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Characterization of Normal and Waxy Corn Starch for Bioethanol Production

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Supporting Information

ABSTRACT: Objectives of this study were to compare ethanol production between normal and waxy corn using a cold fermentation process and to understand effects of starch structures and properties on ethanol production. Ethanol yields positively correlated ($p < 0.01$) with starch contents of kernels of the normal and waxy corn. The average starch−ethanol conversion efficiency of waxy corn (93.0%) was substantially greater than that of normal corn (88.2%). Waxy corn starch consisted of very little amylose and mostly amylopectin that had a shorter average branch chain length than normal corn amylopectin. Regression analyses showed that average amylopectin branch chain lengths and percentage of long branch chains (DP > 37) of waxy corn starch negatively correlated with the starch hydrolysis rate and the ethanol yield. These results indicated that starch structures and properties of the normal and waxy corn had significant effects on the ethanol yield using a cold fermentation process.

KEYWORDS: *bioethanol, cold fermentation, starch−ethanol conversion efficiency, normal corn, waxy corn, starch*

INTRODUCTION

The United States is the largest petroleum-consuming country in the world, and large portions of the petroleum, peaking in 2005 at >60%, have been imported from foreign countries.[1] The nation's heavy dependence on foreign oil supply raised concerns of its energy security, which led to a huge surge of interest in alternative liquid fuels produced from renewable sources. Ethanol is an attractive biorenewable fuel because it can be produced from starchy or sugar-containing crops.[2] Representing 10% of the nation's motor fuel supply in 2011, fuel ethanol has reduced the nation's net import of foreign petroleum to 45% in 2011.[3]

Ethanol is produced almost exclusively from corn in the United States.[2,4] Production of ethanol from corn requires hydrolysis of starch to glucose, and glucose is then fermented by yeast to produce ethanol because yeast cannot utilize starch directly.[5] A conventional process for ethanol production is to gelatinize starch in dry-grind grain, and the gelatinized starch is hydrolyzed to dextrin using thermally stable α-amylases (liquefaction). The resulting dextrin dispersion is cooled to 60 °C and then saccharified with amyloglucosidase to produce glucose, which is the major substrate for ethanol fermentation. In this process, energy used to cook starch increases the production cost and decreases the energy return of ethanol.[6,7] In addition, amylose−lipid complex and retrograded starch formed in the gelatinized starch are resistant to enzyme hydrolysis and reduce the amount of fermentable sugar production and, thus, decrease the ethanol yield.[6,8]

To reduce energy costs and improve the net energy yield, raw-starch (cold) fermentation has been developed.[7,9] In a cold fermentation process, raw starch is hydrolyzed by granular starch hydrolyzing enzymes to produce fermentable sugars without prior cooking and liquefaction, and the sugars are fermented simultaneously by yeast to produce ethanol. Compared with the conventional process, cold fermentation effectively decreases the energy input, simplifies the process, reduces osmotic stress to yeast, and minimizes the occurrence of Maillard reaction, amylose−lipid complex, and retrograded-starch during and after the heating process.[6,9,10] Furthermore, cold fermentation does not require a large capital investment and is more feasible for small-scale or on-farm ethanol production.[7] Raw-starch hydrolysis of the cold fermentation process provides another advantage of displaying a low viscosity of the slurry. Thus, it allows a greater solids loading and a larger production capacity of the equipment for fermentation.[9] An industrial process of cold fermentation using raw-starch hydrolyzing enzymes was recently developed.[10] This innovative technique for ethanol production showed an improved ethanol yield and produced a coproduct, distiller's dry grains (DDG), with better qualities than the conventional process. The protein in the DDG produced using the cold fermentation is not denatured and, thus, retains good properties for other applications, such as biodegradable plastics.[11]

Aiming to improve the ethanol yield, numerous studies have been conducted to evaluate the ethanol fermentation performance using a variety of waxy and nonwaxy cultivars.[12−15] Ondas et al. reported that, using a conventional fermentation process with cooking, waxy wheat and corn showed greater starch−ethanol conversion efficiencies than nonwaxy counterparts.[12] Wu et al. reported a negative correlation between the amylose content of starch and starch−ethanol conversion efficiency

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using a conventional process.[13] Employing isolated maize starch with different amylose contents, Sharma et al. have shown that corn starch with 0% amylose produced ethanol at a higher yield than corn starch with 30 or 70% amylose using both conventional and cold fermentation processes.[14,15] These studies showed that waxy cultivars, containing little amylose in the starch, had a better ethanol fermentation performance than nonwaxy cultivars. Nevertheless, there have been few studies to date comparing ethanol production of dry-grind waxy corn with normal corn using a cold fermentation process and reporting effects of starch structures and properties, including amylopectin structures and starch thermal properties, on the ethanol yield.

This study aimed to compare ethanol yields and starch–ethanol conversion efficiencies of uncooked waxy and normal corn, using a cold fermentation process. Structures and properties of the waxy and normal corn starches were characterized to reveal effects of starch structures and properties on the ethanol yield. Results obtained from this study can be applied to improve ethanol production using cold fermentation by selecting corn varieties with starch of desirable structures and properties.

