

Project Title: Development of GEM line starch to improve nutritional value and biofuel production

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Project Overview

This report serves to document research conducted under a cooperative agreement between ARS and Iowa State University. Specific objectives of this research project are to (1) Develop and characterize hybrids using normal and waxy GEM lines to produce hybrids with improved yield and starch-ethanol conversion efficiency to increase ethanol yield; and (2) Develop healthy foods and healthy food ingredients using GEM lines.

For Objective 1, a normal (B73) and a waxy (08GEMS05044) inbred corn and two hybrids developed by intercrossing between the normal and waxy corn were used in this study. Amylose contents (3.9-26.5%) of the starch negatively correlated with the dosage of the *wx* gene in the corn endosperm. Average amylopectin branch-chain lengths (DP 18.4-19.9), however, showed no correlation with the *wx* gene dosage. The amylose content of the starch was inversely proportional to the gelatinization enthalpy-change (12.0-14.5 J/g), the gelatinization temperature-range (T_c-T_o) (10.2-11.7 °C), and the raw-starch digestibility ($r = -1$, $p < 0.001$), but positively correlated with the percentage retrogradation (38.2-64.9%) and starch pasting-temperature (69.6-75.7 °C) ($r = 1$, $p < 0.001$). These results indicated that amylose molecules enhanced the integrity of starch granules, restricted granule hydration, narrowed gelatinization temperature-ranges, and reduced digestive-rates of the starch. Amylose contents, instead of the amylopectin branch-chain length distribution, showed dominant effects on starch physicochemical properties. Starch of the hybrid, 08GEMS05044×B73, displayed significantly lower amylose content and higher digestibility than the normal corn starch. The hybrid showed potential to improve the yield of ethanol production.

To further investigate the dosage effects of *wx* gene on starch physicochemical properties, an intercross study using two pairs of isogenic lines grown in 2014 was conducted. Starch gelatinization enthalpy change was positively related with the dosage of *wx* gene in the endosperm. Final and setback viscosities of the starch decreased with increase of the *wx* gene dosage, whereas breakdown viscosities increased with increases of the *wx* gene dosage. Following work will be conducted to understand if the *wx* gene has dosage effects on starch molecular structures and digestibility.

For Objective 2, the hybrid popcorn (GEM-07048) produced by crossing a popcorn (Sg1533) with a high-amylose corn (GEMS-0067) was used in this study. The corn kernels of GEM-07048 were separated visually to two groups, normal popcorn with an amylose content of 36.3% and high-amylose popcorn with an amylose content of 61.4%. The high-amylose popcorn displayed the smallest expansion volume (11.8 cm/g) compared with the normal popcorn seeds (19.7 cm/g) and commercial popcorn (34.9 cm/g). The popped kernels of

high-amylose popcorn showed largest resistant starch content (46.3%), indicating its potential as a healthy snack food.

Publications and presentations:

- 1) H. Yangcheng, H. Jiang, M. Blanco and J. Jane. Characterization of starch from intercrossed normal and waxy maize. Poster presentation to Corn Utilization & Technology Conference, Louisville, KY, June 2-4, 2014.
- 2) H. Yangcheng, H. Jiang, M. Blanco and J. Jane. Physicochemical properties of starch produced by intercrossing between normal and waxy maize. Poster presentation to American Association of Cereal Chemists International, Providence, RI, Oct.5-8, 2014.

Objectives

Objective1: Develop and characterize hybrids using normal and waxy GEM lines to produce hybrids with improved yield and starch-ethanol conversion efficiency to increase ethanol yield.

Objective 2: Develop healthy foods and healthy food ingredients using GEM lines.

Characterization of starch from intercrossed normal and waxy maize

Two hybrids were developed by intercrossing between a normal (B73) and a waxy corn (08GEM05044). Dosages of the *wx* gene in endosperms of the inbred and hybrid corn are: 0 (B73), 1(B73×08GEMS05044), 2 (08GEMS05044×B73), and 3 (08GEMS05044) (**Table 1**). Starches of the corn lines were isolated using a wet-milling process, and the isolated starch was used for the study to understand the starch physicochemical properties and to reveal the effects of amylose contents and amylopectin structures on starch properties and digestibility.

