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Starch Characterization and Ethanol Production of Sorghum

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ABSTRACT: This study aimed to characterize and compare the chemical structures, physical properties, and enzymatic hydrolysis rates of five sorghum starches (6B73, 6C21, 6C69, 7R34, and X789) with that of corn starch (B73). Sorghum kernels consisted of 68.7−70.6% starch, more than the B73 corn (67.4%). Sorghum starches displayed higher gelatinization temperatures (66.6−67.4 °C), greater gelatinization enthalpy changes (13.0−14.0 J/g), and greater percentages of retrogradation (60.7−69.1%), but slower enzymatic hydrolysis rates (83.8−87.8% at 48 h) than the B73 corn starch (61.7 °C, 10.1 J/g, 51.5%, and 88.5%, respectively). These differences could result from the sorghum amylopectins consisting of fewer short branch chains (DP 6−12) (12.8−14.0%) than the corn amylopectin (15.0%). The sorghum starches showed greater peak and breakdown viscosities but lower setback viscosities than the B73 corn starch, resulting from the lower amylose content of the sorghum starches. After 96 h of fermentation, most ground sorghums exhibited lower ethanol yields (30.5−31.8%) than the ground B73 corn (31.8%).

KEYWORDS: sorghum starch, corn starch, starch structure, starch property, starch enzymatic hydrolysis, ethanol yield

INTRODUCTION

Sorghum (*Sorghum bicolor* (L.) Moench) is one of the most important cereal crops in the world. Sorghum can replace corn as the main crop in regions where the annual rainfall is <900 mm.[1] Because of its adaptability to a dry climate, sorghum is primarily grown in semiarid and dry areas around the world. Sorghum is consumed as a staple in parts of Asia and Africa and provides protein and energy needed for their population. In the United States, most of the sorghum is used for animal feed, and a small proportion is used for ethanol production.[2]

Similar to other cereal crops, sorghum kernels have large starch contents, ranging from 72.3 to 75.1% among different varieties.[3] Animal feeding studies have shown that sorghum has a lower starch digestibility than other cereal crops.[4−6] The low starch digestibility of sorghum has been attributed to the highly cross-linked prolamine protein matrices surrounding starch granules and the presence of tannin in sorghum kernels.[7−10]

The entrapment of starch granules in the protein matrices also results in lower starch yields from wet-milling of sorghum.[11] In addition, phenolic pigments of sorghum leach from the pericarp, testa, and aleurone tissues and cause the off-color of the isolated sorghum starch.[12] The poor yield and off-color of the sorghum starch are reasons for the lack of commercial wet-milling production of sorghum starch in the United States.[13]

In recent years, commercial production of ethanol in the United States has expanded rapidly. Following this trend, there is a growing interest in using sorghum as an alternative feedstock for ethanol production besides corn. Annual ethanol production from sorghum fermentation has grown steadily since 2004.[14] The ethanol yield has been reported to be positively correlated with the starch content of the sorghum and negatively correlated with the protein content.[1,15] Various methods, including decortication, sonication, protease hydrolysis, and steam-flaking, have been applied to increase the ethanol yield by reducing the interaction between starch and protein in sorghum kernels.[2,14,16,17]

In this study, the compositions of sorghum kernels from five lines (6B73, 6C21, 6C69, 7R34, and X789) and the structures, thermal properties, pasting properties, and enzymatic hydrolysis rates of the sorghum starches were analyzed and compared with that of B73 corn starch. Ethanol production using a cold fermentation process with uncooked sorghums and corn was also conducted and compared. The data obtained in this study can be used to predict value-added utilizations of sorghum in bioethanol, animal feed, and human food.

MATERIALS AND METHODS

Materials. Sorghum kernels of five lines, 6B73, 6C21, 6C69, 7R34, and X789, used in this study were harvested in Mt. Hope, KS, by Sorghum Division of Monsanto in 2004. The B73 corn kernels were provided by Dr. Michael Blanco, Germplasm Enhancement of Maize Project, ARS, USDA. The corn was grown at the North Central Regional Plant Introduction Station Farm (Ames, IA) in 2008.

Porcine pancreatic α-amylase (type VI-B, 21.6 units/mg solid), maltohexaose, and maltoheptaose were purchased from Sigma Chemical Co. (St. Louis, MO). Amyloglucosidase from *Aspergillus niger* (200 U/mL), isoamylase from *Pseudomonas* sp. (1000 U/mL), Total Starch Assay Kit, Starch Damage Assay Kit, and D-Glucose Assay Kit were purchased from Megazyme International Ireland Ltd. (Co. Wicklow, Ireland). Catechin monohydrate was purchased from Enzo Life Sciences International, Inc. (Plymouth Meeting, PA). Raw starch hydrolyzing enzyme (Distillase SSF, 380 GAU/g) was a gift from Genencor International Inc. (Palo Alto, CA). One GAU was defined as the amount of enzyme that would release 1 g of reducing sugars calculated as glucose per hour from soluble starch substrate under the assay condition. Yeast (Ethanol Red) was purchased from Lesaffre Yeast Co. (Milwaukee, WI).