MATERIALS AND METHODS

Materials. Four normal corn lines (08GEM04701–4704) and eight waxy corn lines (08GEM05036–5040, 5042–5044) were developed by the USDA-ARS Germplasm Enhancement of Maize (GEM) Project. The inventory numbers are used throughout the paper, which indicate the seed sources used. The corn lines were selected to represent corn of racial diversities comprising germplasm from seven races and three tropical hybrids originated from seven countries. All of the corn lines were grown at the North Central Regional Plant Introduction Station farm (Ames, IA, USA) in both 2009 and 2010 crop seasons. Pedigree, racial background, and geographic origin of each line are included in the Supporting Information. Ethanol Red dry yeast (>20 × 10^9 living cells/g) was obtained from Lesaffre Yeast Corp. (Milwaukee, WI, USA). Lactrol (virginiamycin) was from Phibro Animal Health Co. (Ridgefield, NJ, USA). Isotab (hop acid) was from Beta Tec Hop Products (Washington, DC, USA). Novozyme 5009 raw-starch hydrolyzing enzymes containing a mixture of fungal α-amylase and amyloglucosidase were from Novozyme (Franklinton, NC, USA). *Pseudomonas* isoamylase (EC 3.2.1.68, 1000 U/mL) and total starch kit were from Megazyme International Ireland (Wicklow, Ireland). All other chemicals were of reagent grade and were obtained from either Sigma-Aldrich Co. (St. Louis, MO, USA) or Fisher Scientific (Pittsburgh, PA, USA) and used without further purification.

Dry Grinding of Corn Kernels. Maize kernels of GEM lines were dried to approximately 12% moisture and were then ground using a Cyclone Mill (UDY Corp., Fort Collins, CO, USA), screening with a steel sieve of 0.5 mm pore size.

Cold Fermentation. Dry-grind corn (35g, dry basis, db) was placed in a polypropylene bottle (125 mL, previously autoclave-sterilized). Distilled water containing liquid urea (0.03%, w/w), lactrol (2 ppm), isotab (40 ppm), and acetate buffer (10 mM, pH 4.2) was added to the dry-grind corn to make a mash of 100 g total weight (35% solid content). The mash was then mixed with 0.5 g dry yeast and raw-starch hydrolyzing enzymes (0.46%, v/w of dry-grind corn as recommended by the manufacturer (Novozyme)). The samples were incubated in a shaker incubator at 29 °C and 160 rpm for 96 h. Aliquots (8.0 mL) were taken from the fermentation broth at 96 h fermentation time and centrifuged at 7010*g* for 10 min. The supernatant was filtered through a nylon membrane filter with 0.2 μm pore size. The ethanol concentration was analyzed using a HPLC system consisting of a Prostar 210 pump (Varian, Walnut Creek, CA, USA), an injection valve (model 7725i) (Rheodyne, Oak Harbor, WA, USA), and a Prostar 355 refractive index detector (Varian), following the procedures previously reported.[16]

Ethanol conversion efficiency was calculated using the equation conversion efficiency (%) = 100% × ethanol yield (w/w)/theoretical yield of ethanol. The theoretical yield of ethanol is 56.73 g ethanol/100 g starch, which is calculated on the basis of 1 g of starch being hydrolyzed into 1.11 g of glucose and 1 mol of glucose fermented to produce 2 mol of ethanol.[4]

Starch Content Assay. The starch content of the corn grain was analyzed using a total starch kit (Megazyme International), following AACC Method 76.13.[17]

Starch Isolation by Wet-Milling. Starch was isolated from corn kernels using a wet-milling method.[18]

Starch Hydrolysis of Uncooked Isolated Starch and Dry-Grind Grain. Dry-grind corn containing 200 mg of starch (db) or isolated starch (200 mg, db) suspended in a sodium acetate buffer (20 mL, 10 mM, pH 4.2) was preincubated at 29 °C for 30 min. Novozyme 5009 raw-starch hydrolyzing enzymes (0.67%, v/w of starch) were then added, and the incubation was continued at 29 °C with constant shaking at 160 rpm for 96 h. Aliquots (0.1 mL) of the hydrolysate were withdrawn at different time intervals and mixed with 1 mL of 66% ethanol (v/v). The mixture was centrifuged at 6600*g* for 5 min, and the supernatant was collected. The glucose content in the supernatant was determined using a glucose oxidase/peroxidase (GOPOD) assay (Megazyme International).

Amylose Content of Starch. Amylose contents of waxy and normal corn starches were determined using Sepharose CL-2B gel permeation chromatography (GPC) followed by a total carbohydrate analysis and using an iodine potentiometric titration method.[18,19]

Branch Chain Length Distributions of Amylopectin. Amylopectin of normal corn starch was separated from amylose and collected using a GPC column packed with Sepharose CL-2B gel (Pharmacia Inc., Piscataway, NJ, USA).[18] The amylopectin was debranched using isoamylase (Megazyme International) following the methods previously reported.[20] The waxy corn starch was debranched without separation of the amylopectin from amylose by GPC because there was no detectable amylose in the GPC profile. The debranched sample was labeled with 8-amino-1,3,6-pyrenetrisulfonic acid (APTS) and analyzed using a fluorophore-assisted capillary electrophoresis (P/ACE MDQ) (Beckman Coulter, Fullerton, CA, USA) following the methods previously reported.[21,22]

Thermal Properties of Starch. The thermal properties of the isolated starch were analyzed using a Diamond Differential Scanning Calorimeter (Perkin-Elmer, Norwalk, CT, USA).[19] Starch gelatinization onset (T_o), peak (T_p), and conclusion temperatures (T_c) and enthalpy change (ΔH) were obtained using Pyris software (Perkin-Elmer). The gelatinized starch samples were stored at 4 °C for 7 days and then analyzed using the same parameters for measuring their percentages retrogradation. The percentage retrogradation was calculated using the equation retrogradation (%) = 100% × ΔH of dissociation of retrograded starch/ΔH of starch gelatinization.