Amylose content of the starch ranged from 3.9 to 26.5%, and was negatively related with the *wx* gene dosage in the endosperm (**Table 1**). Amylopectin molecular weight ranged from 1.4 to 1.7×10^9 (g/mol), and showed a trend of positive correlation with the *wx* gene dosage in the endosperm.

Table 1. Amylose content of starch and molecular weight and gyration radius of amylopectin^a

| Sample | Waxy gene dosage | Amylose (%) | Amylopectin | |
|----------------|------------------|------------------------|--|-------------------------|
| | | | $M_w \times 10^9$ (g/mol) ^b | R_z (nm) ^c |
| 08GEM05044 | 3 | 3.9 \mathbf{d} ±0.0 | 1.7±0.1 | 439.9±11.3 |
| 08GEM05044×B73 | 2 | 21.0 \mathbf{c} ±0.2 | 1.6±0.0 | 430.8±1.5 |
| B73×08GEM05044 | 1 | 24.3 \mathbf{b} ±0.0 | 1.4±0.0 | 412.7±1.8 |
| B73 | 0 | 26.5 \mathbf{a} ±0.3 | 1.5±0.0 | 420.8±3.0 |

^a Different letters following the mean value in the same column indicate significant differences between the mean values ($p < 0.05$). ^b Weight-average molecular weight (M_w). ^c Z-average radius of gyration.

Results of amylopectin branch-chain length distribution are shown in **Table 2**. Average branch-chain length of amylopectin ranged from DP 18.4 to 19.9, and showed no correlation with the *wx* gene dosage.

Starch thermal properties are shown in **Table 3**. Starch onset-gelatinization temperatures (62.3-65.4 °C) negatively correlated ($r = -1$, $p < 0.001$) with the percentage of short branch-chains of amylopectin (30.1-34.5%) (**Table 2**). Gelatinization enthalpy change (12.0-14.5 J/g) negatively correlated ($r = -1$, $p < 0.001$) with the amylose content (**Table 1**), whereas percentage retrogradation of gelatinized starch (38.2-64.9%) positively correlated ($r = 1$, $p < 0.001$) with the amylose content. The amylose molecules complexing with lipids and interacting with amylopectin to preserve integrity of the starch granule and restrict granule swelling contributed to the smaller gelatinization enthalpy change and higher percentage retrogradation of the starch.

Table 2. Molar-based branch-chain-length distribution of maize amylopectin ^a

| Sample | Average CL (DP) ^b | DP ≤ 12 (%) | DP 13-24 (%) | DP 25-36 (%) | DP ≥ 37 (%) |
|----------------|------------------------------|-------------------------|------------------------|------------------------|------------------------|
| 08GEM05044 | 19.4 \mathbf{a} ±0.6 | 30.1 \mathbf{b} ±0.1 | 49.2 \mathbf{a} ±1.6 | 11.0 \mathbf{a} ±0.1 | 9.6 \mathbf{a} ±1.5 |
| 08GEM05044×B73 | 19.9 \mathbf{a} ±0.7 | 30.8 \mathbf{ab} ±1.9 | 46.9 \mathbf{a} ±0.0 | 10.9 \mathbf{a} ±0.4 | 11.4 \mathbf{a} ±1.5 |
| B73×08GEM05044 | 18.4 \mathbf{a} ±0.2 | 34.5 \mathbf{a} ±0.4 | 46.8 \mathbf{a} ±0.3 | 9.9 \mathbf{a} ±0.2 | 8.8 \mathbf{a} ±0.6 |
| B73 | 19.1 \mathbf{a} ±0.3 | 31.8 \mathbf{ab} ±0.6 | 48.8 \mathbf{a} ±0.0 | 10.2 \mathbf{a} ±0.3 | 9.3 \mathbf{a} ±0.4 |

^a Different letters following the mean value in the same column indicate significant differences between the mean values ($p < 0.05$). ^b Average branch-chain length of amylopectin. DP = degree of polymerization.

