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Ethanol Fermentation Performance of Grain Sorghums (*Sorghum bicolor*) with Modified Endosperm Matrices

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We tested 13 sorghum entries (lines and hybrids) with different endosperm matrices for ethanol production using a laboratory dry grind process. Waxy and heterowaxy samples had the highest efficiencies. Free amino nitrogen (FAN) contents in sorghum samples were positively related to the fermentation rate during fermentation (R^2 = 0.8618). Dried distiller's grain with solubles (DDGS) from different sorghums had significantly different crude protein and crude fat contents. Residual starch content in DDGS ranged from 0.60% for the most efficient sample to 2.66% for the least efficient sample. This study showed that the HD lines (TX1, TX3, TX5, TX7, and TX9) with modified endosperm protein matrix have several attributes desirable for ethanol production: easily pasted starch granules, significantly higher FAN content in finished mashes, 30−45% faster ethanol fermentation rate during early stages, and 50−60% higher lysine content in DDGS.

KEYWORDS: Ethanol; fermentation; dry grinding; grain sorghum (*Sorghum bicolor*); DDGS; endosperm matrices

INTRODUCTION

In 2009, approximately 95% of the more than 10 billion gallons of fuel ethanol in the United States was produced from corn, and ~4% was produced from sorghum (*1*). Annual U.S. ethanol production from cereal grains is projected to grow and remain at 15 billion gallons even after lignocellulosic ethanol technology is fully commercialized, which will consume ~30% of the U.S. corn crop each year (*2*). To keep up with such a challenging goal, intensive efforts has been devoted to the development of high-performance corn hybrids, which results in several high-yielding corn varieties and hybrids with excellent agronomic traits for fuel ethanol production (*3*, *4*) and other industrial uses (*5*). Sorghum has been identified as a promising feedstock for bioethanol production because of its lower fertilizer requirement, higher water efficiency, and other favorable agronomic traits (*6*). Similar research efforts have been made in sorghum breeding, but those efforts mostly focused on improvement of sorghum food quality and yield. High-lysine, high-protein-digestibility (HD) sorghum lines have been developed (*7*−*9*). Research on the compounding factors influencing fuel ethanol production from sorghum has been conducted recently, revealing that genotype or cultivar has the most significant effects on ethanol yields. Growing location significantly affected chemical compositions and physical properties of tested sorghum varieties in Kansas and Texas, which led to very different ethanol yields (*10*, *11*).

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We have developed several mutant HD sorghum genotypes with modified endosperm matrices. The original HD lines were derived within a high-lysine sorghum population; additional lines were developed using pedigree breeding approaches (*12*). The increased protein digestibility of these lines is derived from an increase in α-kafirin digestibility. The HD lines are unique in their protein body structure; they have abnormal, highly invaginated kafirin protein bodies. Immunocytochemistry reveals a normal distribution of α- and *β*-kafirins but a reduced presence of the highly folded *γ*-kafirins in the HD lines (*13*). Segregated progeny with HD population lack the kafirin protein body matrices that surround the starch granules and restrict starch granule swelling and pasting. These genotypes have several benefits. First, the grain starch swells and pastes more easily at lower temperatures (*14*). Second, the proteins present have improved feed value with higher bioavailability and 60% more lysine, similar to high-lysine corn lines (*12*).

Several approaches have been used to evaluate ethanol fermentation performance of a limited number of grain sorghums (*11*, *15*, *16*), and results may be helpful for sorghum breeders and the ethanol industry. The goal of this study was to develop a system approach for breeding sorghum cultivars that optimizes the grain's endosperm matrix for bioethanol conversion and grain distillers feed. We generated data that suggest that the waxy characteristic individually improves the endosperm matrix for low-energy-input gelatinization, enzymatic hydrolysis, and total ethanol production. Given the favorable low energy for gelatinization characteristic of the HD trait, we reasoned that it,

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Table 1. Chemical Composition of Texas Sorghum Samples (± SD; %, db)[a]