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Table 1. Compositions of Sorghum and Corn Kernels (db) and Amylose Contents of Starches[a]

	kernel				
sample	starch (%)	protein (%)	lipid (%)	tannin (CE,[b] mg/g)	amylose content of starch (%)
6B73	69.2 ± 0.4 ab	10.9 ± 0.1 a	3.8 ± 0.0 a	4.6 ± 0.2 b	28.7 ± 0.5 c
6C21	68.7 ± 0.0 ab	10.4 ± 0.2 b	3.9 ± 0.0 a	3.1 ± 0.0 c	29.8 ± 0.5 bc
6C69	70.6 ± 0.8 a	9.7 ± 0.0 c	2.7 ± 0.2 c	2.9 ± 0.1 c	31.3 ± 0.4 ab
7R34	69.2 ± 0.3 ab	11.0 ± 0.0 a	3.7 ± 0.0 a	4.3 ± 0.5 b	31.0 ± 0.3 ab
X789	69.0 ± 0.2 ab	10.6 ± 0.0 ab	3.7 ± 0.1 a	6.6 ± 0.5 a	28.6 ± 0.4 c
B73 (corn)	67.4 ± 0.7 b	10.2 ± 0.1 b	3.1 ± 0.1 b	0.0 ± 0.0 d	32.2 ± 0.3 a

[a] Values with the same letter in a column are not significantly different at $p < 0.05$. [b] CE, catechin equivalent.

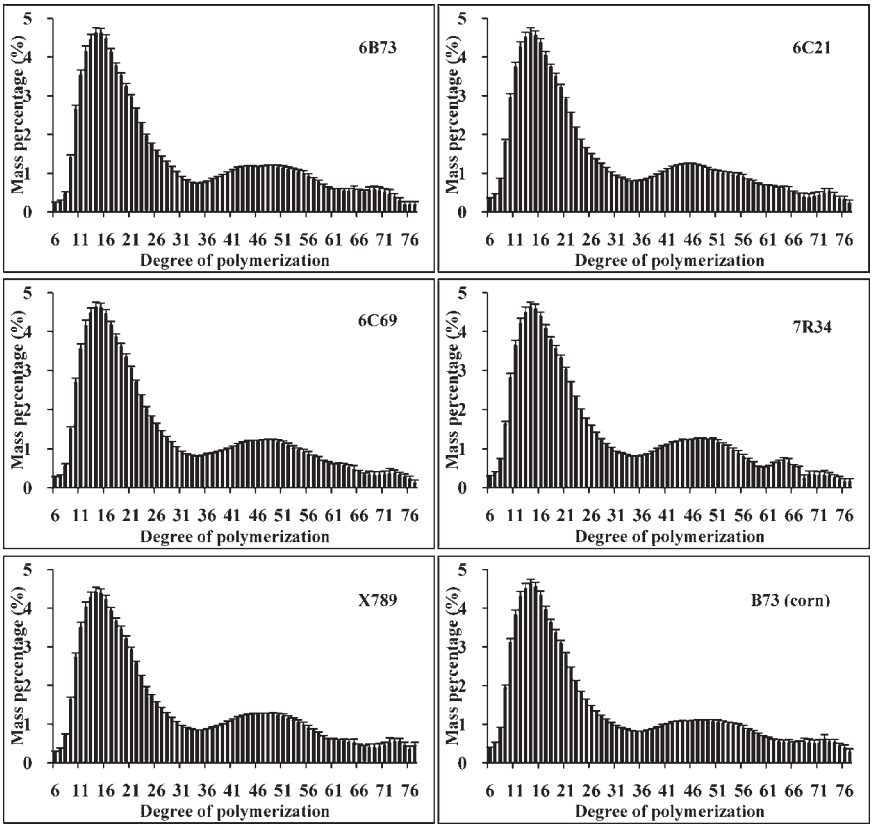

Figure 1. Branch chain length distributions of amylopectins of starches analyzed using fluorophore-assisted capillary electrophoresis.

Lactrol (virginiamycin) was purchased from Phibro Animal Health Co. (Ridgefield, NJ). IsoStab (hop acid) was purchased from Beta Tec Hop Products (Washington, DC).

Starch Isolation. Starch was isolated from sorghum and corn kernels using a wet-milling method.[18]

Dry Grinding of Kernel. The sorghum and corn kernels were dry-ground using a cyclone mill (UDY Corp., Fort Collins, CO) and passed through a 0.5 mm sieve to prepare ground sorghum and corn samples.

Kernel Composition. The ground kernels were used for the analysis of sorghum and corn kernel compositions. The starch content of the kernels was determined using a Total Starch Assay Kit following AACC method 76-13.[19] The nitrogen content of the kernels was determined using a Vario MAX CN Analyzer (Elementar Analysensysteme, Hanau, Germany). The protein content of the kernels was calculated by multiplying the nitrogen content by a conversion factor of 6.25. The lipid content of the kernels was determined using hexanes and Goldfisch Fat Extractors (Labconco Corp., Kansas City, MO) following AACC method 30-25.[19] The tannin content of the kernels was determined following the method of Price et al. with catechin monohydrate as the standard.[20] The starch, protein, lipid, and tannin contents were analyzed in duplicate.

Amylose Content of Starch. The amylose content of starch was determined using an iodine potentiometric titration method.[21] The starch was defatted using an aqueous solution of 85% (v/v) methanol in a Soxhlet extractor for 24 h. The iodine affinity of the defatted starch was determined using an automatic potentiometric titrator (702 SM Tirino, Metrohm, Herisau, Switzerland). The amylose content of the starch was calculated by dividing the iodine affinity by 20.0%.[22] The amylose content of the starch sample was analyzed in duplicate.