Table 3. Thermal properties of maize starches ^a

| Sample | Native starch | | | | Retrogradation (%) ^c |
|----------------|------------------------|---------------------|---------------------|------------------------|---------------------------------|
| | T _o (°C) | T _p (°C) | T _c (°C) | ΔH (J/g) ^b | |
| 08GEM05044 | 65.4 \mathbf{a} ±0.6 | 71.1±0.5 | 77.1±0.5 | 14.5 \mathbf{a} ±0.0 | 38.2 \mathbf{d} ±0.7 |
| 08GEM05044×B73 | 64.1 \mathbf{b} ±0.1 | 69.3±0.3 | 74.7±0.4 | 12.7 \mathbf{b} ±0.1 | 46.2 \mathbf{c} ±0.2 |
| B73×08GEM05044 | 62.3 \mathbf{c} ±0.1 | 67.9±0.1 | 72.8±0.0 | 12.5 \mathbf{b} ±0.1 | 48.9 \mathbf{b} ±1.1 |
| B73 | 63.5 \mathbf{d} ±0.3 | 68.0±0.2 | 73.7±0.6 | 12.0 \mathbf{c} ±0.3 | 64.9 \mathbf{a} ±1.4 |

^a Samples (~3.0 mg, dsb) were mixed with deionized water (~9.0 μl) before the DSC analysis. Different letters following the mean value in the same column indicate significant differences between the mean values ($p < 0.05$). ^b T_o, T_p, T_c and ΔH are onset, peak, conclusion gelatinization temperatures and enthalpy change, respectively. ^c Percentage retrogradation (%) = (ΔH of retrograded starch / ΔH of native starch) × 100%.

Starch pasting profiles are shown in **Figure 1**, and the results are summarized in **Table 4**. Starch pasting temperature (69.6-75.7 °C) was positively related with the amylose content (**Table 1**), whereas the peak viscosity showed no correlation with the amylose content.