Statistical Analysis. The experimental design of this study was a randomized complete block design with the corn variety (normal or waxy) as a fixed effect and the crop season (year 2009 or 2010) as a random block effect. The model statement for the analyses was $Y_{jk} = \mu + V_j + S_k + \varepsilon_{jk}$, where Y_{jk} = the dependent variable, μ = overall mean, V_j = corn variety (j = normal or waxy), S_k = crop season (k = year 2009 or 2010), and ε_{jk} = residual error. Data were analyzed using the PROC MIXED procedure of SAS 9.2 (SAS Institute, Inc., Cary, NC, USA). Average values or means of values presented in the text are least-squares means (LSM) if without further indication. Differences of LSM between normal and waxy corn lines were analyzed using Tukey's procedure. The normality of data for each variable was investigated using the PROC UNIVARIATE procedure. Because all of the variables except data of total starch content were not normally distributed (data not shown), a Spearman correlation test was used to analyze correlations between the ethanol yield, total starch content, and physicochemical properties of the starch. Statistical significance was declared at $p < 0.05$.

Table 1. Total Starch Content, Ethanol Titers, Ethanol Yields, and Starch–Ethanol Conversion Efficiencies of Normal and Waxy Corn[a]

	starch content (%)		ethanol concentration (mL/100 mL)		ethanol yield (g/100 g dry grain)		conversion efficiency[b] (%)	
line	2009 crop year	2010 crop year	2009 crop year	2010 crop year	2009 crop year	2010 crop year	2009 crop year	2010 crop year
				Waxy				
5036	71.1 ± 0.3	72.3 ± 0.2	18.7 ± 0.2	18.9 ± 0.1	37.6 ± 0.5	37.9 ± 0.2	93.1	92.5
5037	71.0 ± 0.6	72.8 ± 0.0	18.1 ± 0.1	18.4 ± 0.1	36.3 ± 0.1	37.0 ± 0.3	90.1	89.6
5038	67.5 ± 0.5	67.3 ± 0.7	18.1 ± 0.1	17.9 ± 0.0	36.3 ± 0.1	35.9 ± 0.1	94.6	94.0
5039	68.0 ± 0.6	69.2 ± 0.0	17.8 ± 0.4	17.9 ± 0.2	35.7 ± 0.8	36.0 ± 0.4	92.5	91.5
5040	66.6 ± 0.3	66.4 ± 0.2	17.6 ± 0.1	17.6 ± 0.3	35.4 ± 0.2	35.3 ± 0.6	93.7	93.8
5042	64.1 ± 0.0	65.3 ± 0.1	17.2 ± 0.2	17.4 ± 0.3	34.6 ± 0.3	34.8 ± 0.5	95.0	94.0
5043	68.9 ± 0.7	66.7 ± 0.2	18.0 ± 0.3	17.8 ± 0.2	36.2 ± 0.5	35.7 ± 0.4	92.5	94.3
5044	68.1 ± 1.0	66.8 ± 0.4	17.9 ± 0.1	17.8 ± 0.1	35.9 ± 0.1	35.6 ± 0.2	92.8	94.0
LSM ± SEM[c]	68.3b ± 0.6		17.9a ± 0.1		36.0a ± 0.3		93.0a ± 0.3	
				Normal				
4701	74.3 ± 0.7	74.1 ± 0.5	18.6 ± 0.2	18.7 ± 0.2	37.2 ± 0.5	37.5 ± 0.4	88.3	89.2
4702	71.5 ± 0.8	70.5 ± 0.4	17.5 ± 0.4	17.5 ± 0.2	35.2 ± 0.8	35.1 ± 0.4	86.8	87.6
4703	68.2 ± 0.5	68.1 ± 0.1	17.1 ± 0.2	17.1 ± 0.6	34.2 ± 0.4	34.3 ± 1.3	88.5	88.8
4704	74.1 ± 0.7	74.1 ± 0.6	18.5 ± 0.3	18.4 ± 0.5	37.2 ± 0.7	37.0 ± 1.1	88.4	87.9
LSM ± SEM	71.9a ± 0.9		17.9a ± 0.2		36.0a ± 0.4		88.2b ± 0.5	

[a]Values are the mean ± standard deviation of two replicates. [b]Conversion efficiency (%) = 100 × ethanol yield (w/w)/theoretical yield of ethanol. [c]Least-squares means (LSM) ± standard errors (SEM). Different letters following the LSM values within the same column indicate statistically different mean values ($p < 0.05$).