entry	pedigree	endosperm phenotype[b]	starch	crude protein	crude fat	crude fiber	ash	FAN (mg/L)	amylose	efficiency at 24 h	efficiency at 72 h
TX 1	P850029	HD	66.6 d	14.47 a	3.89 de	2.80 c	1.89 c	119 b	23.5	73.7	85.5 f
TX 2	BTx635	WT	73.3 a	12.63 c	3.15 h	2.61 de	1.57 ef	44.7 h	27.0	54.5	84.6 g
TX 3	(BTx635*P850029)-CS9-CS1-CS1	HD	67.9 cd	13.17 b	3.78 ef	2.48 e	2.05 b	133 a	24.3	79.5	84.6 g
TX 4	RTx436	WT	72.9 a	10.48 f	3.43 g	1.91 g	1.59 e	78.1 ef	27.5	60.1	86.1 ef
TX 5	(RTx436*P850029)-CS42-CS1-CS1-CS1	HD	73.5 a	11.16 e	2.82 i	2.49 e	1.49 f	64.4 g	24.1	57.6	86.0 ef
TX 6	RTx436*P850029)-CS22-CS1-CS1-CS2	WT	62.9 e	11.75 d	5.52 a	3.69 a	2.55 a	77.8 ef	26.2	68.7	89.0 b
TX 7	(96GCPOB124*P851171)-CS28-CS1-CS1-CS1	HD	68.0 cd	12.64 c	3.78 f	2.69 cd	1.74 d	116 b	25.1	74.2	86.3 cd
TX 8	96GCPOB124	WT	70.4 b	10.35 g	3.34 g	2.70 cd	1.53 ef	72.2 f	24.6	61.4	86.4 de
TX 9	P851171	HD	68.3 bcd	11.22 e	3.15 h	2.32 f	1.35 g	108 c	29.2	76.8	83.7 g
TX 10	ATxARG-1/RTX2907	Waxy	70.3 b	10.38 fg	3.12 h	2.30 f	1.36 g	83.7 e	6.26	62.1	91.4 a
TX 11	ATxARG-1/RTx436	heterowaxy	66.9 d	9.56 h	4.91 b	3.02 b	1.97 bc	102 cd	20.1	66.6	91.8 a
TX 12	ATx2928/RTx436	WT	72.7 a	8.75 i	3.98 d	2.78 c	1.72 d	76.9 ef	26.6	59.8	87.4 c
TX 13	ATx2928/RTx2907	heterowaxy	69.1 bc	9.54 h	4.40 c	3.05 b	1.93 c	97.0 d	20.2	66.7	87.1 d

[a] Means in a column with different letters differ ($P < 0.05$). [b] HD refers to a high protein digestible and nonwaxy endosperm phenotype; WT refers to a normal nonwaxy, average digestible endosperm phenotype; waxy refers to a waxy, average digestible endosperm phenotype; and heterowaxy is grain that segregated for waxy endosperm phenotype with normal protein digestibility.

too, would improve ethanol fermentation efficiency of the sorghum grain. If true, sorghum cultivars that combine a HD trait with a high amylopectin (waxy) starch trait would be ideal for bioethanol conversion.

MATERIALS AND METHODS

Thirteen sorghum inbred lines with different endosperm matrices (HD, waxy, or wild types) were planted in the normal cropping season in 2008 at the Texas Agrilife Research Farm near College Station, TX. Entries were combine harvested and processed in fall 2008; this grain was used for the remainder of the experiments. The samples were ground using a Udy cyclone sample mill with a 0.5 mm screen (UDY Corp., Fort Collins, CO) for analysis and the fermentation test. Physical properties and chemical compositions of the sorghum samples are listed in **Table 1**.

Potassium phosphate monobasic, magnesium sulfate, dextrose, sodium acetate, hydrochloric acid, sodium hydroxide, acetic acid, and dimethyl sulfoxide (DMSO) were purchased from Fisher Scientific (Fairlawn, NJ). Difco yeast extract and Difco peptone were from Becton-Dickinson (Sparks, MD). Maltose, maltotriose, 4-morpholinepropanesulfonic acid (MOPS), and analytical standard glucose were from Supelco (Bellefonte, PA). Standard reference ethanol (SRM 2899a) was purchased from NIST (Gaithersburg, MD). All other chemicals were reagent grade or better.

The hydrolyzing enzymes, Liquozyme (a high-temperature α-amylase produced by *Bacillus licheniformis*) and Spirizyme (a glucoamylase produced by *Aspergillus niger*), were provided by Novozymes (Novozymes North America, Inc., Franklinton, NC). The dry alcohol yeast, Ethanol Red, was provided by Fermentis in vacuum-packed aluminum foil bags (Lesaffre Yeast Corp., Milwaukee, WI).

Analytical Methods. *Free Amino Nitrogen* (*FAN*) *Analysis*. Free amino nitrogen content of finished mashes (after liquefaction and before addition of yeast extract, glucoamylase, and inoculation of activated yeast) was determined by following European Brewery Convention method 8.8.1 (*17*). Finished mash samples were diluted by mixing 100 μL of clear supernatant with 1900 μL of HPLC-grade distilled water in a screw-capped 16 mL test tube before being analyzed.

Total Starch in Original Sorghum Samples and DDGS. Total starches in sorghum samples and corresponding freeze-dried DDGS were determined by using Megazyme K-TSTA kits with modified DMSO procedures (*18*). Starches in the samples were completely solubilized in DMSO and hydrolyzed in two steps into glucose by using thermostable α-amylase (100 °C, pH 6−6.5) and amyloglucosidase (50 °C, pH 4.5).

Apparent Amylose Content. Apparent amylose content of the sorghum samples was analyzed colorimetically using the dual wavelength approach (*19*−*21*). Sorghum flours containing ∼100 mg starch were wetted with 1.0 mL of 95% ethanol and dissolved in 10 mL of 1.0 N NaOH in a 100 mL volumetric flask with continuous shaking (120 rpm) on a rotary shaker at room temperature overnight. The clear dissolved mixture was diluted with distilled water to 100 mL. Two milliliters of the dissolved sample was transferred into another 100 mL volumetric flask, mixed with ∼50 mL of distilled water, and neutralized with 2.0 mL of 0.1 N HCl. After 2.0 mL of colorant iodine solution (0.2% iodine in 0.2% KI solution) was added, the mixture was brought to volume with distilled water. The mixture was left at room temperature for 30 min for color development, and then absorbance values at 510 and 620 nm were read on a spectrophotometer against distilled water and iodine solution blanks.