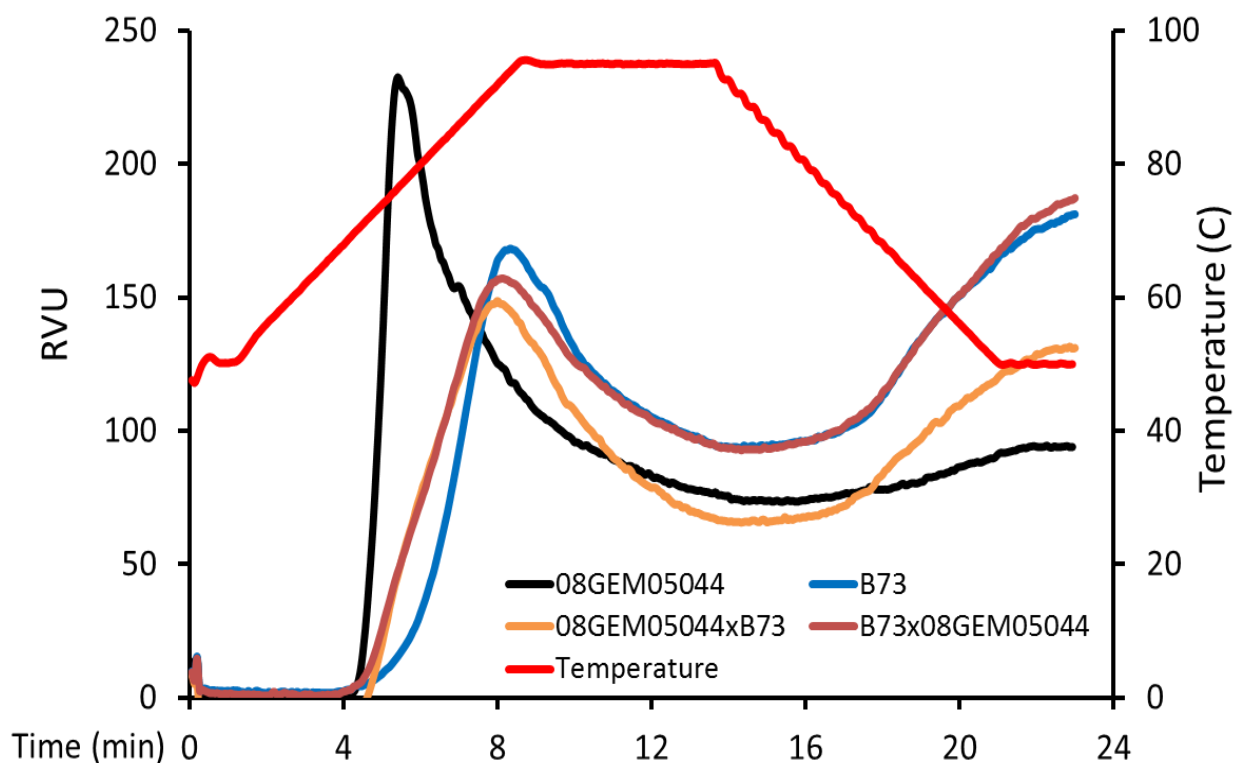


Figure 1. Pasting profiles of maize starches.

Table 4. Pasting properties of maize starch measured using a Rapid Visco-Analyzer ^{a, b}

| Sample | Pasting Temp.(°C) | Peak (RVU) | Hold (RVU) | Final (RVU) | Breakdown (RVU) | Setback (RVU) |
|----------------|------------------------|------------|------------|-------------|-----------------|---------------|
| 08GEM05044 | 69.6 ^c ±0.0 | 232.3±0.1 | 71.1±3.3 | 92.7±2.6 | 161.2±3.2 | 21.6±0.7 |
| 08GEM05044xB73 | 70.6 ^b ±0.3 | 156.8±0.8 | 71.1±3.5 | 141.3±1.6 | 85.6±2.7 | 70.2±1.9 |
| B73x08GEM05044 | 71.1 ^b ±0.7 | 161.3±0.1 | 100.3±8.3 | 188.6±0.6 | 61.0±8.4 | 88.3±8.9 |
| B73 | 75.7 ^a ±0.8 | 165.6±0.8 | 92.6±3.7 | 180.1±3.0 | 73.0±2.8 | 87.5±0.7 |

^a Different letters following the mean value in the same column indicate significant differences between the mean values (p<0.05). ^b Viscosity was reported in Rapid Visco-Units (RVU) where 1 RVU = 12 centipoise.

Starch digestibility of uncooked corn starch was determined using Englyst method (1992), and the results are shown in **Table 5**. The percentages of hydrolyzed starch at 120min

hydrolysis time ranged from 79.6 to 93.4%, and are inversely proportional to the amylose content (**Table 1**).

Table 5. Digestibility of maize starches ^a

| Sample | Digestibility (%) | |
|----------------|------------------------|-------------------------|
| | 30 min | 120 min |
| 08GEM05044 | 58.3 \mathbf{a} ±0.8 | 93.4 \mathbf{a} ±1.3 |
| 08GEM05044×B73 | 40.5 \mathbf{b} ±0.6 | 87.2 \mathbf{b} ±0.9 |
| B73×08GEM05044 | 34.5 \mathbf{c} ±0.8 | 82.9 \mathbf{bc} ±2.5 |
| B73 | 33.6 \mathbf{c} ±0.6 | 79.6 \mathbf{c} ±0.0 |

^a Digestibility of the uncooked maize starch was determined using Englyst's method (1992). Different letters following the mean value in the same column indicate significant differences between the mean values (p<0.05)

Starch of the hybrid (08GEM05044×B73) displayed significantly lower amylose content (21.0%) and higher digestibility (87.2% at 120min) than the normal corn starch (26.5% and 79.6% at 120min, respectively), indicating its potential to improve the yield of ethanol production.