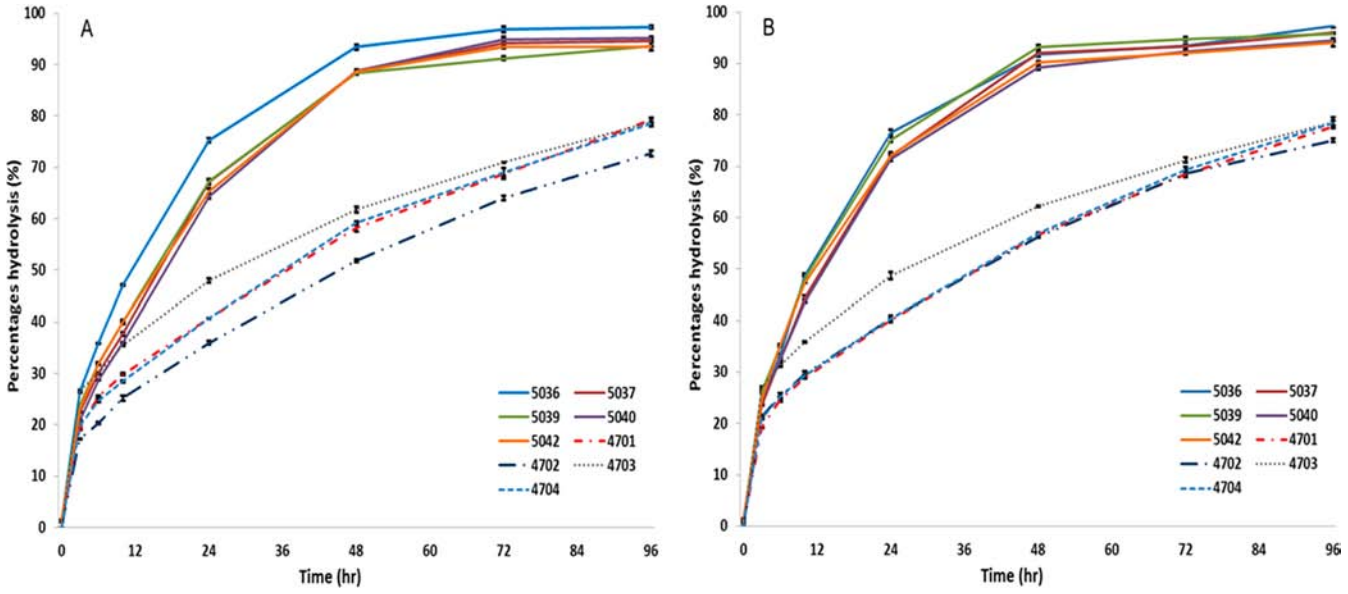

Figure 1. Enzymatic hydrolysis of the starch in the dry-grind grain of normal and waxy corn: (A) dry-grind grain of 2009 crops; (B) dry-grind grain of 2010 crops.

RESULTS AND DISCUSSION

Ethanol yields of the dry-grind normal and waxy corn using a cold fermentation process are shown in Table 1. Ethanol yields of the four normal corn lines ranged from 34.2% (34.2 g/100 g dry grain, line 4703) to 37.5% (line 4701), and those of the eight waxy corn lines ranged from 34.6% (line 5042) to 37.9% (line 5036). Notably, using the cold fermentation process, the fermentation output of both waxy and normal corn lines contained 17.9% ethanol on average (v/v) (Table 1), which was greater than the ethanol titer produced using a typical conventional process (about 13.8%, v/v).[23] The difference was attributed to the limited solids loading of the conventional process (31.1%, w/w, instead of 35%, w/w of the cold fermentation). Cooking dry-grind corn generates a high viscosity of the slurry, making it difficult to pump or stir.[9,10,23] On the contrary, taking advantage of the low viscosity of the slurry, the cold fermentation process allows a larger solids loading and higher production capacity of the equipment.[9]

To understand reasons causing differences in the ethanol yield of GEM corn lines, we analyzed starch contents of kernels of the corn lines (Table 1). Kernels of both the waxy and normal corn lines had wide ranges of starch contents (i.e.,

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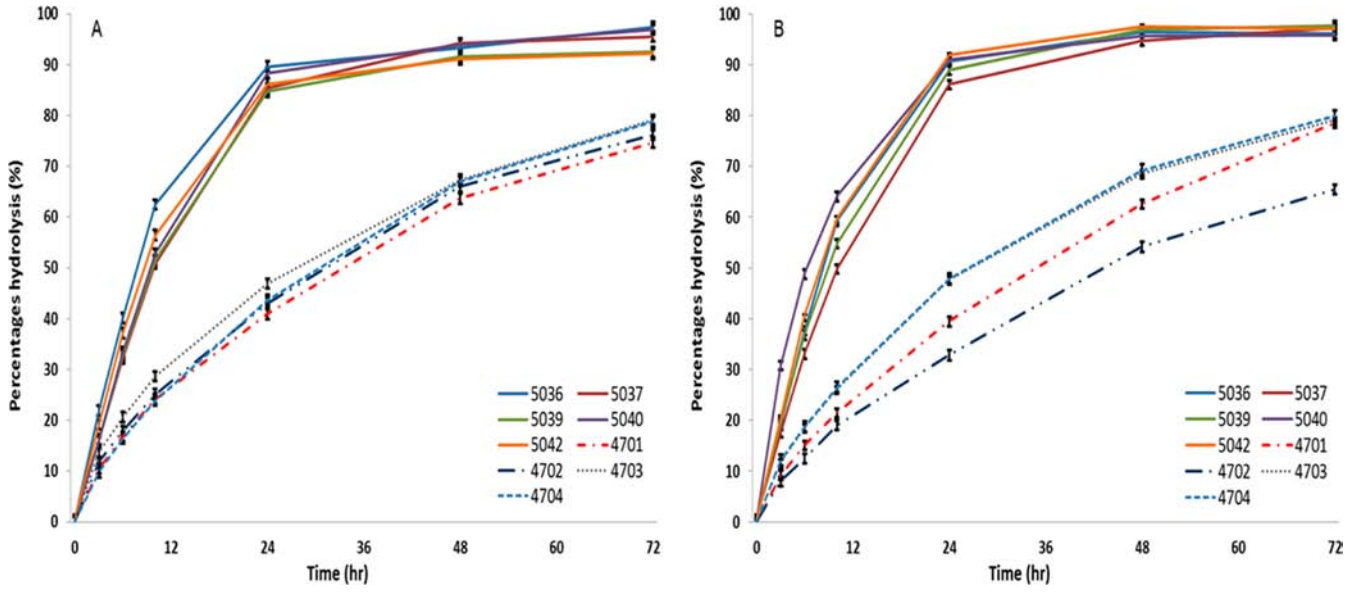

