e-Xtra*

Impact of Deficit Irrigation on Maize Physical and Chemical Properties and Ethanol Yield

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ABSTRACT

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The objective of this research was to study the effect of irrigation levels (five levels from 102 to 457 mm of water) on the physical and chemical properties and ethanol fermentation performance of maize. Twenty maize samples with two crop rotation systems, grain sorghum–maize and maize–maize, were harvested in 2011 and evaluated at the Kansas State University Southwest Research-Extension Center near Garden City, Kansas, under a semiarid climate. Results showed that maize kernel weight, density, and breakage susceptibility decreased as irrigation level decreased. Starch contents of maize samples grown under a low irrigation level were approximately 3.0% lower than those under a high irrigation level. Protein contents ranged from 9.24 to 11.30% and increased as irrigation level decreased. Maize flour thermal and rheological properties were analyzed by differential scanning calorimetry and the Micro Visco-Amylo-Graph-U device. Starch gelatinization temperature increased significantly as irrigation level decreased, whereas starch pasting viscosity decreased as irrigation level decreased. Free amino nitrogen (FAN) was significantly affected by irrigation level: it increased as irrigation decreased. Ethanol fermentation efficiency ranged from 90.96 to 92.48% and was positively correlated with FAN during the first 32 h of fermentation ($r^2 = 0.645$). Deficit irrigation had a negative impact on ethanol yield. The maize with lower irrigation yielded about 4.0% less ethanol (44.14 mL/100 g of maize) than maize with high irrigation (45.92 mL/100 g of maize). Residual starch contents in the distillers dried grains with solubles were in a range of 0.80–1.02%. In conclusion, deficit irrigation had a significant effect on physical properties, chemical composition, ethanol yield, and fermentation efficiency of maize.

Irrigated agriculture is the primary user of water resources globally and consumes 70–80% of total diverted water in the arid and semiarid zones (Fereres and Soriano 2007). Irrigated agriculture used more than 70% of the water withdrawn from the Earth's rivers (Heng 2002). Crop production is highly dependent on water availability, and any shortage has a significant impact on final yields (Kirda 2002; Tognetti et al 2006; Quiroga et al 2011); however, water is a finite resource for which competition is increasing among agricultural, industrial, and domestic sectors. Meeting increased demand for food production and food security with less water availability is a great challenge.

With reduced water resources available for agriculture, scientists and engineers have developed innovative technologies such as deficit irrigation programs aimed at increasing efficient use of irrigation water (Kirda 2002; Tognetti et al 2006; Fereres and Soriano 2007). Water deficits during a specific crop development period significantly affect crop yield (metric tons per hectare); therefore, the yield response to water stress has been studied extensively. Previous research reported that grain yields (metric tons per hectare) decreased as irrigation level decreased (Kirda et al 2005; Fereres and Soriano 2007; Ayana 2011). Pandey et al (2000) studied the effect of deficit irrigation and nitrogen on maize and found that grain yield reduction was proportional to duration of deficit irrigation. Because maize is an important irrigated crop, field research has been conducted on maize over years to study the relationship between irrigation and yields. Klocke et al (2007) studied yield and irrigation for maize in 1986–1998 in west central Nebraska and found that 90% of full irrigation grain yields could be gained by applying only 47% of full irrigation. Klocke et al (2011) conducted a field study of fully irrigated to deficit-irrigated maize in 2005–2009 in southwest Kansas and reported that yield variability increased as irrigation decreased, illustrating a greater income risk with less irrigation.

As water resources continue to decline, deficit irrigation is becoming an important strategy for minimizing agricultural water use. Limited or deficit irrigation may significantly affect not only crop yields but also grain quality and end uses; however, little attention has been paid to the effects on grain quality and end-use quality, such as in the area of ethanol production.

Maize is the number one crop produced in the United States in terms of land area and annual production. Maize is widely used for livestock feed, ethanol production, and food production. The U.S. Department of Agriculture reported in 2010 that feed and residual use accounted for 121,717 thousand metric tons of maize and industrial ethanol accounted for 127,533 thousand metric tons of maize, which was the number one use for maize in that year (U.S. Grains Council 2011). According to the World Agricultural Supply and Demand Estimates of the USDA (2012), 5 billion bushels of maize from the 2011–2012 crop year was used for ethanol and by-products. Maize grain qualities are important and greatly affect end-use product qualities. Some research has been done on grain sorghum to study the effect of irrigation on grain quality and ethanol yield (Wu et al 2008; Miller and Ottman 2010), but little research has been conducted on maize. The objective of this research was to study the effect of deficit irrigation on the physical and chemical properties and ethanol fermentation performance of maize.

