Food Science and Human Nutrition Publications   Food Science and Human Nutrition

12-2009

Characterization of Corn Grains for Dry-Grind Ethanol Production

Sathaporn Srichuwong
Iowa State University

Jelena Gutesa
Iowa State University

Michael Blanco
United States Department of Agriculture

Susan A. Duvick
United States Department of Agriculture

Jay-Lin Jane
Iowa State University, jjane@iastate.edu

Follow this and additional works at: https://lib.dr.iastate.edu/fshn_ag_pubs

Part of the Agronomy and Crop Sciences Commons, Food Microbiology Commons, Food Processing Commons, Human and Clinical Nutrition Commons, and the Molecular, Genetic, and Biochemical Nutrition Commons

The complete bibliographic information for this item can be found at https://lib.dr.iastate.edu/fshn_ag_pubs/177. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.

Characterization of Corn Grains for Dry-Grind Ethanol Production

Abstract

The objectives of this study were to understand how the composition of corn kernels and starch structure affected the enzyme hydrolysis of starch in dry-grind corn and the ethanol yield from yeast fermentation. Four selected corn inbred lines were used in this study. Starch in uncooked dry-grind corn samples showed greater enzyme digestibility than did the uncooked starch isolated from the same source by wet-milling process. The greater digestibility of starch in uncooked dry-grind corn correlated with a physical damage of starch granules. In contrast, starch in cooked dry-grind corn samples displayed less enzyme digestibility than did the cooked isolated starch. The difference could be attributed to interference caused by non-starch components in the dry-grind corn. The entrapment of starch in protein matrix and the formation of amylose-lipid helical complexes and/or retrograded starch may decrease the enzyme digestibility of starch in cooked dry-grind corn. Lab-scale ethanol production showed that ethanol yield after 72 h fermentation of the four corn inbred lines ranged between 34.3 and 38.0 g ethanol/100 g dry-grind corn. The conversion efficiency at 72 h of fermentation ranged between 86.8 % and 90.3 % of the theoretical ethanol yield. The highest ethanol yield was found in the corn line containing the largest starch content and the smallest amounts of lipid and protein.

Disciplines

Agronomy and Crop Sciences | Food Microbiology | Food Processing | Food Science | Human and Clinical Nutrition | Molecular, Genetic, and Biochemical Nutrition

Comments

This article is published as S. Srichuwong, J. Gutesa, M. Blanco, S. A. Duvick, C. Gardner, and J. Jane, "Characterization of corn grains for dry-grind ethanol production." *Journal of ASTM International,* (2010). Paper ID JAI102568.

Authors

Sathaporn Srichuwong, Jelena Gutesa, Michael Blanco, Susan A. Duvick, Jay-Lin Jane, and C. A. Gardner

Sathaporn Srichuwong,[1] *Jelena Gutesa,*[1] *Michael Blanco,*[2] *Susan A. Duvick,*[2] *Candice Gardner,*[2] *and Jay-Lin Jane*[3]

Characterization of Corn Grains for Dry-Grind Ethanol Production

ABSTRACT: The objectives of this study were to understand how the composition of corn kernels and starch structure affected the enzyme hydrolysis of starch in dry-grind corn and the ethanol yield from yeast fermentation. Four selected corn inbred lines were used in this study. Starch in uncooked dry-grind corn samples showed greater enzyme digestibility than did the uncooked starch isolated from the same source by wet-milling process. The greater digestibility of starch in uncooked dry-grind corn correlated with a physical damage of starch granules. In contrast, starch in cooked dry-grind corn samples displayed less enzyme digestibility than did the cooked isolated starch. The difference could be attributed to interference caused by non-starch components in the dry-grind corn. The entrapment of starch in protein matrix and the formation of amylose-lipid helical complexes and/or retrograded starch may decrease the enzyme digestibility of starch in cooked dry-grind corn. Lab-scale ethanol production showed that ethanol yield after 72 h fermentation of the four corn inbred lines ranged between 34.3 and 38.0 g ethanol/100 g dry-grind corn. The conversion efficiency at 72 h of fermentation ranged between 86.8 % and 90.3 % of the theoretical ethanol yield. The highest ethanol yield was found in the corn line containing the largest starch content and the smallest amounts of lipid and protein.

KEYWORDS: corn, dry-grind ethanol production, starch, enzyme hydrolysis

Introduction

Corn is the primary crop used for fuel ethanol production in the United States. Endosperm and germ are the major components of corn kernel, which comprise ~82% and 11 % of the kernel dry weight, respectively. Starch makes up 70–73 % of the kernel dry weight. Starch granules are packed within a protein matrix. Protein in corn consists of prolamins (60 %), glutenins (34 %), albumins (3 %), and globulins (3 %) [1]. The protein matrix is composed of amorphous protein material and protein bodies, mainly prolamins (zein). Corn oil is present exclusively in the germ. Triglycerides and free fatty acids are the predominant storage lipids in corn kernels [2]. The pericarp and tip cap contain 80 % of the kernel total fiber [3].