Amino Acid Composition of DDGS. Samples were weighed and then placed in about 0.5 mL of 6 N HCl along with the internal standard and hydrolyzed at 110 °C for 20 h. An aliquot, usually 10 or 20 μL, of that HCl was diluted up to 250 μL with 0.4 M borate buffer to dilute the sample and raise the pH. After precolumn derivatization with *o*-phthalaldehyde (OPA) and 9-fluorenylmethyl chloroformate (FMOC), 1 μL of this diluent was injected into an HPLC system with a C18 column (Hypersil AA-ODS, 2.1 × 200 mm, 5 μm). Mobile phase A was 20 mM sodium acetate buffer with 0.018% (v/v) triethylamine, 0.05 mM EDTA, and 0.3% tetrahydrofuran, pH adjusted to 7.2 using acetic acid. Mobile phase B was 100 mM sodium acetate:acetonitrile:methanol (20:40:40, v/v). The elution conditions were from 100% A to 60% B in 17 min at 0.45 mL/min. Amino acid derivatives were detected with a fluorescent detector at 340/450 nm (excitation/emission) for primary amino acids and 266/305 nm for secondary amino acids. Human serum albumin was used as a control, and norvaline and sarcosine were used as internal standards.

Methods for analyses of crude protein, lipid, and ash were AOAC 990.03, 920.39, and 942.05, respectively. Crude fiber was analyzed by the filter bag technique using the ANKOM A200 (http://www.ankom.com/media/documents/CrudeFiber_1108_A200.pdf).

Physical Properties of Sorghum Samples. *RVA Test*. RVA tests were performed on a model S4A RVA analyzer with Thermocline for Windows ver. 3.10 software (Newport Scientific, Warriewood, NSW, Australia) using Standard Procedure 1 (holding at 50 °C for 1 min, heating to 95 at 10 °C/min, holding at 95 °C for 2 min, and cooling to 50 °C).

DSC Analysis. DSC analyses of selected sorghum flour samples were conducted on a PerkinElmer Diamond DSC by weighing ∼9 mg of flour into stainless steel pans on a PerkinElmer autobalance (model AD6; PerkinElmer Life and Analytical Sciences, Shelton, CT). The flour was then mixed with distilled water to form a slurry with a moisture content of 75%. The temperature program was holding at 30 °C for 3 min and then ramping to 180 at 10 °C/min.

Starch Crystallinity of Selected Sorghums Using Wide-Angle X-ray Diffraction (*WAXD*). Sorghum starch from selected sorghum samples was prepared by following a laboratory wet milling procedure described by Wang and Chung (*22*). The starches were examined with an Advanced Polymers Beamline (X27C) in the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory. Experimental setups at the X27C beamline followed those reported by Chen et al. (*23*, *24*). The wavelength used was 0.1371 nm, and the sample-to-detector distance was 97.33 mm. A 2D MAR-CCD (MAR USA, Inc.) X-ray detector was used

for data collection. Polar software (Precision Works NY, Inc.) was used to process data.

Ethanol Fermentation. Ground samples containing 30.00 g of dry mass were mixed with 100 mL of preheated (~60−70 °C) enzyme solution (containing 1.0 g/L KH_2PO_4 and 200 μL/L Liquozyme) in a clean 250 mL Erlenmeyer flask to form an evenly suspended slurry. The temperature program for mashing and the procedures and conditions for simultaneous saccharification and fermentation (SSF) were the same as described by Wu et al. (*11*).

Ethanol concentration in the finished beer was determined by HPLC with a Rezex RCM column and RI detector after distillation as described by Wu et al. (*25*). The fermentation efficiency was calculated on the basis of the theoretical ethanol yield of 56.72 g from 100.0 g of dry starch.

Statistical Analysis. Differences in each trait among lines were determined using the LSD LINE option of PROC GLM. Simple correlations of physical and chemical traits were determined using PROC CORR (SAS 9.1.3 service pack 4 for Windows. SAS Institute Inc., Cary, NC).

RESULTS AND DISCUSSION

Major chemical components differed among the 13 sorghum entries (**Table 1**). Starch content of the tested samples was similar to that of most sorghum and corn cultivars (~70%), except TX6 had only 62.9% starch. Three HD samples, TX1, TX3, and TX7, and one normal sorghum, TX2, had significantly higher (> 12%) crude protein content than the rest of the samples. Among all samples, TX10 had the lowest amylose content (6.26% of total starch), and TX9 had the highest amylose content (29.2% of total starch).

