch also displayed the largest enthalpy changes in melting amylose-lipid complex, which suggested that the intermediate components of the starch had greater facilities to form helical complex with lipids.

Table 4. Gelatinization properties of high-amylose maize starches

Pedigree	Native starch			
	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
GUAT209:S13//OH43ae/H99ae 1-2-1[a]	65.7±0.5[b]	87.3±12.1	106.0±2.3	5.0±1.1
GUAT209:S13//OH43ae/H99ae 4-4-2-1	66.3±1.2	90.4±2.3	107.7±0.8	5.8±1.0
GUAT209:S13//OH43ae/H99ae 4-4-2-1	66.7±0.9	86.5±9.4	106.4±0.9	4.8±0.7
H99ae	65.1±0.7	81.2±3.5	92.3±3.4	13.9±1.5
OH43ae	67.2±0.3	79.9±0.5	99.8±0.6	10.3±1.5
B89ae	64.5±1.0	81.2±1.2	98.3±0.6	12.5±0.5
B84ae	65.7±0.9	78.2±1.4	88.0±0.7	13.9±1.8

[a] Samples (~6.0 mg,dsb) and deionized water (~18.0 mg) were used for the analysis; T_0, T_p, T_c and ΔH are onset, peak, conclusion temperature, and enthalpy change, respectively.
[b] Values were calculated from three replicates; ±Standard deviation.

Table 5. Melting of amylose-lipid complex of the high-amylose maize starch

Pedigree	Amylose-lipid complex			
	T_0 (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
GUAT209:S13//OH43ae/H99ae 1-2-1[a]	76.9±6.4[b]	99.4±0.8	104.5±1.4	2.2±0.7
GUAT209:S13//OH43ae/H99ae 4-4-2-1	81.7±6.4	94.4±4.4	104.6±0.8	1.9±0.4
GUAT209:S13//OH43ae/H99ae 4-4-2-1	73.4±2.7	95.8±3.9	103.2±0.3	2.5±0.1
H99ae	77.5±2.8	97.9±0.6	104.9±0.9	1.2±1.2
OH43ae	78.3±2.4	95.9±3.5	107.4±1.1	2.5±0.1
B89ae	85.5±5.1	97.7±0.5	104.8±0.3	0.7±0.3
B84ae	84.3±6.0	97.8±5.3	105.4±4.2	0.4±0.5

[a, b] The same as that given in Table 4.

After subjecting to α-amylase hydrolysis at 100ºC for 30 min following the AOAC method, the remaining starch was viewed under a light microscope. The micrographs showed that RS located mainly at the periphery of the starch granule and retained partial birefringence (data not shown). M_w of the RS obtained from the three new GEM line starches were 2.6 – 3.3 X 10^4 (DP 160-204), which were larger than that of H99ae, B89ae, and B84ae (1.4 – 1.7 x 10^4, DP 86-105). Examples of the chromatograms of the RS obtained from the new GEM line starches and that obtained from the other inbred line high-amylose maize starch are shown in Figure 1. The RS produced from the new GEM line starches consisted of more large molecules than that produced from the other inbred line high-amylose starches. The structures of these large molecules are being investigated to reveal the mechanism of the large RS contents of the new GEM line starches.

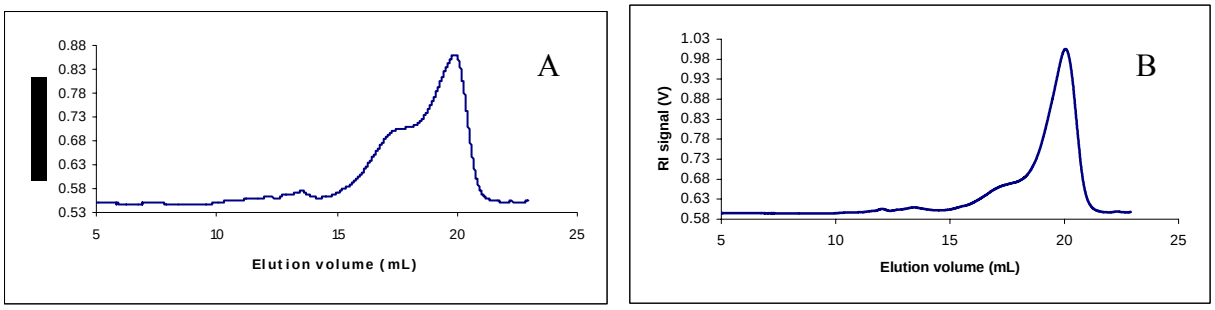

Figure 1. HPSEC chromatograms of resistant starch produce by enzyme hydrolysis of the native starch at boiling temperature. A. Resistant starch of GUAT209:S13//OH43ae/H99ae 1-2-1 starch, and B. Resistant starch from H99ae starch. The resistant starch was separated using Shodex OH pak KB-806 and KB-804 analytical columns and KB-G guard column (Showa Denko K.K., Tokyo, Japan).

Objective 2. To produce easily digestible normal cornstarch to be used for feed of small-animals

GEM lines of AR17056:N2025-574-001-B-B-B-B, AR17056:N2025-574-001-B-B-B-B-B, DKB844:S1601-289-001-B-B-B-B-B, DKB844:S1601-289-001-B-B-B-B, DKB844:S1601-289-001-B-B-B-B × AR17056:N2025-574-001-B-B-B, and (DKB844:1601-289-001-B-B-B-B × AR17056:N2025-574-001-B-B-B)-B were grown in Ames in 2006, and kernels have been provided by

Dr. Mike Blanco. Starch of these cross lines are in the process of being separated, and the enzyme digestibility of granular starch of each line is being analyzed.