In general, modern dry-grind corn ethanol plants can convert one bushel of corn grain to 2.7–2.8 gal of ethanol (equivalent to 31.7–32.9 g ethanol/100 g corn grain) and 17 lb (7.7 kg) of distiller's dried grains with solubles [4]. Dry-grinding of corn kernels breaks the hard pericarp and disrupts the protein matrix, releasing free starch granules with some physical damages. Starch in dry-ground corn is cooked and then hydrolyzed to glucose and fermentable sugars by α-amylase and amyloglucosidase (AMG). Following subsequent yeast fermentation, sugars are converted to ethanol and carbon dioxide.

It is known that enzymatic digestibility of raw starch granules varies among corn varieties [5,6], which could be attributed to the differences in fine structure of starch, including amylose content and branch-chain-length of amylopectin [7,8]. Physical damage of starch granules exposes amorphous materials, resulting in greater digestibility of starch [9,10]. In the conventional dry-grind ethanol production process, the efficiency of enzymatic conversion of starch to glucose is affected by the degree of starch gelatinization and formation of enzyme resistant molecules. A greater degree of starch gelatinization increases starch accessibility for enzyme hydrolysis. It has been shown that starch gelatinization properties are strongly correlated with starch molecular structure [11]. In dry-grind corn, the protein matrix obstructs water

TABLE 1—*Background information of corn inbred lines.*

Sample Code	Inventory	Pedigree	GEM Code	Country	Race
A	05GEM06031	DKB844:S1601-073-001-B-B-B-B-B	GEMS-0115	Mexico	Tropical hybrid
B	05GEM02989	BR51721:N2012-098-002-B	GEMN-0156	Brazil	Dente Amarelo
C	05GEM06000	GEMS-0002	GEMS-0002	United States	Mixed
D	06GEM01778	BR52051(SE32):S17-B-023–001-B-B-Sib-B-B-B	GEMS-0003	Brazil	Dente Amarelo

penetration, resulting in increased gelatinization temperature of starch. The presence of protein has been reported to decrease the starch digestibility of cooked sorghum flours [12–14]. The formation of amylose-lipid helical complexes also decreases enzyme hydrolysis of starch [15–17].

The objectives of this study were to understand the enzyme digestibility of dry-grind corn and the isolated starch and to evaluate the performance of new corn lines from the Germplasm Enhancement of Maize (GEM) Project for ethanol production. The influence of the molecular structures of starch, grain composition, and physical damage of starch granules is discussed in relation to enzyme digestibility and ethanol yield. The results obtained are useful for improving ethanol production from dry-grind corn.

Materials and Methods

Materials

Corn inbred lines 05GEM06031, 06GEM01778, 05GEM02989, and 05GEM06000 designated as A, B, C, and D, respectively, were provided by United States Department of Agriculture-Agriculture Research Service GEM Project located at the North Central Regional Plant Introduction Station in Ames, IA. Background information of each inbred line was shown in Table 1.

Corn grains for ethanol fermentation experiments were ground to pass through a 2 mm screen in a MicroHammer mill IV (GlenMills, Inc., NJ). Samples for other analyses were ground to pass through a 0.5 mm screen using a Cyclone Mill (UDY Corp., CO). Crystalline *Pseudomonas* isoamylase (EC 3.2.1.68), with specific activity 66 000 units/mg protein, was purchased from Hayashibara Shoji, Inc. (Okayama, Japan). α-amylase from porcine pancreas (Type I-A in 2.9 M NaCl, 1122 units/mg protein), α-amylase from *Aspergillus oryzae* (Type X-A, 200 units/mg protein), thermostable α-amylase from *Bacillus licheni-formis* (829 units/mg protein and 29 mg protein/mL) and AMG from *Aspergillus niger* (300 units/mL) were purchased from Sigma-Aldrich (St. Louis, MO). AMG from *Aspergillus niger* (200 U/mL) included in a total starch kit (Megazyme International, Wicklow, Ireland; catalog no. K-TSTA) was also used. All enzymes were used without further purification. Ethanol Red™ dry yeast ($>20\times10^9$ living cells/g) was obtained from Lesaffre yeast corporation (Milwaukee, WI). Yeast inoculum was prepared by dispersing 0.4 g of dry yeast in 6 mL of deionized water. The culture was mixed and incubated at 30°C for 15 min. The yeast culture had a living cells $\sim 1.3\times10^9$ per mL. All chemicals were reagent grade and used without further treatments.

Protein and Lipid Contents of Dry-Grind Corn

Nitrogen content of dry-grind corn was determined using a macro-Kjeldahl method. Protein content was calculated by multiplying the nitrogen content by 6.25. Lipid was extracted from dry-grind corn with hexanes using Goldfish extractor for 6–7 h. Solvent was removed by evaporation in a hood for 16 h. Lipid content was determined gravimetrically and reported as gram lipid per 100 g dry matter.

Starch Content and Starch Damage Assays

Starch content of dry-grind corn was determined using the total starch kit (Megazyme International, Wicklow, Ireland; catalog no. K-TSTA) and reported as gram starch per 100 g dry matter. Starch damage of the dry-grind corn was determined enzymatically using a starch damage kit (AACC method 76-31, Megazyme International, Wicklow, Ireland; catalog no. K-TSTA) with modification, in which the fungal

α-amylase in the kit was replaced by α-amylase from *Aspergillus oryzae* (200 U/mL; Sigma-Aldrich, MO).