Fermentation results (**Table 1**) from the laboratory dry-grind process showed that TX10 and TX11 had the highest efficiencies (91.4% and 91.8%, respectively) and TX9 had the lowest fermentation efficiency (83.7%). As previously reported (*11*, *25*, *26*), desirable characteristics for ethanol production include high starch content, rapid liquefaction, low viscosity during liquefaction, high fermentation speed, and high fermentation efficiency. Tannin, high mash viscosity, and amylose−lipid complex had negative effects on ethanol production. Waxy varieties always performed better than nonwaxy varieties of the same crop types (*15*, *26*). Findings from the current study agree with previously reported results on ethanol production from corn, pearl millets, sorghum, and wheat.

Although only 13 sorghum samples were tested for ethanol fermentation, correlation between starch content and ethanol yield was similar to that reported by Wu et al. (*26*). Ethanol yield was positively correlated with starch content (R^2 = 0.6299; **Figure 1**). An interesting finding was that TX5, a HD endosperm sorghum, and TX6, a normal endosperm sorghum, underwent a similar breeding process but had totally different starch contents. Among all tested samples, TX5 had the highest starch content and TX6 had the lowest starch content. Amylose content has been demonstrated to be a negative factor for ethanol production. Fermentation efficiency and ethanol yield decrease as amylose content increases. Efficiency results in this study followed a similar trend. Waxy and heterowaxy samples (TX10 and TX11) had the highest fermentation efficiencies and ethanol yield, and the sample with highest amylose content (TX9) had the lowest fermentation efficiency (83.7%) and ethanol yield (362 L/ton, or 2.43 gal/bu). Fermentation efficiency of TX7, the hybrid developed from TX9, was also among the lowest of all samples. Starch content together with fermentation efficiency determines the final ethanol yield of a hybrid or inbred. This explains why TX10, which has modest starch content, had the highest ethanol yield of all tested samples. Having only high starch content or high fermentation efficiency cannot guarantee high ethanol yield. TX11 and TX6 had high efficiency because of their significantly higher contents of sucrose and fructose (data not shown), which were not accounted in the total starch assay but contributed to

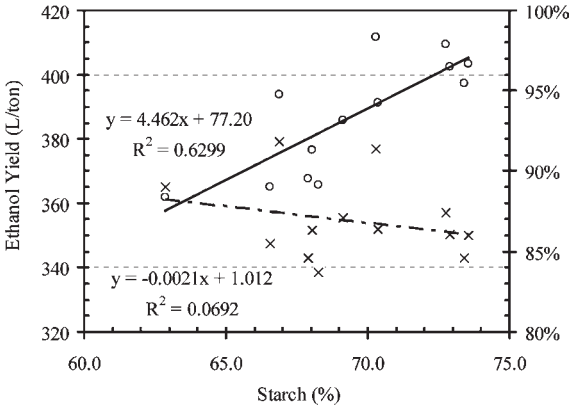

Figure 1. Relationship between ethanol yield, fermentation efficiency, and starch content.

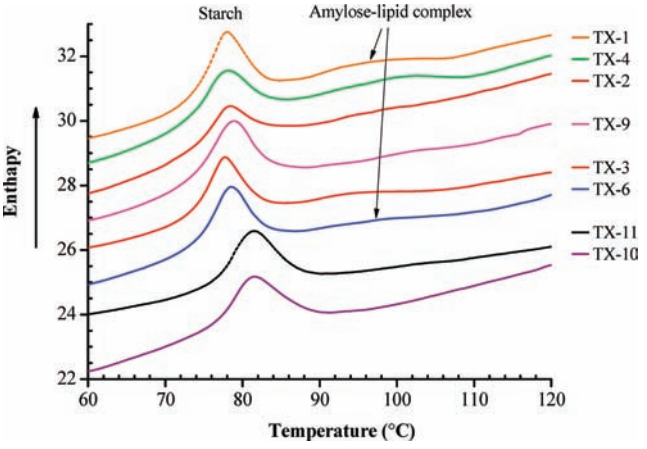

Figure 2. DSC thermograms of selected sorghum samples show the major starch gelatinization peak and amylose−lipid complex peaks.

ethanol production during fermentation. Ethanol yields from TX 6 and TX11 were pretty low (362 and 394 L/ton respectively) because their starch contents were relatively low (62.9% for TX6 and 66.9% for TX11, the average of all 13 samples was 69.5%).

Figure 2 shows that most sorghum samples except the waxy and heterowaxy ones (TX10 and TX11) had a significant amylose−lipid complex peak beside the major starch gelatinization peak. It has long been recognized that formation of the amylose−lipid complex can significantly reduce starch digestibility (*27*, *28*). The more amylose−lipid content a starch contains, the slower and less completely the starch will be hydrolyzed, which will ultimately result in lower ethanol yield and fermentation efficiency. As shown in **Figure 2**, dissociation temperatures for amylose−lipid complexes are around 95−105 °C, which is higher than the 85 °C mashing temperature. The lipid-complexed amylose will have poor or slow access for amylase hydrolysis, which, consequently, will result in increased residual starch in DDGS. The residual starch content in DDGS observed in this study positively confirms this hypothesis (**Table 1**). The amylose−lipid peaks were especially large in the thermograms of TX1, TX2, TX3, TX4, and TX9, which all have low fermentation efficiencies.