To further investigate the dosage effects of *wx* gene on starch physicochemical properties, an intercross study using isogenic normal and waxy GEM lines was conducted. Two pairs of isogenic lines were grown in 2014, including waxy1 (*wx1*) and normal1 (N1) and waxy2 (*wx2*) and normal2 (N2). The pedigree information of these four lines and their F1 progeny obtained through reciprocal crossing is shown in **Table 6**.

Table 6. Pedigree information of GEM corn lines

| Sample | Pedigree | <i>wx</i> gene dosage |
|-----------------|--|-----------------------|
| <i>wx1</i> | DKXL370:N11a20-036-002-B <i>wx</i> | 3 |
| <i>wx1</i> x N1 | DKXL370:N11a20-036-002-B <i>wx</i> /DKXL370:N11a20-036-002-B-B-B-B | 2 |
| N1 x <i>wx1</i> | DKXL370:N11a20-036-002-B-B-B-B/DKXL370:N11a20-036-002-B <i>wx</i> | 1 |
| N1 | DKXL370:N11a20-036-002-B-B-B-B | 0 |
| <i>wx2</i> | AR16035:S02-615-001-B <i>wx</i> | 3 |
| <i>wx2</i> x N2 | AR16035:S02-615-001-B <i>wx</i> /AR16035:S02-615-001-B-B-B-B | 2 |
| N2 x <i>wx2</i> | AR16035:S02-615-001-B-B-B-B/AR16035:S02-615-001-B <i>wx</i> | 1 |
| N2 | AR16035:S02-615-001-B-B-B-B | 0 |

Starch thermal properties are shown in **Table 7**. Starch gelatinization enthalpy changes increased with increase of the *wx* gene dosage in the endosperm. Onset gelatinization

temperatures showed no correlation with the *wx* gene dosage. The conclusion temperatures, however, showed a trend of increase with increasing *wx* gene dosage.

Table 7. Starch thermal properties of GEM corn lines

| | T _o (°C) | T _p (°C) | T _c (°C) | ΔH (J/g) |
|----------|---------------------|---------------------|---------------------|----------|
| wx1 | 65.8±0.1 | 71.9±0.4 | 77.7±0.2 | 14.6±0.2 |
| wx1 x N1 | 65.6±0.2 | 70.8±0.1 | 75.3±0.2 | 14.1±0.0 |
| N1 x wx1 | 65.7±0.1 | 70.7±0.2 | 75.8±0.1 | 13.3±0.1 |
| N1 | 65.4±0.4 | 69.4±0.5 | 74.0±0.7 | 13.2±0.0 |
| wx2 | 61.7±0.2 | 68.9±0.0 | 74.7±0.1 | 14.5±0.0 |
| wx2 x N2 | 62.1±0.1 | 67.7±0.2 | 73.1±0.4 | 13.9±0.0 |
| N2 x wx2 | 62.7±0.1 | 67.3±0.1 | 72.2±0.2 | 13.1±0.0 |
| N2 | 61.8±0.1 | 66.8±0.1 | 71.5±0.2 | 12.8±0.1 |

Starch pasting properties are shown in **Table 8**. The final and setback viscosities decreased with increase of the *wx* gene dosage, whereas breakdown viscosities increased with increase of the *wx* gene dosage. Pasting temperatures and peak viscosities, however, showed no correlation with the *wx* gene dosage.