Branch Chain Length Distribution of Amylopectin. Amylopectin was fractionated from the isolated starch and purified by repeating the 1-butanol complex method.[23] The purified amylopectin was then

Table 2. Branch Chain Length Distributions of Amylopectins of Starches[a,b]

	branch chain length distribution				
sample	DP 6−12 (%)[c]	DP 13−24 (%)	DP 25−36 (%)	DP >36 (%)	av DP
6B73	12.8 ± 0.5 b	42.7 ± 1.0	13.1 ± 0.0	31.4 ± 1.5	20.8 ± 0.4
6C21	14.0 ± 0.1 ab	42.0 ± 0.5	13.1 ± 0.0	30.9 ± 0.5	20.3 ± 0.2
6C69	13.1 ± 0.3 b	43.3 ± 1.5	13.6 ± 0.4	30.0 ± 1.6	20.5 ± 0.2
7R34	13.7 ± 0.4 ab	42.8 ± 0.4	13.3 ± 0.0	30.1 ± 0.8	20.4 ± 0.2
X789	13.3 ± 0.5 b	41.3 ± 0.6	13.6 ± 0.2	31.9 ± 1.3	20.8 ± 0.3
B73 (corn)	15.0 ± 0.3 a	41.3 ± 0.4	13.1 ± 0.1	30.7 ± 0.9	20.2 ± 0.4

[a] Values with the same letter in a column are not significantly different at $p < 0.05$. [b] Analyzed using fluorophore-assisted capillary electrophoresis. [c] Mass basis.

Table 3. Damaged Starch Contents of Isolated Starches and Ground Kernels[a,b]

	damaged starch (%)	
sample	isolated starch	ground kernel
6B73	0.3 ± 0.0 a	6.2 ± 0.2 ab
6C21	0.9 ± 0.3 a	4.4 ± 0.1 bc
6C69	0.0 ± 0.0 a	2.0 ± 0.7 c
7R34	0.4 ± 0.0 a	5.1 ± 1.0 b
X789	0.9 ± 0.3 a	8.7 ± 0.8 a
B73 (corn)	0.7 ± 0.2 a	2.4 ± 0.6 c

[a] Values with the same letter in a column are not significantly different at $p < 0.05$. [b] Determined using a Starch Damage Assay Kit.

debranched using isoamylase.[24] The debranched chains were labeled with 8-amino-1,3,6-pyrenetrisulfonic acid, and the chain length distribution was analyzed using a fluorophore-assisted capillary electrophoresis (P/ACEMDQ, Beckman Courter, Fullerton, CA).[25] Maltohexaose and maltoheptaose were used as the reference standards for the analysis. The sample was analyzed in duplicate.

Starch Damage. Damaged starch contents of the isolated starch and ground kernels were determined using a Starch Damage Assay Kit following AACC method 76-31.[19]

Starch Granule Morphology. Granule morphology of the isolated starch was studied using a scanning electron microscope (SEM, JEOL JAM-5800LV, Tokyo, Japan) following the method of Jane et al.[26]

Starch Crystallinity. X-ray diffraction patterns of isolated starches were obtained using a diffractometer (D-500, Siemens, Madison, WI) with copper Kα radiation.[27] The percentage crystallinity was calculated as % crystallinity = $100 \times A_c/(A_c + A_a)$, where A_c and A_a are the crystalline and amorphous areas in the X-ray diffractogram, respectively.[28]

Thermal Property of Starch. Gelatinization and retrogradation properties of the isolated starch were analyzed using a differential scanning calorimeter (Diamond DSC, Perkin-Elmer, Norwalk, CT). The sample was prepared and analyzed following the method of Song and Jane.[21] The thermal transition parameters were determined using Pyris software (Perkin-Elmer). The percentage starch retrogradation was calculated as % retrogradation = $100 \times \Delta H$ of dissociation of retrograded starch/ΔH of starch gelatinization, where ΔH is the enthalpy change of the thermal transition. The analysis was done in duplicate.

Pasting Property of Starch. The pasting property of isolated starch was analyzed using a Rapid Visco-Analyzer (RVA, Newport Scientific, Sydney, Australia). A suspension (28.0 g) containing 8% starch (w/w, dry base, db) was equilibrated at 50 °C for 1 min, heated to 95 °C at a rate of 6 °C/min, held at 95 °C for 5 min, and then cooled to 50 °C at a rate of 6 °C/min. The rotating speed for the paddle was 160 rpm except that 960 rpm was used for the first 10 s. The analysis was done in duplicate.

Enzymatic Hydrolysis of Starch. Enzymatic hydrolysis of the isolated starch and that of starch in the ground kernels (≤0.5 mm) were carried out following the method of Setiawan et al.[29] with modification. Starch (1%, w/v) was hydrolyzed into soluble sugars using porcine pancreatic α-amylase (PPA, 1000 units/g starch, db) at 37 °C with shaking (100 rpm). The supernatant containing soluble sugars was separated by centrifugation and was collected and further digested into glucose using amyloglucosidase. The concentration of glucose released was quantified using a D-Glucose Assay Kit containing glucose oxidase and peroxidase. The percentage of starch hydrolysis was calculated as % starch hydrolysis = 100 × total mass of glucose released/initial dry mass of starch × (162/180). The analysis was done in duplicate.