All sorghum starches gave an A-type X-ray diffraction pattern (**Figure 3**). The degree of crystallinity clearly differentiated waxy starches from nonwaxy starches (**Table 2**). It is generally believed that side chains of amylopectin molecules form crystalline lamellae in the starch granules; branch regions of amylopectin molecules and connections between crystalline lamellae are amorphous (*29*). Most amylose molecules are dispersed in the amorphous regions

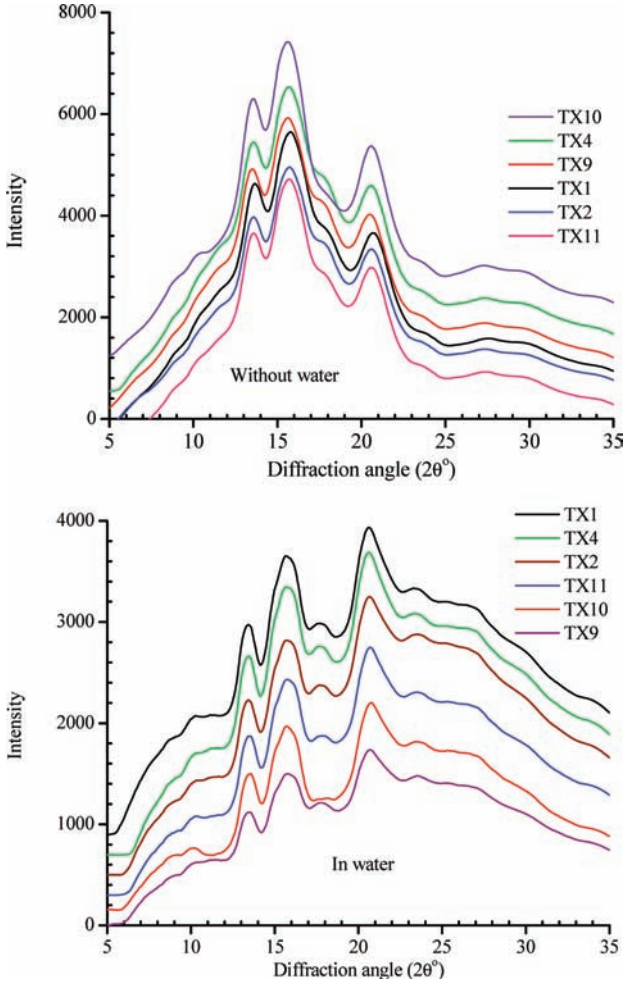

Figure 3. X-ray diffraction patterns of sorghum starch samples with and without water.

Table 2. Degree of Crystallinity (%) of Selected Sorghum Starches from WAXD Data

sample	pedigree	crystallinity (%)
TX 1	P850029	27.8
TX 2	BTx635	26.8
TX 4	RTx436	28.1
TX 9	P851171	27.5
TX 10	ATxARG-1/RTx2907	34.9
TX 11	ATxARG-1/RTx436	28.1

of starch granules (*30*). Therefore, starches with less amylose (waxy starch) will have a higher degree of crystallinity than those with more amylose (normal and high-amylose starches (*31*)). In this study, waxy starch from TX10 had the highest degree of crystallinity, and starch from TX9 had the lowest degree of crystallinity. It should be noted that even though the waxy sorghum starch (TX10) had a higher degree of crystallinity, it had high fermentation efficiency (**Table 1**). This is because despite its high degree of crystallinity, waxy cereal starch swells more and granules are fragmented after cooking (*32*). Starch granular swelling is considered a property of amylopectin, and amylose acts as a diluent (*33*). As a result, a waxy starch is easier to be hydrolyzed by α-amylase after cooking. Pasting properties are normally used to study possible behaviors of starchy materials in food and feed production process and have been adapted to evaluate several feedstocks for ethanol production (*15*). RVA pasting graphs of samples tested in this study are shown in **Figure 4**.

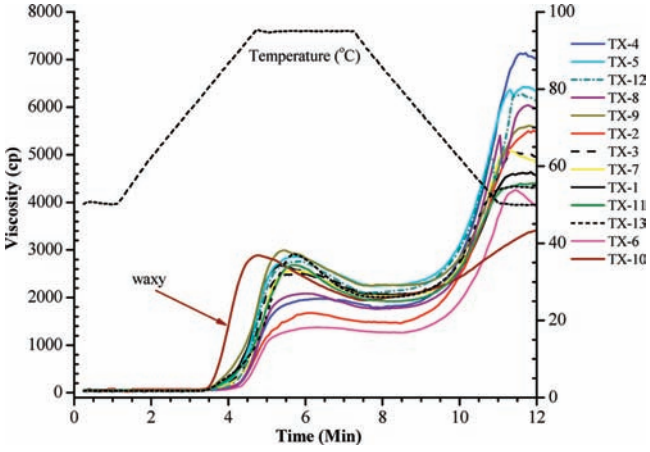

Figure 4. RVA pasting graphs of tested sorghum samples using the 13 min standard procedure.