Figure 2. Enzymatic hydrolysis of the isolated starch of normal and waxy corn: (A) isolated starches of 2009 crops; (B) isolated starches of 2010 crops.

Table 2. Amylose Content of the Normal and Waxy Corn Starch[a]

	line	amylose (%) iodine titration[b]	amylose (%) GPC[c]
		2009 Crop Year	
waxy	5036	0.9 ± 0.0	ND[d]
	5037	1.3 ± 0.0	ND
	5039	1.5 ± 0.1	ND
	5040	1.4 ± 0.4	ND
	5042	2.2 ± 0.0	ND
normal	4701	27.9 ± 0.1	34.9 ± 1.4
	4702	28.3 ± 0.1	34.9 ± 0.1
	4703	28.2 ± 0.2	32.4 ± 1.3
	4704	28.0 ± 0.6	31.0 ± 0.3
		2010 Crop Year	
waxy	5036	1.6 ± 0.0	ND
	5037	2.1 ± 0.0	ND
	5039	2.5 ± 0.2	ND
	5040	2.1 ± 0.1	ND
	5042	4.6 ± 0.4	ND
normal	4701	28.3 ± 0.2	34.3 ± 0.8
	4702	29.0 ± 0.0	34.6 ± 0.0
	4703	30.4 ± 0.5	31.0 ± 0.4
	4704	28.5 ± 0.1	33.4 ± 1.3
waxy	LSM ± SEM[e]	2.0b ± 0.5	
normal		28.5a ± 0.6	

[a]Values are the mean ± standard deviation of two replicates. [b]Determined using iodine potentiometric titration. [c]Determined using gel permeation chromatography (GPC) followed by total carbohydrate analysis. [d]Not detectable. [e]Least-squares means (LSM) ± standard errors (SEM). Different letters following the LSM values within the same columns indicate statistically different mean values ($p < 0.05$).

64.1−72.8%, waxy corn; 68.1−74.3%, normal corn) (Table 1). The average starch content of the waxy corn lines (68.3%) was significantly lower ($p < 0.01$) than that of the normal corn lines (71.9%). The kernel-starch contents positively correlated with ethanol yields for both the waxy ($r = 0.96$, $p < 0.01$) and normal ($r = 0.88$, $p < 0.01$) corn lines.

The average starch−ethanol conversion efficiency of the waxy corn lines was 93.0%, whereas that of the normal corn lines was 88.2%. The conversion efficiency from starch to ethanol cannot reach 100% because of sugars consumed for yeast growth and proliferation and losses in byproduct formation, such as glycerol and succinate.[24,25] The waxy corn showed significantly greater conversion efficiency than the normal corn ($p < 0.01$), suggesting that there might be an incomplete hydrolysis of normal corn starch to glucose during the cold fermentation process.

To reveal what structural features of starch were responsible for the differences in the ethanol yield and starch−ethanol conversion efficiency, we isolated and characterized starches of selected lines. Five waxy corn lines (lines 5036, 5037, 5039, 5040, and 5042) were selected of the eight waxy corn lines to represent different ranges of ethanol yield using the cold fermentation (e.g., lines 5036 and 5042 gave the largest and smallest ethanol yields, respectively). Starches of all four normal corn lines and five waxy corn lines grown in both 2009 and 2010 crop seasons were isolated and characterized for their structures and properties, including starch hydrolysis rates, amylose contents, amylopectin branch chain length distributions, and starch thermal properties.

Enzymatic hydrolysis rates of starch in dry-grind corn samples using the raw-starch hydrolyzing enzymes (Novozyme 5009) are shown in Figure 1. The dry-grind waxy corn samples displayed substantially greater hydrolysis rates than the normal corn samples. After 96 h of hydrolysis, >90% starch in the dry-grind waxy corn was hydrolyzed to glucose, whereas <80% starch in the dry-grind normal corn was hydrolyzed to glucose. These results suggested that the normal corn starch was less readily hydrolyzed by the raw-starch hydrolyzing enzymes and, therefore, reduced the starch−ethanol conversion efficiency (Table 1).