Ethanol Production. A cold fermentation process was used to produce ethanol from ground kernels. A ground sample (35 g, db) was placed in a polypropylene bottle. An aqueous solution (5 mL), containing 30 mg of urea, 0.2 mg of Lactrol, and 4 mg of IsoStab, and an acetate buffer solution (5 mL, 200 mM, pH 4.2) were added to the sample. Deionized water was added to make a total mass of 100 g. The mash was stirred for 0.5 h at 27 °C before the addition of raw starch hydrolyzing enzyme (72.4 GAU) and yeast (0.50 g) and then incubated at 27 °C with shaking (160 rpm). Aliquots (8.0 mL) were removed from the fermentation broth at time intervals of 24, 48, 72, and 96 h and centrifuged at 7200*g* for 10 min to collect supernatant. After filtration through a membrane filter (0.2 μm), the ethanol concentration of the supernatant was analyzed using an HPLC system consisting of a Prostar 210 pump (Varian, Walnut Creek, CA), an injection valve (5 μL sample loop, model 7725i, Rheodyne), and a Prostar 355 refractive index detector (Varian). A Shodex SH-G guard colum (WAT034243) and an ion exclusion column (WAT010295, Waters, Milford, MA) were used to separate ethanol from other components. The columns were maintained at 75 °C using a Prostar 510 column oven (Varian), and the detector was set at 30 °C. The mobile phase was a sulfuric acid solution (0.5 mM) at 1.0 mL/min. Ethanol was quantified using a standard curve.

Ethanol yield was calculated as % ethanol yield = 100 × total mass of ethanol produced/initial dry mass of ground kernels. Ethanol conversion efficiency was calculated as % ethanol conversion efficiency = 100 × actual yield of ethanol/theoretical yield of ethanol, where the theoretical yield of ethanol is 56.73 g ethanol/100 g starch, on the basis of 1 g of starch being hydrolyzed into 1.11 g of glucose and 1 mol of glucose being fermented to produce 2 mol of ethanol. The analysis was done in duplicate.

Statistical Analysis. Statistical significance was evaluated using one-way ANOVA and multiple comparison using Tukey's adjustment with a 5% significance level. Correlations between the kernel compositions, structure, and properties of the sorghum starches were analyzed using the Pearson correlation test. The statistical analyses were conducted in SAS (version 9.1, SAS Institute, Inc., Cary, NC).