TX10 had a typical waxy starch RVA curve with easy swelling and low final viscosity characteristics. Pasting graphs for TX2, TX4, and TX8 were characteristic of starches with higher-than-normal amylose content, which feature lower peak viscosity, no obvious set back, and higher-than-normal final viscosity. Pasting graphs of the other samples were similar to those of normal sorghum samples reported by Zhao et al. (*15*). It is interesting that lines previously identified as HD (TX1, TX3, TX5, TX7, and TX9) had earlier and higher peak viscosity at lower temperatures than normal sorghum lines (**Figure 4**). This could be due to the reduced or more porous protein highway matrix surrounding the starch granules in the HD lines. Sorghum kafirins have been shown to form a highly cross-linked matrix around starch granules during cooking (*34*), and this effect has been shown to be reduced in HD lines (*35*). Additionally, HD lines are noted for having a reduced γ-kafirin on the periphery of the kafirin protein bodies (*13*). Compared with other prolamin proteins, γ-kafirin is the most hydrophobic (*7*). Thus, a reduction in its level may improve liquid access to starch granules and, consequently, lower the gelatinization temperature of starch in the HD lines. However, no close correlation was found between RVA viscosities (peak, hold, and final) and fermentation efficiencies (R^2 = 0.109, 0.007, and 0.230 respectively). Results from the pasting test (easily pasting and low final viscosity; high final viscosity for high amylose samples) agree with those from other tests, including amylose analysis (**Table 1**) and DSC test (**Figure 2**) on sorghum flours, and WAXD analysis (**Table 2**) on sorghum starch samples.

The tested samples showed very diverse fermentation kinetics in the laboratory SSF dry-grind process (**Figure 5**). All 13 samples can be classified into one of three categories according to fermentation rate: fast, medium, or slow. The HD lines (TX1, TX3, TX7, and TX9) are in the fast fermentation group. Fermentation efficiencies for these HD lines at 24 h were 75−80% of their theoretical values (**Table 1**), which were 84−92% of their final efficiencies. The fermentation process for these HD lines was essentially completed in approximately 36 h. TX2 belongs to the slow fermentation category and needs about 60 h to complete the fermentation process. All other samples are in the medium group and can finish fermentation in 48 h. The difference in fermentation rate among sorghum samples could be explained by their initial FAN content. The fast-fermenting group (HD lines) had the highest initial FAN content (more than 100 mg/L), whereas the slowest fermenting sample (TX2) had the lowest initial FAN content (44.7 mg/L); all other samples had an initial FAN content intermediate to these values. The importance of FAN in ethanol fermentation has been well investigated (*36*, *37*). The FAN

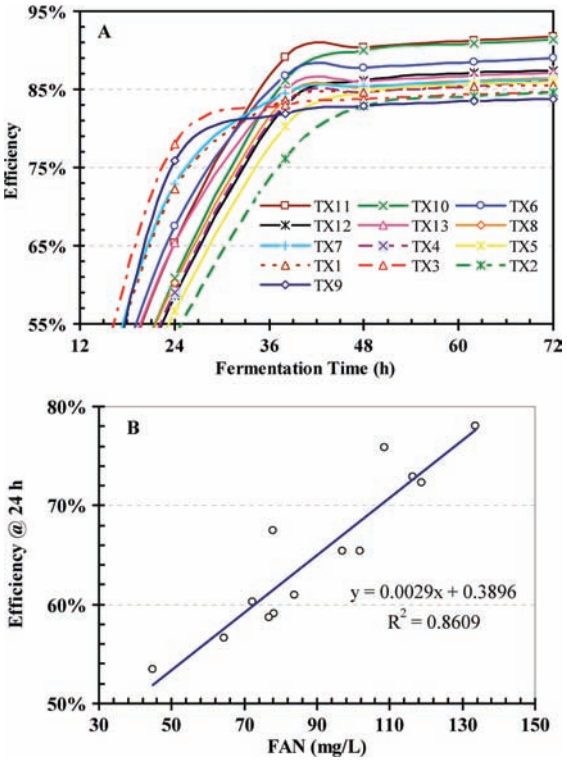

Figure 5. Kinetics of ethanol fermentation process (**A**) and correlation between FAN and 24 h fermentation efficiency (**B**).