Isolation of Starch by Wet-Milling

Corn kernels were soaked in a sodium metabisulfite solution (0.45 % *w*/*v*) containing 0.1 M NaCl for 16 h at 4°C. The germ was separated from endosperm. The endosperm was milled with the sodium metabisulfite solution in a micro-blender for 2 min. The ground sample was filtered through a nylon screen with a pore size of 53 μm. Crude starch was then isolated by centrifugation and washed several times with distilled water. Starch was collected by centrifugation, resuspended in 0.1 M aqueous NaCl solution containing 10 % toluene, and stirred for 1 h using a magnetic stirrer at a high speed to remove protein. This step was repeated until the toluene layer became clear and contained no protein. The starch sediment was washed three times with water and twice with ethanol and dried at 30°C for 48 h.

Amylose Content and Branch-Chain-Length Distribution of Amylopectin

Amylose content of starch was determined using gel permeation chromatography (GPC) following the method of Song and Jane [18]. The amylose content was calculated by dividing the total carbohydrate content of the amylose peak by that of the total starch eluted. The fractions containing amylopectin were pooled, evaporated, and precipitated with the absolute ethanol. The precipitated amylopectin pellet was vacuum-dried and kept in a desiccator at room temperature.

The isolated amylopectin was analyzed for the chain-length distribution using fluorophore-assisted carbohydrate electrophoresis (FACE) [19,20] with modification. Amylopectin was dispersed in 90 % dimethyl sulfoxide solution, precipitated with ethanol, and centrifuged at 6750*g* for 15 min. Amylopectin was dispersed in water to give 2 mg amylopectin/mL water. The mixture was heated to a boiling temperature for 30 min and subsequently cooled to the room temperature. 80 μL of the mixture was added with 19 μL acetate buffer solution (50 mM and *p*H of 3.5) containing 0.02 % sodium azide and digested with 60–120 units isoamylase at 40°C for 12 h. The digested sample was heated in a boiling water bath for 10 min to inactivate the isoamylase. An aliquot of 50 μL was evaporated in a centrifugal vacuum evaporator to obtain dry amylopectin. The reductive amination reaction of reducing ends was performed by adding 2 μL of 0.2 M 8-amino-1,3,6-pyrenetrisulfonic acid (Sigma-Aldrich Co., St. Louis, MO) in 15 % acetic acid and 2 μL of 1 M sodium cyanoborohydride to the dry sample. The mixture was incubated at 40°C for 18 h and then mixed with 46 μL deionized water. An aliquot of 10 μL was diluted to 200 μL with deionized water. Analysis of chain-length distribution of amylopectin was conduced using a Beckman P/ACE capillary electrophoresis instrument with a N-CHO coated capillary (50 μm diameter and 50 cm length) and a Separation Gel Buffer-N (ProteomeLab™ Carbohydrate labeling and analysis kit: Beckman Coulter, Inc., CA). The sample was introduced by pressure injection, 5 s at 0.5 psi. Separation was performed at 23.4 kV with the reversed polarity at 25°C. Maltohexaose and maltoheptaose were used as references.

Confocal Laser Scanning Microscopy

The internal structure of starch granules was examined using a confocal laser scanning microscopy (CLSM) following the method described by Jane and co-workers [8].

Thermal Properties of Starch

Thermal properties of starch were determined using a differential scanning calorimeter (DSC-7, Perkin–Elmer, Norwalk, CT) [11]. Wet-milled starch (3 mg, db) was mixed with 9 μL water and scanned at a rate of 10°C/min over a temperature range from 25 to 110°C in a sealed aluminum pan. An empty pan was used as the reference. The data were calculated using Pyris software (Perkin–Elmer, Norwalk, CT).

Enzyme Digestibility of Dry-Grind Corn and Wet-Milled Starch

Enzyme digestibility of cooked and uncooked dry-grind corn and wet-milled starch was investigated. Starch (100 mg) or flour containing 100 mg of starch was suspended in 9.8 mL of sodium acetate buffer

TABLE 2—*Composition of corn grains (g/100 g dry matter).*

Corn Inbred Line	Starch	Lipid	Protein
A	70.7 ± 0.66	3.2 ± 0.05	12.1 ± 0.07
B	68.9 ± 0.53	4.7 ± 0.08	12.3 ± 0.19
C	75.2 ± 0.39	2.8 ± 0.12	9.4 ± 0.14
D	69.7 ± 0.20	3.0 ± 0.07	13.3 ± 0.12

(0.1 M and pH of 5.2 in saturated benzoic acid). To study the enzymatic digestibility of the cooked sample, the suspension was heated in a boiling water bath for 25 min with mechanical stirring prior to the enzyme digestion. Raw or cooked samples were equilibrated at 37°C for 10 min, and 0.2 mL of the specific enzyme solution, containing 1 U of porcine pancreatic α-amylase (PPA) (Sigma-Aldrich, MO) and 5 U of AMG (Megazyme International, Wicklow, Ireland), was added to the slurry. Enzyme digestion was carried out at 37°C, and 1 mL aliquots of hydrolyzate were withdrawn at different time intervals. Enzyme reactions were stopped by adding 1 mL of aliquot into 10 mL of 70 % ethanol to achieve a final ethanol concentration of ~64% [21]. The glucose content of the hydrolyzates was determined using a glucose oxidase/peroxidase assay (Megazyme International Irelands. Ltd. Co., Wicklow, Ireland; catalog no. K-GLUC). Enzymatic digestibility (%) was calculated as glucose content/initial starch content $\times 162/180 \times 100$.