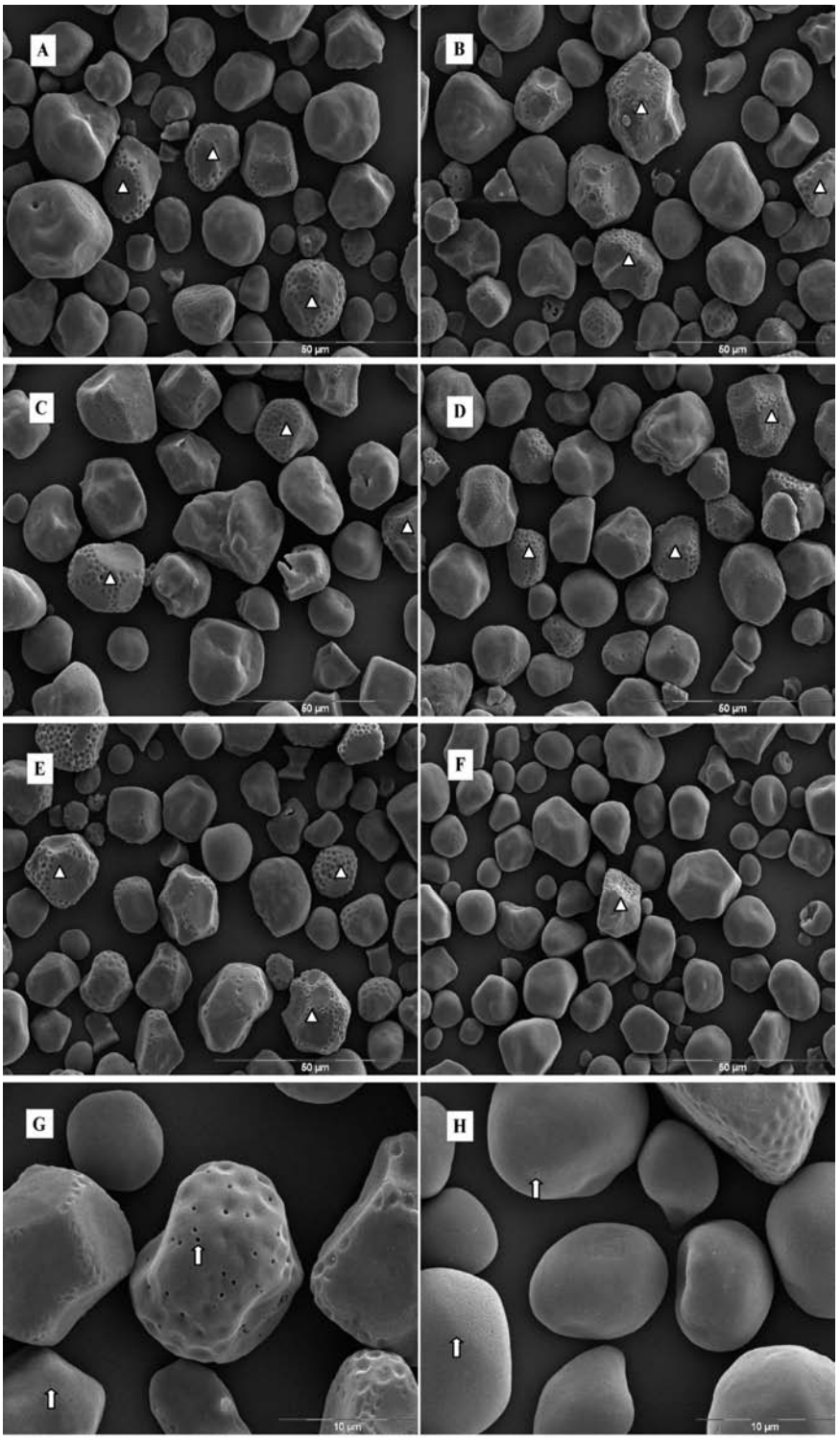

Figure 2. Scanning electronic micrographs of sorghum and corn starch granules (A) 6B73, (B) 6C21, (C) 6C69, (D) 7R34, (E) X789, and (F) B73 (corn) (all at 1500× magnification) and (G) 6B73 and (H) B73 (corn) (both at 5000× magnification). Triangles mark starch granules with indentations on the surface; arrows mark pinholes observed on the granule surface.

RESULTS AND DISCUSSION

Starch, protein, lipid, and tannin contents of sorghum and corn kernels and amylose contents of the starches are shown in Table 1. Kernels of all the sorghum lines consisted of more starch (68.7−70.6%) than that of the B73 corn (67.4%). Sorghum line 6C69 had the largest starch content (70.6%), whereas line 6C21 had the least (68.7%) (Table 1). Protein contents of the sorghum samples ranged from 9.7 to 11.0%, which were comparable with that of the B73 corn (10.2%) (Table 1). Lipid contents of most sorghum samples ranged from 3.7 to 3.9% with the exception of

6C69 (2.7%), compared with 3.1% for the B73 corn (Table 1). Tannin contents of sorghum samples ranged from 2.9 to 6.6 mg/g (catechin equivalent), whereas the B73 corn had no tannin (Table 1). The sorghum starches had less amylose (28.6−31.3%) than the B73 corn starch (32.2%) (Table 1).

Branch chain length distributions of amylopectin molecules fractionated from the sorghum and corn starches are shown in Figure 1, and the results are summarized in Table 2. Amylopectins of the sorghum starches had smaller proportions (12.8−14.0%) of short branch chains (DP 6−12), but larger proportions (41.3−43.3%) of branch chains of DP 13−24 than that of the B73 corn starch (15.0 and 41.3%, respectively) in general. Average branch chain lengths of the sorghum amylopectins varied between DP 20.3 and 20.8, which were longer than that of the B73 corn starch amylopectin (DP 20.2) (Table 2).

Damaged starch contents of the isolated starches and the ground samples are shown in Table 3. For all of the tested samples, ground kernels displayed substantially larger contents of damaged starch (2.0−8.7%) than the starches isolated by wet-milling (0−0.9%) (Table 3). This difference was attributed to the fact that dried kernels with a low moisture content (about 11%) were at a glassy state during dry-grinding and thus required a large force to break apart. Consequently, some weak starch granules yielded to the force and became damaged.[30] Among the ground samples, X789 and 6B73, with the least amylose contents of their starches (28.6 and 28.7%, respectively), displayed the greatest percentages of damaged starch (8.7 and 6.2%, respectively), whereas 6C69 sorghum and B73 corn, having the largest amylose contents of their starches (31.3 and 32.2%, respectively), showed the least damaged starch (2.0 and 2.4%, respectively) (Tables 1 and 3). Damaged starch contents of the ground sorghums negatively correlated with the amylose contents of starches ($r = -0.83$, $p = 0.08$), although the correlation was not significant. Among the wet-milled starches, X789 and 6C21samples displayed the largest damaged starch content (0.9%) (Table 3). Both starches consisted of low amylose contents (28.6 and 29.8%, respectively) (Table 1). Also, starch of line 6C21 consisted of amylopectin with the largest proportion of short branch chains (DP 6−12) and the shortest average

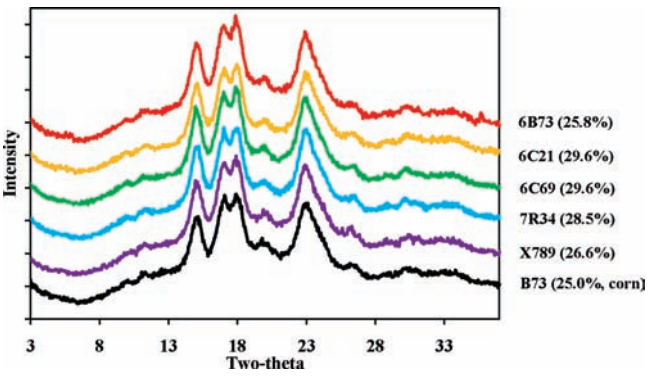

Figure 3. X-ray diffraction patterns of isolated starches. Percentage crystallinity is given in parentheses.

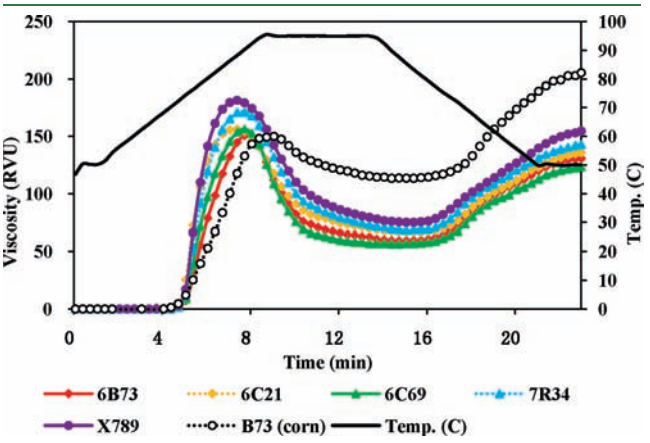

Figure 4. Pasting profiles of isolated starches measured using a Rapid Visco-Analyzer with 8% (dsb, w/w) starch suspension.