content should be ~150−300 mg/L to ensure complete fermentation in 72 h. The FAN amount added in our dry-grind process was equivalent to ~100 mg/L. Because TX2 sorghum had an initial FAN content of 44.7 mg/L, the added FAN was barely sufficient to increase total FAN to the 150 mg/L minimal requirement for ethanol fermentation. It is not surprising that the fermentation rate of TX2 lagged behind those of the other samples. Because FAN amount in the finished mash is very important for yeast's propagation and its subsequent performance in ethanol fermentation, behavior of the fermentation system (in our case, the airlock-sealed, inoculated mashes in Erlenmeyer flasks) in the first 24 h could predicate fermentation rate. As indicated in **Figure 5B**, a good correlation (R^2 = 0.8609) exists between initial FAN amounts (mg/L, before addition of yeast extract) and fermentation efficiencies at 24 h. The HD lines have been well documented to have a higher pepsin protein digestibility than normal sorghum endosperms. This is thought to result from a diminished level of the cysteine-rich γ-kafirin content on the periphery of the protein bodies in HD lines, which conveys better access to the abundant α- and β-kafirins in the protein body interior (*13*). This feature may explain the increase in FAN content during the early stages of fermentation. On the basis of this feature, we may adjust the starting FAN amount to suit the intended purposes. If the purpose is to estimate potential ethanol yield of a feedstock, we can add 200 or even 300 mg/L of FAN to the finished mash, which will mask any possible differences in initial FAN from the tested sample. The fermentation process will complete in a shorter-than-normal time. If the purpose is to evaluate overall performance of feedstocks in the ethanol fermentation process (both fermentation rate and final ethanol yield), we usually add only ~100 mg/L of FAN to the finished mashes (there will be ~100 mg/L FAN in the finished mashes from 30 g dry mass samples per 100 mL). A brief look at the crude protein and FAN values in **Table 1** may give an impression of a proportional relationship between crude protein and initial FAN level in the finished mash. The actual correlation coefficient between crude protein and initial FAN is low (R^2 = 0.1298). Therefore, the proper way to use initial FAN level to predict fermentation rate is to sample the finished mash and determine FAN content.

Table 3. Chemical Compositions of DDGS from Tested Texas Sorghum Samples (%)[a]

entry	residual starch	crude protein	net protein[b]	crude fat	crude fiber	ash
TX 1	0.60	38.63 bc	26.1 abcd	8.58 de	6.68 abcd	5.92 c
TX 2	2.66	42.21 a	30.2 ab	4.24 f	4.75 f	4.75 d
TX 3	0.78	38.19 bc	28.3 abc	8.17 e	5.95 cdef	5.84 c
TX 4	1.38	33.67 def	22.1 d	10.1 bcd	5.72 def	6.78 abc
TX 5	1.54	36.01 cde	25.8 abcd	7.99 e	6.32 bcde	6.40 bc
TX 6	1.05	30.77 f	20.4 d	12.2 a	7.47 a	6.77 abc
TX 7	0.88	36.50 bcd	24.6 bcd	9.20 cde	6.17 cde	6.23 c
TX 8	0.68	33.13 ef	20.3 d	8.85 de	7.71 a	6.31 bc
TX 9	0.72	33.04 ef	24.6 bcd	10.9 abc	6.10 cde	6.58 abc
TX 10	0.75	39.59 ab	30.9 a	7.93 e	5.26 ef	5.96 c
TX 11	0.71	32.57 f	23.8 cd	11.5 ab	6.12 cde	6.63 abc
TX 12	0.86	31.31 f	22.5 d	11.5 ab	6.84 abc	7.34 ab
TX 13	0.65	30.70 f	22.4 d	11.3 ab	7.40 ab	7.47 a

[a] Means in a column with different letters differ ($P < 0.05$). [b] Sum of all the amino acid contents.

Another important product from ethanol production is DDGS, which accounts for approximately one-third of the original feedstock weight and up to 20% of an ethanol refinery's profit margin. DDGS currently is marketed as feed ingredient, and DDGS with higher feed quality will definitely help ethanol refineries sell their byproduct and realize their profit margin. Major components of DDGS from this study are listed in **Table 3**. Amino acid compositions of DDGS proteins are in **Table 4**. Most of the DDGS samples had residual starch content of less than 1% (db), which indicates that starches in those samples were well hydrolyzed into fermentable sugars and utilized during fermentation. However, a few DDGS samples (from TX2, TX4, and TX5) had residual starch content of more than 1%, especially the DDGS from TX2, which had a residual starch content of 2.66%. This indicates that starches in these samples were less efficiently utilized. Further study of the residual starches in these samples is needed to determine the possibility of more efficient utilization of starches for ethanol production.

Protein content is a critical quality parameter for DDGS. It is obvious that the crude protein values (N × 6.25) and net protein contents (sum of amino acid content) in **Table 3** do not agree with each other. The sum of amino acid contents of all DDGS samples was significantly lower than the crude protein values of the same samples. There may be several reasons for this difference. First, the crude protein content of DDGS obtained by multiplying the nitrogen content with a nitrogen factor of 6.25 normally overestimated the actual protein content of DDGS by 5−20% because the actual nitrogen-to-protein conversion factor for corn and sorghum is around 5.65 (*38*−*40*). Second, nonprotein nitrogen, which could account for 11−19% of nitrogen in whole sorghum meal, may be another major cause of the higher crude protein content of DDGS compared with the sum from amino acid analysis (*41*). Third, not all amino acids in the DDGS protein have been detected by the traditional acid hydrolysis procedures for amino acid analysis (*42*). Therefore, the sum of detected amino acids underestimates the protein content of a sample because of the total destruction of tryptophan and incomplete derivatization of amino acids (especially sulfur-containing amino acids and proline). Amino acid composition, especially lysine content, is another important quality parameter for feed-use DDGS. Normal