Ethanol Production of Dry-Grind Corn

Dry-ground corn (50 g) was mixed with 200 mL of distilled water. The slurry was placed in the temperature-controlled water bath shaker at 85°C. The liquefaction process was conducted by adding 0.5 mL of thermostable α-amylase from *Bacillus licheniformis* (Sigma-Aldrich, MO) and holding the slurry at 85°C for 90 min with an agitation speed of 120 rpm. The slurry temperature was cooled down to ~60°C and adjusted to pH 4.2 using 1 N H_2SO_4. The saccharification was performed at 60°C at 120 ppm for 2 h, with addition of 0.5 mL of AMG from *Aspergillus niger* (Sigma-Aldrich, MO). The mixture was cooled to 37°C. Ammonium sulfate (0.03 % w/v) was added as the nitrogen source for yeast growth. The mixture was then inoculated with 5 mL of yeast culture. Fermentation was conducted statically at 30°C for 72 h.

After 72 h, a fermented beer was added with cold distilled water to a total volume of 1 L. Aliquot was withdrawn from the diluted beer and centrifuged at 5500g at 4°C for 15 min to obtain a clear supernatant. Supernatant was filtered using 0.2 μm membrane filter prior to analysis of ethanol concentration using Waters High performance liquid chromatography (Millipore Corporation, Milford, MA) equipped with a Waters Model 401 refractive index detector. The separation was done using an Aminex HPX-87H anion-exchange column (Bio-Rad, Richmond, CA) maintained at 65°C. high-performance liquid chromatography (HPLC)-grade water containing 0.012N H_2SO_4 was used as a mobile phase and eluted at a rate of 0.8 mL/min.

The conversion efficiency was calculated from the theoretical yield of 56.73 g of ethanol produced from 100 g starch (1 g of starch is hydrolyzed into 1.11 g of glucose, and 1 mol of glucose is converted into 2 mol of ethanol).

Results and Discussion

Composition of Corn Grains

Starch, lipid, and protein contents of the four corn inbred lines are shown in Table 2. Starch contents varied from 68.9 % to 75.2 %. Line C had the largest starch content. Lipid (3.0–4.7 %) and protein (9.4–13.3 %) contents of the samples were in agreement with the values reported in the literature [3,22]. Line B had the largest lipid content of 4.7 %, whereas line D had the largest protein content of 13.3 %.

Structural Features of Starch

Amylose contents of wet-milled starches were determined using GPC (Table 3). Line C (29.5 % amylose) and line D (20.7 % amylose) showed the largest and the smallest amylose content, respectively. It is noted that intermediate components of starch may cause an overestimation of the amylose content when it is determined using GPC [11].

TABLE 3—*Amylose content and branch-chain-length distribution of amylopectin.*

Corn Inbred Line	Amylose Content (%)	Avg. CL of Amylopectin	Amylopectin Chain-Length Distribution			
			DP6-12	DP13-24	DP25-36	DP > 36
A	24.1	17.9	34.9	47.1	10.8	7.2
B	24.3	19.3	26.2	53.6	11.2	9.0
C	29.5	19.6	25.9	52.2	12.6	9.3
D	20.7	20.2	25.3	51.5	12.2	11.1

Amylopectin branch-chain-length distributions determined by FACE are shown in Fig. 1. The profiles showed that amylopectins of all four corn lines displayed a similar peak chain-length at DP12, which was similar to the previous report on normal corn amylopectin, analyzed using a high performance anion-exchange chromatography with an AMG reactor and a pulsed amperometric detector [11]. Summary of chain-length distribution (Table 3) showed that amylopectin of line A had the largest proportion of DP6-12 (34.5 %) and the shortest average chain-length (DP17.9). Line D showed the longest average chain-length (DP20.2), followed by line C (DP19.6), line B (DP19.3), and line A (DP17.9).

The internal structures of starch granules were examined using CLSM (Fig. 2). The CLSM micrographs showed voids and cracked internal granule structures for all corn lines. These features reflected the loosely packed starch molecules organized by amylopectin of short branch-chains [8].

Starch Gelatinization

Thermal properties of wet-milled starches were determined using DSC. Results showed that gelatinization temperatures varied with corn inbred lines (Table 4). The ranges of onset (T_o), peak (T_p), conclusion (T_c) temperatures and enthalpy change were 61.9–66.1°C, 68.3–71.4°C, 73.2–76.3°C, and 11.4–12.1 J/g, respectively. The values agreed well with the previous report on normal corn starch [11].