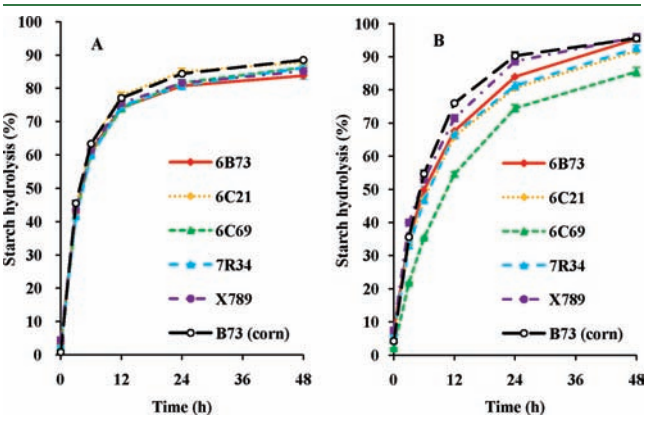

Figure 5. Enzymatic hydrolysis of isolated starches (A) and of starches in ground kernels (B). PPA was used for the hydrolysis of uncooked starch and ground kernels at 37 °C, pH 6.9, with 100 rpm shaking. % starch hydrolysis = 100 × total mass of glucose released/initial dry mass of starch × (162/180).

Table 4. Thermal Properties of Isolated Starches[a,b]

	gelatinization of starch				dissociation of retrograded starch				
sample	T_o (°C)[c]	T_p (°C)	T_c (°C)	ΔH (J/g)	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	retrogradation[d] (%)
6B73	66.7 ± 0.6 a	69.9 ± 0.3 a	74.4 ± 0.6	13.6 ± 0.1 a	39.3 ± 0.5	51.1 ± 1.2	62.5 ± 0.1	9.1 ± 0.5 a	66.9
6C21	67.1 ± 0.0 a	70.7 ± 0.1 a	75.5 ± 0.2	13.8 ± 0.4 a	39.6 ± 0.3	51.7 ± 0.0	62.6 ± 0.1	8.4 ± 0.2 a	60.7
6C69	67.4 ± 0.7 a	71.1 ± 0.7 a	75.3 ± 1.2	13.1 ± 0.6 a	41.0 ± 0.7	52.3 ± 0.1	63.4 ± 0.1	8.1 ± 0.9 a	61.8
7R34	66.6 ± 0.0 a	70.3 ± 0.1 a	74.4 ± 0.3	14.0 ± 0.1 a	40.1 ± 1.1	52.4 ± 0.0	64.2 ± 0.1	8.9 ± 0.5 a	63.7
X789	66.6 ± 0.1 a	70.5 ± 0.1 a	74.6 ± 0.2	13.0 ± 0.0 a	38.1 ± 0.7	51.0 ± 0.3	62.8 ± 0.7	9.0 ± 0.5 a	69.1
B73 (corn)	61.7 ± 0.0 b	68.9 ± 0.7 b	74.3 ± 0.1	10.1 ± 0.2 b	44.2 ± 0.9	53.4 ± 0.0	62.4 ± 0.1	5.1 ± 0.7 b	51.5

[a] Values with the same letter in a column are not significantly different at $p < 0.05$. [b] Measured using a differential scanning calorimeter. [c] T_o = onset temperature, T_p = peak temperature, T_c = conclusion temperature, and ΔH = enthalpy change. [d] % retrogradation = 100 × ΔH of dissociation of retrograded starch/ΔH of starch gelatinization.

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Table 5. Enzymatic Hydrolysis of Isolated Starches and of Starches in Ground Kernels[a,b]

	starch hydrolysis[c] (%)					
	isolated starch			ground kernel		
sample	0 h[d]	24 h	48 h	0 h[d]	24 h	48 h
6B73	3.3 ± 0.0 ab	80.8 ± 0.9 b	83.8 ± 0.2 c	7.2 ± 0.1 a	84.0 ± 0.3 b	95.4 ± 0.7 ab
6C21	2.2 ± 0.0 ab	85.0 ± 0.3 a	87.8 ± 1.0 ab	5.4 ± 0.1 b	80.6 ± 0.6 b	91.7 ± 0.2 c
6C69	2.6 ± 0.3 ab	81.7 ± 1.0 b	86.1 ± 1.4 abc	2.0 ± 0.2 d	74.5 ± 0.9 c	85.5 ± 1.3 d
7R34	3.1 ± 1.5 ab	80.9 ± 0.3 b	86.2 ± 0.6 abc	6.4 ± 0.2 a	81.3 ± 0.6 b	92.6 ± 0.2 bc
X789	4.3 ± 0.5 a	81.6 ± 0.4 b	85.2 ± 0.2 bc	7.4 ± 0.0 a	88.6 ± 1.2 a	95.9 ± 0.8 a
B73 (corn)	0.8 ± 0.5 b	84.4 ± 0.4 a	88.5 ± 0.1 a	4.2 ± 0.5 c	90.3 ± 1.1 a	95.9 ± 1.0 a

[a] Values with the same letter in a column are not significantly different at $p < 0.05$. [b] PPA was used for the hydrolysis of uncooked starch and ground kernels at 37 °C and pH 6.9 with 100 rpm shaking. [c] % starch hydrolysis = 100 × total mass of glucose released/initial dry mass of starch × (162/180). [d] Soluble sugar determined using a D-Glucose Assay Kit before the addition of PPA.