Table 4. Amino Acid Composition of DDGS from Tested Sorghum Samples (as Weight Percentage of Net Protein)[a]

aa[b]	TX1	TX2	TX3	TX4	TX5	TX6	TX7	TX8	TX9	TX10	TX11	TX12	TX13
							Essential						
His	2.6 a	2.5 a	2.3 a	2.4 a	2.6 a	2.4 a	2.2 a	3.1 a	3.1 a	2.9 a	3.2 a	3.2 a	3.1 a
Ile	5.4 a	5.9 a	5.6 a	5.2 a	4.8 a	5.4 a	5.6 a	5.8 a	4.8 a	5.0 a	5.1 a	5.1 a	4.8 a
Leu	15.6 ab	17.5 a	15.4 bc	15.1 bc	14.3 bc	14.8 bc	14.8 bc	14.7 bc	14.6 bc	15.1 bc	14.0 bc	13.3 c	13.7 bc
Lys	3.9 a	1.9 b	3.4 ab	2.8 ab	4.0 a	3.1 ab	3.0 ab	3.1 ab	3.1 ab	2.7 ab	3.6 ab	4.2 a	4.3 a
Met	2.1 a	1.9 a	2.0 a	3.7 a	2.0 a	3.6 a	3.6 a	5.5 a	1.9 a	1.7 a	2.1 a	2.1 a	1.9 a
Phe	6.7 a	7.3 a	7.0 a	7.0 a	6.3 a	6.9 a	6.9 a	7.1 a	6.4 a	6.6 a	6.3 a	6.2 a	6.3 a
Thr	4.3 ab	4.2 b	4.5 ab	4.6 ab	4.4 ab	4.4 ab	4.6 ab	4.7 a	4.2 b	4.2 b	4.4 ab	4.5 ab	4.5 ab
Val	5.9 a	6.1 a	6.1 a	4.1 a	5.3 a	4.4 a	4.5 a	3.1 a	5.3 a	5.1 a	5.7 a	5.7 a	5.4 a
							Nonessential						
Arg	5.5 abc	3.9 e	5.0 cd	5.3 abcd	5.7 abc	5.6 abc	5.1 bcd	5.7 abc	5.2 bcd	4.6 de	5.6 abc	6.1 a	6.0 ab
Ala	10.1 a	10.5 a	9.8 a	10.0 a	9.1 a	9.6 a	10.1 a	9.3 a	9.1 a	9.1 a	8.8 a	8.6 a	8.9 a
Asx	9.6 ab	8.1 b	9.6 ab	9.5 ab	8.8 ab	9.4 ab	10.1 a	9.4 ab	8.2 b	8.2 b	8.3 ab	8.4 ab	8.7 ab
Glx	7.3 a	7.3 a	7.3 a	7.5 a	11.3 a	8.6 a	6.7 a	7.3 a	13.7 a	14.2 a	12.2 a	12.1 a	11.0 a
Gly	3.8 ab	2.6 d	3.5 abcd	3.8 ab	3.5 acd	3.7 ab	3.9 a	3.5 abcd	2.9 bcd	2.7 cd	3.4 abcd	3.7 abc	3.6 abc
Pro	9.1 bc	10.7 a	9.1 bc	9.2 b	8.7 bcd	8.8 bc	9.1 bc	7.9 d	8.5 bcd	8.9 bc	8.4 bcd	8.2 cd	8.6 bcd
Ser	5.7 a	5.3 a	5.5 a	5.9 a	5.4 a	5.5 a	5.7 a	5.7 a	5.2 a	5.1 a	5.1 a	5.2 a	5.5 a
Tyr	2.4 a	4.2 a	3.9 a	4.1 a	3.7 a	3.9 a	4.0 a	4.2 a	3.9 a	3.9 a	3.8 a	3.5 a	3.6 a

[a] Means (averages of duplicate) in a row with different letters differ ($P < 0.05$). [b] Amino acid.

grain sorghum has a lysine content of 0.59% (*43*), common corn DDGS has an average lysine content of 0.85% (*44*), and high-lysine sorghum varieties could have 1.5−2.5% lysine (*45*). DDGS from this study had an average of 2.4% lysine. In contrast, the lysine content of DDGS from TX1, TX3, and TX5 was ∼3.5%, or roughly 50% higher than those from normal sorghums. FAN from yeast extract may partially contribute to the change in amino acid composition and increase in essential amino acid content.

Good fermentation characteristics (high yield, high efficiency, fast fermentation) and high-quality DDGS are breeding goals for high-performance sorghum hybrids for ethanol production. HD lines (TX1, TX3, TX5, TX7, and TX9) with modified endosperm protein matrix have several attributes desirable for ethanol production. Use of such sorghum hybrids will allow ethanol refineries to achieve higher profits by lowering processing cost (less energy and enzyme cost), enhancing production capacity (faster fermentation), and increasing ethanol yield (higher starch content and fermentation efficiency means more ethanol from the same amount of feedstock). Ideal sorghum lines for bioethanol production would be the ones with high starch content combined with HD and waxy endosperm traits.

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