Enzymatic hydrolysis rates of isolated starch granules using the raw-starch hydrolyzing enzymes are shown in Figure 2. Compared with the dry-grind corn, interestingly, the isolated

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Table 3. Amylopectin Branch Chain Length Distribution[a] of Normal and Waxy Corn Starch[b]

	line	DP < 12	DP 13−24	DP 25−36	DP > 37	av CL[c]
			2009 Crop Year			
waxy	5036	22.7 ± 0.4	47.7 ± 0.7	14.0 ± 0.7	15.5 ± 0.4	21.1 ± 0.3
	5037	23.4 ± 0.1	45.9 ± 0.4	13.9 ± 0.2	16.9 ± 0.7	21.5 ± 0.4
	5039	22.1 ± 0.2	48.0 ± 0.1	13.9 ± 0.5	16.1 ± 0.4	21.6 ± 0.0
	5040	23.6 ± 0.7	45.7 ± 0.5	13.5 ± 0.2	17.2 ± 1.0	21.7 ± 0.5
	5042	22.1 ± 0.0	45.5 ± 0.3	13.5 ± 0.3	18.9 ± 0.0	22.4 ± 0.0
normal	4701	21.8 ± 0.2	38.4 ± 1.3	17.1 ± 1.0	22.6 ± 0.4	23.1 ± 0.0
	4702	14.6 ± 0.3	42.6 ± 0.7	18.9 ± 0.6	24.0 ± 1.0	24.4 ± 0.3
	4703	20.5 ± 0.5	45.0 ± 1.0	14.9 ± 0.6	19.6 ± 0.8	22.4 ± 0.2
	4704	20.2 ± 0.3	42.0 ± 0.0	15.3 ± 0.3	22.4 ± 0.0	23.5 ± 0.0
			2010 Crop Year			
waxy	5036	21.4 ± 0.3	47.7 ± 0.6	15.2 ± 0.1	15.6 ± 0.0	21.4 ± 0.2
	5037	22.7 ± 0.2	47.0 ± 0.5	13.9 ± 0.1	16.4 ± 0.6	21.5 ± 0.1
	5039	20.9 ± 0.5	46.8 ± 0.4	14.8 ± 0.8	17.5 ± 0.1	22.2 ± 0.1
	5040	20.2 ± 0.2	45.2 ± 0.6	14.5 ± 0.2	20.1 ± 0.6	23.1 ± 0.2
	5042	20.6 ± 3.1	44.4 ± 3.2	14.8 ± 0.6	20.2 ± 0.8	23.1 ± 0.1
normal	4701	18.4 ± 0.5	40.5 ± 0.1	17.7 ± 0.4	23.4 ± 0.1	23.9 ± 0.1
	4702	20.5 ± 0.1	44.8 ± 0.1	14.8 ± 0.2	19.9 ± 0.1	22.7 ± 0.2
	4703	19.3 ± 0.2	43.3 ± 0.1	15.5 ± 0.5	21.8 ± 0.2	23.3 ± 0.0
	4704	21.0 ± 0.4	43.0 ± 0.9	15.4 ± 0.8	20.6 ± 0.5	22.9 ± 0.2
waxy	LSM ± SEM[d]	22.0a ± 0.5	46.4a ± 0.5	14.2b ± 0.3	17.4b ± 0.5	22.0b ± 0.2
normal		19.5b ± 0.6	42.5b ± 0.6	16.2a ± 0.4	21.8a ± 0.6	23.3a ± 0.2

[a]Values are the mean ± standard deviation of two replicates. Different letters within the same columns indicate statistically different mean values ($p < 0.05$). [b]Molar basis. [c]Average branch chain length of amylopectin. [d]Least squares means (LSM) ± standard error (SEM). Different letters following the LSM values within the same columns indicate statistically different mean values ($p < 0.05$).

starch displayed a smaller hydrolysis rate during the first 3−6 h. After 10 h, however, the starch hydrolysis rate of the isolated starch became greater than that of the dry-grind corn. The greater hydrolysis rate of starch in the dry-grind corn at the first 3−6 h could be attributed to the presence of damaged starch granules resulting from mechanical force used for the dry-grinding process and the presence of endogenous amylases in the dry-grind corn.[26] Starch granules in the dry-grind corn, however, are embedded in the protein matrix and endosperm cell wall material as previously reported.[27] The protein matrix and cell wall material reduce the accessibility of starch granules to the amylases and, therefore, decrease the hydrolysis rate of the starch in dry-grind corn during the later stage of hydrolysis.

Normal corn starch consisted of 27.9−30.4% amylose, determined using iodine potentiometric titration, and 31.0−34.9% amylose, determined using GPC with total carbohydrate analysis (Table 2). The difference in the amylose content obtained between the two methods could be attributed to the presence of low molecular weight amylopectin, which was eluted with amylose in the GPC profiles.[28] Amylose contents of waxy corn starch samples determined using the iodine potentiometric-titration ranged from 0.9 to 4.6% but were not detectable using the GPC analysis (Table 2). It is known that long branch chains of amylopectin can also form a single helical complex with iodine during the potentiometric titration and result in greater amylose contents of starch.[29] In this study, the amylose content of waxy corn starch positively correlated with percentages of long branch chains (DP > 37) (Table 3) of the amylopectin ($r = 0.73$, $p < 0.05$). These results indicated that the iodine potentiometric titration results of the waxy corn starch could correspond to the long branch chains of amylopectin instead of amylose.