MATERIALS AND METHODS

Cropping System and Irrigation Protocol

Twenty maize samples were grown in a five-year rotation of maize-maize-wheat-sorghum-sunflower (maize–maize) and sunflower-maize-maize-wheat-sorghum (GS–maize) starting in 2005

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*The ***e*-Xtra** logo stands for "electronic extra" and indicates that Figures 1–3 and 6–8 appear in color online.

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and continuing through 2011 (Table I). GS–maize and maize–maize samples treated with five irrigation levels (457, 356, 254, 178, and 102 mm of water) were evaluated for physical and chemical properties and ethanol fermentation performance. The prior year's irrigation treatment effects carried over to the same irrigation treatment in the following year. Each crop was present every year in five cropping blocks, which were replicated over the five years. The irrigation variable was achieved by increasing the number of days between irrigation events rather than applying a percentage of full irrigation during each irrigation event. The irrigation treatment protocol was designed to include operational constraints of commercial center-pivot irrigation systems in the Great Plains region, where pumping capacities limit the frequency of irrigation events. Cultural practices, hybrid selections, planting techniques, and fertilizer and herbicide applications were the same across irrigation treatments and followed the requirements of no-till management (Klocke et al 2011). This research was conducted at the Kansas State University Southwest Research-Extension Center near Garden City, Kansas. The climate is semiarid, with long-term average annual precipitation of 477 mm, mean summer growing season daytime high temperature of 29°C (30 year average May through August), open-pan evaporation (April through September) of 1,810 mm, and a frost-free period of 170 days. During the study, average annual precipitation was 495 mm.

Sample Preparation

All samples were screened with a Gamet sieve shaker (Dean Gamet Manufacturing, Minneapolis, MN, U.S.A.) with a 6.35 mm screen and were hand cleaned to remove large foreign materials. For ethanol fermentation, the cleaned samples were ground into fine flour by passing through a 0.5 mm screen on a UDY cyclone mill (UDY Corporation, Fort Collins, CO, U.S.A.).

Physical Properties of Maize Kernels

Maize density was determined with an air-comparison pycnometer (model MVP–1, Quantachrome Corporation, Syosset, NY, U.S.A.), as described by Pomeranz et al (1984). Kernel breakage susceptibility was tested with a Stein breakage tester (model CK2) following AACC International Approved Method 55-20.01. The 1,000-kernel weights were obtained from the kernel weight of 1,000 whole, sound kernels. Maize test weight was determined by AACCI Approved Method 55-10.01.

Microstructures of maize endosperm were examined with a Hitachi S-3500N scanning electron microscope (SEM) with an S-6542 absorbed electron detector (Hitachinaka, Ibaraki, Japan). Samples were coated with 4 nm of a 60% gold and 40% palladium mixture in a Denton vacuum chamber (Desk II, Moorestown, NJ, U.S.A.) before SEM examination. Images were taken from enlarged floury endosperm with 500× magnification.

Chemical Composition of Maize

Total starch was analyzed following AACCI Approved Method 76-13.01. Crude protein, fat, and ash were analyzed following AOAC approved methods 990.03, 920.39 (corresponding to AACCI Approved Method 30-20.01), and 942.05 (corresponding to AACCI Approved Method 08-03.01), respectively (AOAC International 1999), and crude fiber was analyzed with the A200 filter bug technique (Ankom Technology 2006). Free amino nitrogen (FAN) was determined through the European Brewery Convention method (EBC 1987) with modification. Around 150 mg of maize flour was mixed with 1.5 mL of deionized distilled water in a 2.5 mL microcentrifuge tube, vortexed five times in 10 min, and then centrifuged at 10,644 × *g* for 20 min. An aliquot of 1.0 mL of supernatant was diluted with 4.0 mL of distilled water; it then was ready for FAN analysis.

TABLE I
Crop Rotation Used in This Study[z]

Crop Year	GS–Maize	Maize–Maize
2005–2006	sunflower–maize	maize–maize
2006–2007	maize–maize	maize–wheat
2007–2008	maize–wheat	wheat–sorghum
2008–2009	wheat–sorghum	sorghum–sunflower
2009–2010	sorghum–sunflower	sunflower–maize
2010–2011	sunflower–maize	maize–maize

[z] Maize used in this study was the second crop listed for the 2010–2011 crop year. GS = grain sorghum.