Starch gelatinization is an endothermic reaction that corresponds to the dissociation of semi-crystalline structure of starch, which is constructed by double helical structures of amylopectin. In this study, the lowest gelatinization temperature of line A coincided with its shortest amylopectin branch-chain-length (Table 3). The results supported that double helical structures formed by short amylopectin branch-chains were dissociated at low temperature [11,23,24].
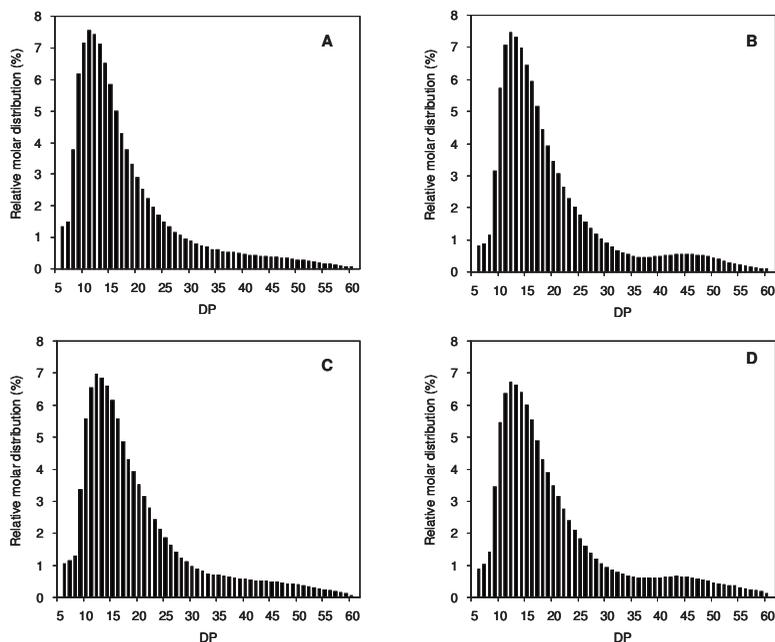

FIG. 1—*Molar-based branch-chain-length distributions of amylopectins isolated from corn inbred lines A, B, C, and D.*
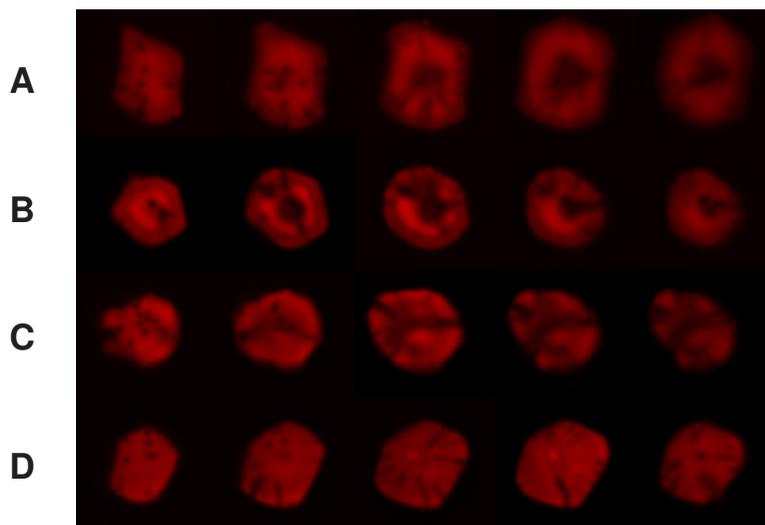
FIG. 2—*CLSM images of starch granules from corn inbred lines A, B, C, and D.*

Enzymatic Digestibility of Uncooked Dry-Grind Corn and Wet-Milled Starch

Enzymatic digestibility of starch in dry-grind corn was studied and compared with that of isolated starch. Glucose yield produced from PPA and AMG hydrolysis of uncooked corn samples is shown in Fig. 3. Results showed that starch in dry-grind corn was hydrolyzed faster than the isolated starch from wet-milling. After 48 h of enzyme hydrolysis, 95.6–99.8 % of starch in dry-grind corn samples was hydrolyzed to glucose, which was much higher than that obtained from the isolated starches (66.2–68.4 %).

It is known that semi-crystalline starch molecules are hydrolyzed more slowly than dispersed amorphous molecules. It has been proposed that the A-type polymorph starch granules, e.g., normal corn starch, have weak points that are susceptible to enzyme hydrolysis [25,26]. CLSM images also showed cracks and voids in the A-type polymorph starch. These loosely packed internal structures would enhance greater hydrolytic activity on starch granules [8].

Greater enzyme digestibility of starch in dry-grind corn resulted from physical damage of starch granules. During the dry-grind process, the endosperm is broken along cell wall and across cells, resulting

TABLE 4—*Thermal properties of wet-milled starches.*

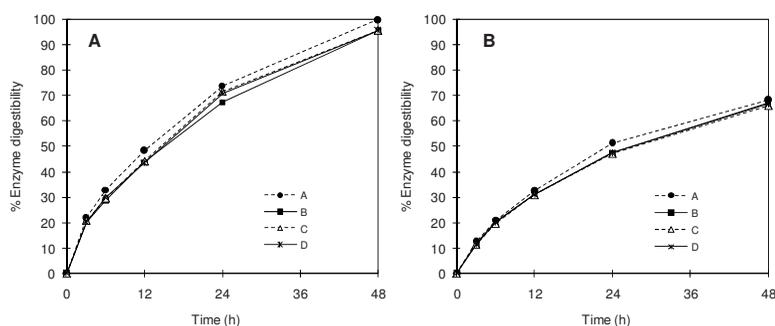| Corn Inbred Line	Thermal Properties			
	T_o (°C)	T_p (°C)	T_C (°C)	ΔH (J/g)
A	61.9 ± 0.3	68.3 ± 0.1	73.2 ± 0.4	11.4 ± 0.5
B	66.1 ± 0.5	71.4 ± 0.3	76.3 ± 0.7	12.1 ± 0.9
C	63.2 ± 0.5	69.1 ± 0.4	74.4 ± 0.1	11.8 ± 0.2
D	65.3 ± 0.2	70.6 ± 0.2	75.0 ± 0.5	12.1 ± 0.6