Differences in amylose contents of waxy and normal corn starch contributed to the different starch hydrolysis rates during the cold fermentation process. Amylose molecules of the normal corn starch are known to intertwine with amylopectin and restrict the swelling of the granule,[30] and amylose molecules are more concentrated at the periphery of starch granules, forming a hard shell on the surface,[30,31] which reduces the enzymatic hydrolysis rate of normal corn starch. On the contrary, waxy corn starch has little amylose (Table 2) and a loosely packed peripheral structure revealed by confocal laser light scanning microscopy,[30] which renders the starch granule more susceptible to enzymatic hydrolysis.

Amylopectin branch chain length distributions of the selected waxy corn and normal corn starches are shown in Table 3. Amylopectin of the waxy corn starch displayed a significantly ($p < 0.01$) shorter average branch chain length (DP 22.0) than that of the normal corn starch (DP 23.3). The difference in branch chain lengths between the normal and waxy corn starch was the result of the amylopectin of the waxy corn starch consisting of significantly ($p < 0.01$) smaller percentages of long branch chains of DP > 37 (mean = 17.4%) and larger percentages of short branch chains of DP < 12 (mean = 22.0%) than that of the normal corn starch (DP > 37, mean = 21.8%; and DP < 12, mean = 19.5%). These results agreed with previous studies.[29] The differences could be partially attributed to the lack of extra-long branch chains of amylopectin in the waxy corn starch. The extra-long branch chains were synthesized by the granular bound starch synthase I (GBSS I) that was missing in the waxy corn.[32]

For normal corn lines, there was no clear correlation between amylopectin branch chain lengths and starch hydrolysis rates, indicating that amylopectin branch chain lengths of normal corn starch played a minor role in starch hydrolysis compared

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Table 4. Thermal Properties of the Normal and Waxy Corn Starch[a]

		native starch				retrogradated starch				
	line	T_o[b] (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)[a]	T_p (°C)	T_c (°C)	ΔH (J/g)	retro[c] (%)
					2009 Crop Year					
waxy	5036	62.3 ± 0.2	69.6 ± 0.1	76.1 ± 0.8	14.8 ± 0.1	43.3 ± 2.1	58.0 ± 0.2	67.5 ± 0.8	4.7 ± 0.1	32.0 ± 0.1
	5037	63.6 ± 0.2	69.8 ± 0.7	75.0 ± 0.7	15.6 ± 0.1	42.2 ± 0.3	56.0 ± 2.6	64.2 ± 2.1	5.2 ± 1.3	33.5 ± 1.3
	5039	64.1 ± 0.7	69.7 ± 0.8	74.9 ± 1.3	15.7 ± 0.1	41.2 ± 0.1	54.5 ± 0.1	62.7 ± 0.0	6.1 ± 1.2	39.0 ± 1.2
	5040	64.3 ± 0.4	70.2 ± 0.1	76.8 ± 0.3	15.5 ± 0.0	40.3 ± 1.7	54.8 ± 0.2	62.9 ± 0.0	6.5 ± 0.1	41.9 ± 0.1
	5042	65.0 ± 0.5	72.9 ± 0.4	79.1 ± 0.3	15.9 ± 02	43.0 ± 0.8	55.1 ± 0.0	64.3 ± 0.4	7.9 ± 0.4	49.8 ± 0.4
normal	4701	56.8 ± 0.9	66.0 ± 0.3	72.0 ± 0.4	11.2 ± 0.0	35.6 ± 0.9	48.4 ± 0.8	60.9 ± 0.3	6.0 ± 0.0	53.0 ± 0.0
	4702	62.4 ± 0.4	68.6 ± 0.5	73.9 ± 0.4	12.3 ± 0.1	37.7 ± 0.4	50.4 ± 0.7	62.2 ± 0.4	7.3 ± 0.0	59.3 ± 0.6
	4703	64.2 ± 0.1	69.6 ± 0.1	74.5 ± 0.2	11.7 ± 0.0	39.2 ± 0.0	50.6 ± 0.5	61.5 ± 0.3	6.6 ± 0.0	56.4 ± 0.2
	4704	59.2 ± 0.0	67.0 ± 0.0	73.6 ± 0.0	10.8 ± 0.0	39.8 ± 0.2	51.7 ± 0.2	61.6 ± 0.4	5.7 ± 0.0	53.4 ± 0.2
					2010 Crop Year					
waxy	5036	65.1 ± 0.2	71.4 ± 0.1	77.3 ± 0.3	15.7 ± 0.3	43.1 ± 0.4	54.0 ± 0.2	61.4 ± 0.1	6.5 ± 0.3	41.3 ± 1.4
	5037	65.4 ± 0.0	71.3 ± 0.0	77.5 ± 0.0	15.4 ± 0.1	42.0 ± 0.2	54.7 ± 0.4	64.6 ± 0.2	6.6 ± 0.1	43.0 ± 0.9
	5039	64.5 ± 0.4	71.4 ± 0.5	77.7 ± 0.5	15.2 ± 0.3	41.7 ± 0.7	54.3 ± 0.0	63.6 ± 0.2	6.6 ± 0.1	43.6 ± 0.1
	5040	57.7 ± 0.3	69.6 ± 0.9	79.9 ± 0.3	15.1 ± 0.0	41.4 ± 0.3	54.6 ± 0.0	63.4 ± 0.3	6.8 ± 0.1	44.8 ± 0.0
	5042	66.6 ± 0.3	74.1 ± 0.0	79.2 ± 0.1	15.9 ± 0.1	43.0 ± 0.2	54.9 ± 0.0	64.0 ± 0.0	7.2 ± 0.0	45.4 ± 0.0
normal	4701	61.7 ± 0.1	69.0 ± 0.1	75.1 ± 0.1	11.4 ± 0.0	40.6 ± 0.8	52.2 ± 1.2	62.7 ± 0.2	6.3 ± 0.1	55.0 ± 0.4
	4702	65.4 ± 0.2	70.8 ± 0.1	76.1 ± 0.3	12.3 ± 0.2	40.7 ± 0.1	52.3 ± 0.1	63.0 ± 0.0	6.9 ± 0.0	56.4 ± 0.5
	4703	67.1 ± 0.1	72.0 ± 0.1	76.9 ± 0.2	12.1 ± 0.2	41.7 ± 0.8	52.6 ± 0.2	63.1 ± 0.6	6.5 ± 0.1	53.4 ± 0.1
	4704	62.8 ± 0.1	69.6 ± 0.1	75.6 ± 0.1	12.2 ± 0.0	41.5 ± 0.6	52.6 ± 0.0	63.1 ± 0.2	6.4 ± 0.0	52.0 ± 0.2
waxy	LSM ± SEM[d]	63.9a ± 1.0	71.0a ± 0.9	77.4a ± 1.1	15.5a ± 0.1					41.4b ± 1.4
normal		62.5a ± 1.0	69.1b ± 1.0	74.7b ± 1.1	11.8b ± 0.2					54.9a ± 1.5