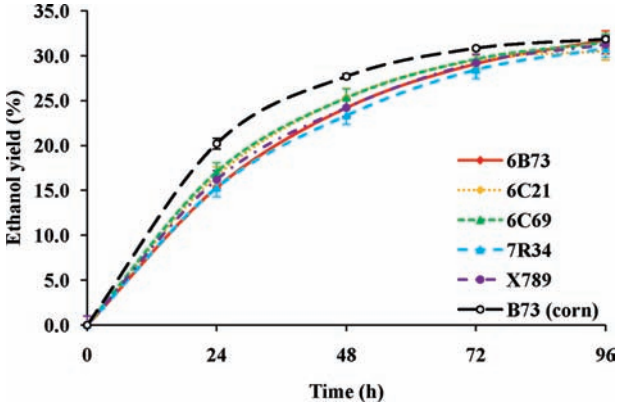

Figure 6. Ethanol yields of ground kernels using cold fermentation. % ethanol yield = 100 × total mass of ethanol produced/initial dry mass of ground kernels.

branch chain length among the sorghums (Table 2). These results suggested that amylose in the starch granules played a role of holding the integrity of starch granules.[31] Starch granules consisting of less amylose and amylopectin with more short branch chains were more fragile and easily damaged.[32,33]

Morphology and surface structures of isolated sorghum and corn starch granules studied using SEM are shown in Figure 2. Both sorghum and corn starch granules displayed polygonal and irregular shapes with diameters of 4−35 μm, but the B73 corn starch had more granules with diameter of <10 μm. The sorghum starch showed more indentations on the surface of starch granules than the B73 corn starch, resulting from the presence of protein bodies located between starch granules (Figure 2A−F).[34,35] Another distinct feature of the sorghum starch was that more granules displayed large pinholes than the B73 corn starch, indicating more severe endogenous amylase hydrolysis of the sorghum starch (Figure 2G,H). Similar characteristic pinholes of sorghum starch granules have been reported by Huber and BeMiller.[36]

X-ray diffraction patterns of the sorghum and corn starches are shown in Figure 3. All starch samples displayed the A-type diffraction pattern. Percentages of crystallinity of the sorghum starches ranged from 25.8 to 29.6%, which were greater than that of the B73 corn starch (25.0%).

Gelatinization and retrogradation properties of the sorghum and corn starches are shown in Table 4. The sorghum starches had significantly higher onset (66.6−67.4 °C) and peak (69.9−71.1 °C) gelatinization temperatures, greater gelatinization enthalpy changes (13.0−14.0 J/g), and greater percentages of retrogradation (60.7−69.1%) than the B73 corn starch (61.7 °C, 68.9 °C, 10.1 J/g, and 51.5%, respectively) (Table 4).

The differences in the percentages of crystallinity and the thermal property between sorghum and corn starches resulted from the different branch chain length distributions of their amylopectins (Table 2). The large proportion of the short branch chains (DP 6−12) of the amylopectin of B73 corn starch led to a defective crystalline structure, which resulted in a smaller percentage crystallinity, a lower gelatinization temperature, and a smaller gelatinization enthalpy change.[31,37] During retrogradation, the starch molecules reassociate to form double helices. Having fewer short branch chains of DP 6−12 and longer average branch chain lengths of the amylopectins, sorghum starch retrograded more quickly. The percentages retrogradation of the sorghum starches positively correlated with their average amylopectin branch chain lengths ($r = 0.92$, $p = 0.03$). The results agreed with previously reported data showing that long branch chains of amylopectin reassociated more promptly to form double helices, whereas short branch chains (DP6−12) retarded the retrogradation.[31,38,39]

Pasting properties of the sorghum and corn starches are shown in Figure 4. Although the pasting temperatures of the sorghum and corn starches were similar, the peak temperatures of the sorghum starches were lower than that of the B73 corn starch. The sorghum starches generally displayed greater peak and breakdown viscosities but smaller setback viscosities than the B73 corn starch. These differences could be attributed to the greater amylose content of the B73 corn starch (Table 1), which restricted the swelling of starch granules during heating but facilitated gel network formation when the starch paste cooled.[31,40,41]

Enzymatic hydrolysis of the isolated starches and that of starch in the ground kernels without cooking are shown in Figure 5, and the results are summarized in Table 5. There were soluble sugars present in both the isolated starch and ground kernels. All of the sorghum starches and most ground sorghum kernels (except 6C69) displayed greater soluble sugar contents than the B73 corn counterparts. The soluble sugars were likely produced by endogenous enzyme hydrolysis,[29] which was evidenced by pinholes on the surface of starch granules (Figure 2).