FIG. 3—*Enzyme digestibility (%) of uncooked dry-grind corn (a) and uncooked wet-milled starch (b) from corn inbred lines A, B, C, and D. A mixture of porcine pancreas α-amylase (1 U) and AMG (5 U) was used for the study.*
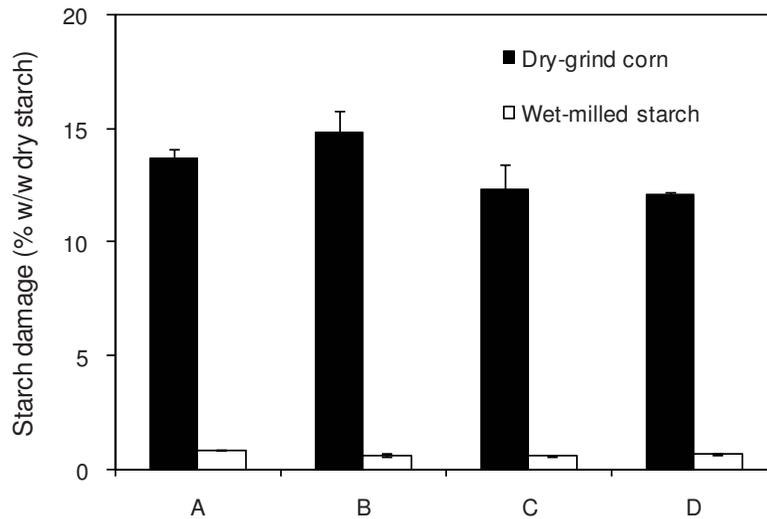
FIG. 4—*Starch damage (% w/w dry starch) of dry-grind corn and wet-milled starch from corn inbred lines A, B, C, and D.*

in the damage of starch granules [3]. Percentages of starch damage of dry-grind corn and isolated starch are shown in Fig. 4. Wet-milled starch was damaged very little (less than 0.84 %), whereas dry-grind corn samples contained significant amounts of damaged starch (12.0–17.5 %). The physically damaged starch granules lost the protection of a tough surface layer composed of a larger proportion of amylose entangled with amylopectin [7], resulting in exposure of the loosely packed hilum of the granule [27]. Line A showed a greater enzyme digestibility of starch than the other corn lines for both dry-grind corn and wet-milled starch. This could be attributed to an inferior crystalline structure organized by short amylopectin branch-chains [7].

Starch Digestibility of Cooked Dry-Grind Corn and Wet-Milled Starch

Starch digestibility of cooked dry-grind corn is of interest for conventional dry-grind ethanol production processes. Results of enzymatic hydrolyses of cooked samples are shown in Fig. 5. The cooking process enhanced enzymatic hydrolysis of starch as indicated by the shorter time required to achieve maximal hydrolysis, compared with the uncooked samples (Fig. 3). The results confirm that amylolytic activity was greater when gelatinized starch was used as the substrate.

Unlike the uncooked samples (Fig. 3), cooked wet-milled starches had greater enzyme digestibility (86.9–92.9 %) than their dry-grind corn counterparts (83.5–87.6 %). This suggested that protein and lipid content of dry-grind corn might interfere with starch hydrolysis. The protein matrix could block water penetration, delay gelatinization of starch, and reduce starch digestibility. This effect would be more severe in large particles of the dry-grind corn [28]. The formation of disulphide-bonded zein oligomers has been also reported in corn flake structures during cooking at 100°C [29]. When starch molecules are surrounded

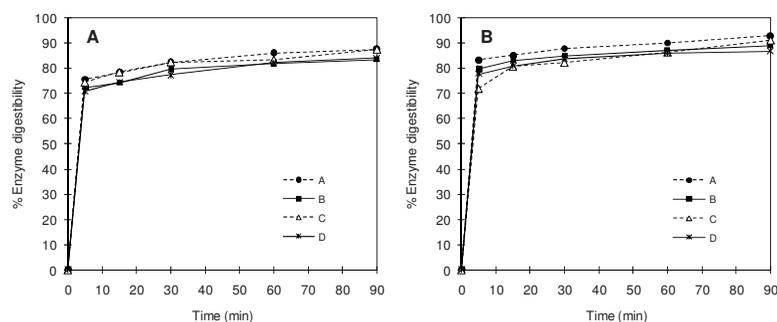

FIG. 5—*Enzyme digestibility (%) of cooked dry-grind corn (a) and cooked wet-milled starch (b) from corn inbred lines A, B, C, and D. A mixture of porcine pancreas α-amylase (1 U) and AMG (5 U) was used for the study.*

TABLE 5—*Ethanol yield and conversion efficiency at 72 h fermentation.*

Corn Inbred Line	Ethanol Yield (g/100 g Dry Matter)	Conversion Efficiency (%)
A	36.2 ± 0.6	90.3 ± 1.6
B	34.6 ± 0.2	88.4 ± 0.6
C	38.0 ± 0.7	89.1 ± 1.7
D	34.3 ± 0.9	86.8 ± 2.4

with hydrophobic disulphide-bonded proteins, the accessibility of starch to enzyme digestion may be obstructed. The addition of reducing agents (e.g., 2-mercaptoethanol, sodium metabisulfite, and ascorbic acid) to prevent or reduce the disulphide-bond formation between proteins resulted in improved enzymatic digestion of starch in cereal flours [12,13].