Table 8. Starch pasting properties of GEM corn lines

| | Pasting Temp.(°C) | Peak (RVU) | Hold (RVU) | Final (RVU) | Breakdown (RVU) | Setback (RVU) |
|----------|-------------------|------------|------------|-------------|-----------------|---------------|
| wx1 | 71.0±0.3 | 217.3±4.0 | 78.3±1.6 | 101.8±2.9 | 138.9±2.4 | 23.4±1.3 |
| wx1 x N1 | 73.8±0.3 | 147.8±2.2 | 73.6±2.4 | 142.1±2.2 | 74.2±0.2 | 68.5±0.2 |
| N1 x wx1 | 72.3±0.6 | 150.2±2.5 | 75.1±1.2 | 148.7±1.5 | 75.1±1.2 | 73.5±0.3 |
| N1 | 71.4±0.3 | 157.6±1.2 | 90.4±0.8 | 182.8±0.6 | 67.2±1.9 | 92.5±1.4 |
| wx2 | 69.0±0.3 | 221.0±0.7 | 72.0±1.7 | 102.1±0.6 | 148.9±1.1 | 30.1±1.1 |
| wx2 x N2 | 72.3±0.0 | 149.9±2.7 | 74.1±0.6 | 146.8±1.6 | 75.8±2.1 | 72.7±2.2 |
| N2 x wx2 | 72.5±0.2 | 143.6±1.4 | 84.9±1.7 | 169.8±2.1 | 58.7±0.3 | 84.9±3.8 |
| N2 | 71.3±0.3 | 148.3±0.7 | 95.6±0.4 | 202.2±2.0 | 52.5±0.1 | 106.6±2.4 |

Future work will be conducted to understand if the *wx* gene has dosage effects on the starch content, content of amylose, molecular size of amylose and amylopectin, branch-chain length of amylopectin, and starch digestibility. Relationships between the physical properties and the structures of the starches will be investigated.

Kernel popping properties and starch digestibility of high-amylose popcorn

GEM-07048 (Pedigree: (GEMS-0067/Sg1533-B)-B) was produced by intercrossing between a popcorn line (Sg1533) and a high-amylose corn (GEMS-0067). Kernels of GEM-07048 were visually separated to two groups, high-amylose popcorn (HAPC) and normal popcorn (NPC).

Kernel starch content, starch amylose content, and expansion volume of popped kernels are shown in **Table 9**. The HAPC of GEM-07048 showed a significantly greater amylose-content (61.4%) than the NPC of GEM-07048 (36.3%).

Table 9. Kernel-starch content, starch amylose content, and expansion volume of popcorn samples

| Sample ^a | Starch (%) | Amylose (%) | Expansion volume (cm/g) |
|---------------------|------------|-------------|-------------------------|
| NPC | 62.3 ± 0.3 | 36.3 ± 0.5 | 19.7 ± 2.2 |
| HAPC | 63.6 ± 0.2 | 61.4 ± 0.9 | 11.8 ± 1.8 |
| CPC | 60.8 ± 0.8 | 30.7 ± 0.4 | 34.9 ± 2.3 |

^aNPC, GEM-07048 normal popcorn; HAPC, GEM-07048 high-amylose popcorn; CPC, Commercial popcorn.

The expansion volume of GEM-07048 kernels was smaller than commercial popcorn (34.9 cm/g). The expansion volume of HAPC of GEM-07048 (11.8 cm/g) was even lower than that of the NPC kernels (19.7 cm/g). The amylose content was negatively correlated with the expansion volume ($r = 0.862$, $p < 0.05$).

Starch digestibility of popcorn kernels after microwave popping is shown in **Table 10**. The popped kernels of HAPC showed largest resistant starch content (46.3%) and slowly-digestible starch content (19.3%) than the other two popcorn samples. The amylose content (**Table 9**) was positively correlated with resistant starch content and negatively correlated with rapidly-digestible starch content.

Table 10. The rapidly-digestible starch (RDS), slowly-digestible starch (SDS) and resistant starch (RS) contents of popped kernel

| Sample ^a | RDS (%) | SDS (%) | RS (%) |
|---------------------|------------|------------|-------------|
| NPC | 77.5 ± 1.7 | 7.9 ± 2.1 | 14.6 ± 0.5 |
| HAPC | 34.4 ± 0.1 | 19.3 ± 1.1 | 46.3 ± 1.0 |
| CPC | 85.9 ± 1.4 | 2.6 ± 3.0 | 11.5 ± 1.6a |

^aNPC, GEM-07048 normal popcorn; HAPC, GEM-07048 high-amylose popcorn; CPC, Commercial popcorn.

These results indicated that high-amylose popcorn is a good source of resistant starch and slowly-digestible starch, which is potentially a healthy snack food. Further efforts, however, are needed to improve the popping characteristics of high-amylose popcorn.