[a]Values are the mean ± standard deviation of two replicates. Different letters within the same columns indicate statistically different mean values ($p < 0.05$). [b]T_o= onset gelatinization temperature, T_p= peak temperature, T_c= conclusion temperature, ΔH = enthalpy change. [c]Retrogradation (%) = 100 × ΔH of dissociation of retrograded starch/ΔH of starch gelatinization. [d]Least-squares means (LSM) ± standard error (SEM). Different letters following the LSM values within the same columns indicate statistically different mean values ($p < 0.05$).

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with the amylose content. For waxy corn lines, however, because of the low amylose content of the starch (Table 2) and limited effects of the amylose, amylopectin structures had significant effects on starch hydrolysis and ethanol yield. Regression analyses showed that average branch chain lengths of amylopectin had significant negative correlations with the ethanol yield ($r = -0.91$, $p < 0.01$) and the starch hydrolysis rate ($r = -0.72$, $p < 0.05$). Percentages of the long branch chains of DP > 37 negatively correlated with the ethanol yield ($r = -0.85$, $p < 0.01$) and the starch hydrolysis rate ($r = -0.63$, $p < 0.05$) for the waxy corn samples. It is known that starch with amylopectin having more short branch chains (DP < 12) displays less crystallinity.[33] On the contrary, starch with amylopectin possessing more medium and long branch chains has a more stable crystalline structure and less void space in the granule, which reduces the rate of α-amylase penetration into the starch granule and decreases the rate of starch hydrolysis.[30,34]

The thermal properties of the selected waxy and normal corn starches are shown in Table 4. The average peak gelatinization temperature (T_p, 71.0 °C) and conclusion gelatinization temperature (T_c, 77.4 °C) of the waxy corn starch were significantly higher ($p < 0.05$) than that of the normal corn starch (average T_p = 69.1 °C and T_c = 74.7 °C, respectively). These results were in agreement with previous studies.[35] The average gelatinization enthalpy change of waxy corn starch (15.5 J/g) was significantly ($p < 0.01$) larger than that of the normal corn starch (11.8 J/g) (Table 4) because the waxy corn starch lacked amylose (Table 2).[29] Average percentage retrogradation of the waxy corn starch (41.4%) was significantly less than that of the normal corn starch (54.9%). The differences were attributed to the amylose molecules and long branch chains of amylopectin (DP > 37) (Table 3) in the normal corn starch granules, which restricted granule swelling and facilitated starch retrogradation.[30] Percentage retrogradation of the waxy corn starch positively correlated with the average branch chain length of amylopectin ($r = 0.85$, $p < 0.01$) and negatively correlated with the ethanol yield ($r = -0.76$, $p < 0.05$).

In conclusion, the waxy corn displayed significantly greater starch–ethanol conversion efficiencies than the normal corn using the cold fermentation process. The difference was attributed to the greater starch hydrolysis rate of the waxy corn than that of the normal corn. The results showed that waxy corn could produce ethanol at a greater yield using the cold fermentation process if the waxy corn had the same starch content as the normal corn. For the waxy corn samples, amylopectin branch chain lengths of the starch showed significant negative correlations with the starch hydrolysis rate and the ethanol yield. For the normal corn samples, however, amylose content rather than amylopectin branch chain lengths played the major role in the raw starch hydrolysis rate and starch–ethanol conversion efficiency. The amylose content of the normal corn starch and the amylopectin branch chain length of the waxy corn starch can be used to predict the ethanol production in the cold fermentation process.

■ ASSOCIATED CONTENT

Supporting Information

Additional tables. This material is available free of charge via the Internet at http://pubs.acs.org.

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