In addition to protein, lipids in corn grain could also decrease enzymatic conversion of starch to glucose. It is known that linear molecules or long branch-chains of starch can form stable helical complexes with lipids during starch gelatinization. The complexes with lamellae crystallites are highly resistant to enzyme digestion [30]. In addition to starch-lipid complexes, the formation of retrograded starch that is insoluble and resistant to enzyme hydrolysis may also occur during the processes. All these factors would result in the incomplete enzymatic conversion of cooked starch to glucose during the conventional dry-grind ethanol production process.

Ethanol Production from Dry-Grind Corn

Fermentation of saccharified corn slurry was conducted to investigate the performance of the corn inbred lines on ethanol productivity. Results obtained after 72 h fermentation are listed in Table 5. Ethanol produced from the four GEM inbred lines varied between 34.6 and 38.0 g ethanol per 100 g dry-grind corn. The values were equivalent to 2.7–3.0 gal ethanol/bushel of corn. The conversion efficiencies ranged from 86.8 % to 90.3 % of the theoretical yield, which were in the range of normal gravity fermentation of *Saccharomyces cerevisiae* [31]. It was found that in general, corn lines that contained smaller amounts of lipid and protein exhibited higher conversion efficiency. The largest ethanol yield of 38.0 g per 100 g dry-grind corn was found in line C, which had the largest starch content and the smallest lipid and protein content. The highest conversion efficiency (90.3 %) shown in line A could be attributed to its low starch gelatinization temperature, which could result in greater starch hydrolysis during the liquefaction step. Line D, having the largest protein content, produced the least ethanol yield and conversion efficiency.

Summary

Enzyme digestibility of dry-grind corn and ethanol productivity varied among corn inbred lines. The variations were attributed to the differences in starch structure and grain composition. In uncooked dry-grind corn samples, enzyme digestibility of starch was accelerated by physical damage of starch granules and also affected by amylopectin branch-chain-length distribution. In cooked dry-grind corn samples, the presence of protein and lipid decreased the enzyme digestibility of starch and subsequently reduced the conversion efficiency of starch to ethanol. This information is of interest for plant breeders to select maize germplasm for improved ethanol productivity and broadening the germplasm base for starch traits.

References

[1] Salamini, F. and Soave, C., "Zein: Genetics and Biochemistry," *Maize for Biological Research*, W. F. Sheridan, Ed., University of South Dakota Press, Charlottesville, 1982, p. 155.
[2] Morrison, W. R., "Lipids in Cereal Starches: A Review," *J. Cereal Sci.*, Vol. 8, 1988, pp. 1–15.
[3] Watson, S. A., "Structure and Composition," *Corn: Chemistry and Technology*, S. A. Watson and P. E. Ramstad, Eds., American Association of Cereal Chemists, Inc., St. Paul, MN, 1987, p. 53.
[4] Kohl, S., "Overview of Ethanol Production," *Ethanol Today*, Jul., 2003, pp. 36–37.0002-7820
[5] Jane, J., Ao, Z., Duvick, S. A., Wiklund, M., Yoo, S.-H., Wong, K.-S., and Gardner C., "Structures of Amylopectin and Starch Granules: How Are They Synthesized?" *J. Appl. Glycosci.*, Vol. 50, 2003,

pp. 167–172.

[6] Perera, C., Lu, Z., Sell, J., and Jane, J., "Comparison of Physicochemical Properties and Structures of Sugary-2 Cornstarch with Normal and Waxy Cultivars," *Cereal Chem.*, Vol. 78, 2001, pp. 249–256.

[7] Jane, J., "Structure of Starch Granules," *J. Appl. Glycosci.*, Vol. 54, 2007, pp. 31–36.

[8] Jane, J., Atichokudomchai, N., and Suh, D. S., "Internal Structures of Starch Granules Revealed by Confocal Laser-Light Scanning Microscopy," *Starch: Progress in Structural Studies, Modifications and Applications*, P. Tomasik, V. P. Yuryev, and E. Bertoft, Eds., Polish Society of Food Technologists, Cracow, 2004, p. 147.

[9] Karkalas, J., Tester, R. F., and Morrison, W. R., "Properties of Damaged Starch Granules. I. Comparison of a Micromethod for the Enzymic Determination of Damaged Starch with the Standard AACC and Farrand Methods," *J. Cereal Sci.*, Vol. 16, 1992, pp. 237–251.

[10] Tester, R. F., Morrison, W. R., Gidley, M. J., Kirkland, M., and Karkalas, J., "Properties of Damaged Starch Granules. III. Microscopy and Particle Size Analysis of Undamaged Granules and Remnants," *J. Cereal Sci.*, Vol. 20, 1994, pp. 59–67.

[11] Jane, J., Chen, Y. Y., Lee, L. F., McPherson, A. E., Wong, K. S., Radosavljevic, M., and Kasemsuwan, T., "Effects of Amylopectin Branch Chain Length and Amylose Content on the Gelatinization and Pasting Properties of Starch," *Cereal Chem.*, Vol. 76, 1999, pp. 629–637.