For the isolated starch samples, B73 corn starch and 6C21sorghum starch showed the greatest hydrolysis rates, which could be attributed to the larger proportions of short branch chains (DP

Table 6. Ethanol Yield and Conversion Efficiency of Ground Kernels Using Cold Fermentation[a]

	ethanol yield (%)[b]				
sample	24 h	48 h	72 h	96 h	ethanol conversion efficiency at 96 h[c] (%)
6B73	15.3 ± 0.1 b	24.2 ± 0.5 bc	29.1 ± 0.7 ab	31.8 ± 0.4 a	80.9 ± 1.0 ab
6C21	16.6 ± 0.1 b	25.3 ± 0.0 b	29.5 ± 0.1 ab	30.5 ± 0.1 b	78.3 ± 0.1 b
6C69	17.1 ± 0.0 b	25.3 ± 0.2 b	29.6 ± 0.0 ab	31.5 ± 0.0 ab	78.6 ± 0.0 b
7R34	15.3 ± 0.7 b	23.3 ± 0.5 c	28.4 ± 0.3 b	30.8 ± 0.2 ab	78.5 ± 0.5 b
X789	16.2 ± 0.9 b	24.2 ± 0.8 bc	29.2 ± 0.7 ab	31.2 ± 0.4 ab	79.8 ± 0.9 b
B73 (corn)	20.2 ± 0.6 a	27.7 ± 0.3 a	30.8 ± 0.2 a	31.8 ± 0.3 a	83.3 ± 0.7 a

[a] Values with the same letter in a column are not significantly different at $p < 0.05$. [b] % ethanol yield = 100 × total mass of ethanol produced/initial dry mass of ground kernels. [c] % ethanol conversion efficiency = 100 × actual yield of ethanol/theoretical yield of ethanol.

6−12) of their amylopectins (Table 2). Amylopectin with greater proportions of short branch chains results in more porous starch granules, which are more susceptible to enzymatic hydrolysis.[37,42,43] Percentages hydrolysis of the isolated sorghum starches by PPA for 48 h positively correlated with the proportions of short branch chains (DP 6−12) of amylopectins (r = 0.89, p = 0.04) and negatively with the average amylopectin branch-chain lengths (r = −0.91, p = 0.03).

Contrary to the view proposed in the literature that the highly cross-linked prolamine protein matrices in sorghum kernels limited the enzymatic hydrolysis of starch,[7,8] in this study the ground kernels displayed greater percentages of starch hydrolysis (91.7−95.9%) after 48 h of incubation with PPA than the isolated starches (83.8−88.5%) except 6C69. The differences could be attributed to the presence of more damaged starch (Table 3) and endogenous amylases in the ground kernels.[29] When starch granules became damaged, they were more easily hydrolyzed by enzymes.[44]

Among the ground sorghum samples, the X789 sorghum exhibited the greatest starch hydrolysis rate by PPA despite the fact that its isolated starch displayed a relatively slower hydrolysis rate, whereas the 6C69 sorghum, with the least damaged starch content, showed the slowest starch hydrolysis rate (Figure 5; Tables 3 and 5). Percentages of hydrolysis of starch in the ground sorghum kernels after 48 h of incubation positively correlated with the percentages of damaged starch (r = 0.93, p = 0.02), but did not show significant correlation with the tannin contents of the kernels (r = 0.79, p = 0.11).

Ethanol yields obtained from ground sorghum and corn samples are shown in Figure 6, and the results are summarized in Table 6. The production of ethanol from ground sorghums was slower than that of the B73 corn. The ground sorghums 6C21 and 6C69, with the least tannin in the kernels, displayed the greatest ethanol production rates of the sorghum samples (Figure 6; Tables 1 and 6). These results suggested that the slower ethanol production rates of ground sorghum samples could be partially attributed to the presence of tannin in their kernels (Table 1). In the fermentation broth with a high solid content (35%, w/w, db), tannin that leached into the solution could interfere with the starch enzymatic hydrolysis and yeast growth and, therefore, slowed the ethanol production rates of the ground sorghum samples. The delaying effects of tannin on enzymatic hydrolysis of starch and yeast growth have been reported before.[9,10,45] After 96 h of fermentation, the ethanol yields of sorghum samples (30.5−31.8%) were less than or equal to that of the B73 corn (31.8%), despite that sorghum kernels having greater starch contents than the B73 corn (Tables 1 and 6). The ethanol conversion efficiency of sorghums (78.3−80.9%) was also substantially lower than that of the B73 corn (83.3%) (Table 6).

Among the sorghum lines, 6B73 and 6C69, consisting of the greatest starch contents, showed the greatest ethanol yields at 96 h (31.8 and 31.5%, respectively), and 6C21, which had the least starch content, displayed the smallest ethanol yield (30.5%) (Table 1, 6). There was no significant difference in ethanol conversion efficiency between sorghum samples.

In conclusion, the isolated sorghum starches had higher gelatinization temperatures, greater gelatinization enthalpy changes, and greater percentages of retrogradation, but slower hydrolysis rates using PPA than the B73 corn starch. The differences were attributed to the fewer short branch chains (DP 6−12) of the sorghum amylopectins. Most of the ground sorghum and corn kernels displayed greater percentages of starch hydrolysis than their respective isolated starches after hydrolysis for 48 h. This could be attributed to the presence of more damaged starch and endogenous amylases in the ground kernels. After being subjected to cold fermentation for 96 h using raw starch hydrolyzing enzyme, most of the ground sorghum samples exhibited smaller ethanol yields and conversion efficiency than the ground B73 corn, despite the greater starch contents of the sorghum kernels. The data obtained in this study are useful for the utilization of sorghum in ethanol production as well as in feed and food processing, particularly for those regions where sorghum is grown as the major crop.

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