[12] Elkhalifa, A.E. O., Chandrashekar, A., Mohamed, B. E. and Tinay, A. H. E., "Effect of Reducing Agents on the In Vitro Protein and Starch Digestibilities of Cooked Sorghum," *Food Chem.*, Vol. 66, 1999, pp. 323–326.

[13] Ezeogu, L. I., Duodu, K. G., and Taylor, J. R. N., "Effects of Endosperm Texture and Cooking Conditions on the In Vitro Starch Digestibility of Sorghum and Maize Flours," *J. Cereal Sci.*, Vol. 42, 2005, pp. 33–44.

[14] Zhang, G. and Hamaker, B. R., "Low α-Amylase Starch Digestibility of Cooked Sorghum Flours and the Effect of Protein," *Cereal Chem.*, Vol. 75, 1998, pp. 710–713.

[15] Holm, J., Bjoerck, I., Ostrowska, S., Eliasson, A. C., Asp, N. G., Larsson, K. et al., "Digestibility of Amylose-Lipid Complexes In-Vitro and In-Vivo," *Starch*, Vol. 35, 1983, pp. 294–297.

[16] Jane, J. and Robyt, J. F., "Structure Studies of Amylose-V Complexes and Retrograded Amylose by Action of Alpha-Amylases, and a New Method for Preparing Amylodextrins," *Carbohydr. Res.*, Vol. 132, 1984, pp. 105–118.

[17] Sievert, D., Czuchajowska, Z., and Pomeranz, Y., "Enzyme-Resistant Starch. III. X-Ray Diffraction of Autoclaved Amylomaize VII Starch and Enzyme-Resistant Starch Residues," *Cereal Chem.*, Vol. 68, 1991, pp. 86–91.

[18] Song, Y. and Jane, J., "Characterization of Barley Starches of Waxy, Normal, and High Amylose Varieties," *Carbohydr. Polym.*, Vol. 41, 2000, pp. 365–377.

[19] O'Shea, M. G. and Morell, M. K., "High Resolution Slab Gel Electrophoresis of 8-Amino-1,3,6-Pyrenetrisulfonic Acid (APTS) Tagged Oligosaccharides Using a DNA Sequencer," *Electrophoresis*, Vol. 17, 1996, pp. 681–686.

[20] O'Shea, M. G., Samuel, M. S., Konik, C. M., and Morell, M. K., "Fluorophore-Assisted Carbohydrate Electrophoresis (FACE) of Oligosaccharides: Efficiency of Labeling and High-Resolution Separation," *Carbohydr. Res.*, Vol. 307, 1998, pp. 1–12.

[21] Englyst, H. N., Kingman, S. M., and Cummings, J. H., "Classification and Measurement of Nutritionally Important Starch Fractions," *Eur. J. Clin. Nutr.*, Vol. 46, 1992, pp. S33–S50.

[22] Belyea, R. L., Rausch, K. D., and Tumbleson, M. E., "Composition of Corn and Distillers Dried Grains with Solubles from Dry Grind Ethanol Processing," *Bioresour. Technol.*, Vol. 94, 2004, pp. 293–298.

[23] Srichuwong, S., Sunarti, T. C., Mishima, T., Isono, N., and Hisamatsu, M., "Starches from Different Botanical Sources I: Contribution of Amylopectin Fine Structure to Thermal Properties and Enzyme Digestibility," *Carbohydr. Polym.*, Vol. 60, 2005, pp. 529–538.

[24] Noda, T., Takahata, Y., Sato, T., Suda, I., Morishita, T., Ishiguro, K. et al., "Relationships Between Chain Length Distribution of Amylopectin and Gelatinization Properties Within the Same Botanical Origin for Sweet Potato and Buckwheat," *Carbohydr. Polym.*, Vol. 37, 1998, pp. 153–158.

[25] Fannon, J. E., Hauber, R. J., and BeMiller, J. N., "Surface Pores of Starch Granules," *Cereal Chem.*, Vol. 69, 1992, pp. 284–288.

[26] Huber, K. C. and BeMiller, J. N., "Visualization of Channels and Cavities of Corn and Sorghum Starch Granules," *Cereal Chem.*, Vol. 74, 1997, pp. 537–541.

[27] Hasjim, J., Srichuwong, S., Scott, M. P., and Jane, J., "Kernel Composition, Starch Structure, and Enzyme Digestibility of Opaque-2 Maize and Quality Protein Maize," *J. Agric. Food Chem.*, Vol. 57, 2009, pp. 2049–2055.

[28] Chandrashekar, A. and Kirleis, A. W., "Influence of Protein on Starch Gelatinization in Sorghum," *Cereal Chem.*, Vol. 65, 1988, pp. 457–462.

[29] Batterman-Azcona, S. J. and Hamaker, B. R., "Changes Occurring in Protein Body Structure and A-Zein During Cornflake Processing," *Cereal Chem.*, Vol. 75, 1998, pp. 217–221.

[30] Biliaderis, C. G., Page, C. M., and Maurice, T. J., "On the Multiple Melting Transitions of Starch/ Monoglyceride Systems," *Food Chem.*, Vol. 22, 1986, pp. 279–295.

[31] Jeffries, T. W., "Ethanol Fermentation on the Move," *Nat. Biotechnol.*, Vol. 23, 2005, pp. 40–41.