Thermal Properties

Thermal properties were analyzed with a TA DSC Q200 instrument. Five samples with different irrigation levels were selected from each GS–maize and maize–maize samples. Maize samples were weighed accurately (≈5–8 mg) into stainless steel pans with a microbalance. Deionized distilled water was added carefully with a micropipette into the sample pan. The weight ratio of water to dry flour was 2:1. The pans were sealed and allowed to rest overnight at room temperature. An empty sealed pan was used as a reference. Samples were characterized in an inert environment that used nitrogen with a gas flow rate of 50 mL/min and were heated from 0 to 140°C at a heating rate of 10°C/min. Enthalpies are reported on a dry flour weight basis. Onset temperature (T_o), peak temperature (T_p), conclusion temperature (T_c), and enthalpy of gelatinization (ΔH_{gel}) were calculated.

Pasting Properties

A Brabender Micro Visco-Amylo-Graph-U device (model 803222, Brabender GmbH & Co. KG, Duisburg, Germany) was used to test pasting properties of maize flour. Five samples with different irrigation levels were selected from each GS–maize and maize–maize samples. Ten grams of flour (14% moisture content) and 105 g of distilled water were mixed in the testing bowl at room temperature; the slurry was heated from 30 to 95°C at the heating rate of 8.0°C/min; then the hot paste was held at 95°C for 5 min, cooled to 30°C at the cooling rate of 8.0°C/min, and held at 30°C for 1 min. The total process took 22 min 16 sec. The test speed of the stirrer was at 300 rpm, and the measurement sensitivity range was at 250 cmg.

Ethanol Fermentation

Whole maize flour with moisture content in a range of 6.48–7.67% (30.00 g, dry mass) was weighed into a clean 250 mL Erlenmeyer flask and was mixed with 100 mL of preheated (around 60–70°C) enzyme solution containing 0.1 g of KH_2PO_4 and 20 µL of Liquozyme α-amylase (Novozymes, Franklinton, NC, U.S.A.). Samples were evenly wetted and thoroughly suspended. Flasks were transferred to a 70°C rotary water-bath shaker operating at around 180 rpm. The temperature of the water bath was raised to 90°C for about 30 min and then lowered to 86°C and kept at that temperature for 60 min. Flasks were removed from the water-bath shaker. Material sticking on the inner surface of the flasks was pushed back into the mashes with a spatula. The inner surface and spatula were rinsed with 3–5 mL of distilled water. After the mashes cooled down to room temperature (≈25–30°C), the pH of the mashes was adjusted to around 4.2 with 2N HCl.

Before the simultaneous saccharification and fermentation process, the dry yeast was activated by adding 1.0 g of active dry yeast into 19 mL of preculture broth (containing 20 g of glucose, 5.0 g of peptone, 3.0 g of yeast extract, 1.0 g of KH_2PO_4, and 0.5 g of $MgSO_4{\cdot}7H_2O$ per liter) and incubated in an incubator at 38°C for around 30 min at 200 rpm.

An aliquot of 1.0 mL of activated yeast culture, 100 µL of Spirizyme glucoamylase (Novozymes), and 0.30 g of yeast extract were added into each flask. Flasks were sealed with an S-airlock filled with mineral oil. Fermentation was conducted at 30°C in an incubator shaker operating at 150 rpm for 72 h. The fermentation was monitored by measuring weight loss resulting from evolution of CO_2 during fermentation ($C_6H_{12}O_6 \rightarrow 2\ C_2H_6O + 2\ CO_2 \uparrow$) at 4, 8, 18, 24, 32, 44, 56, and 72 h of fermentation.

After 72 h of fermentation, finished mash was transferred to a 500 mL distillation flask. The Erlenmeyer flask was washed with 100 mL of distilled water. Two drops of antifoam agent (Antifoam 204, a mixture of organic polyether dispersions, Sigma-Aldrich, St. Louis, MO, U.S.A.) were added to the distillation flask before the flask was placed on the heating unit to prevent foaming during distillation. The distillates were collected into a 100 mL volumetric flask immersed in ice water. When the distillates in the volumetric flask approaching the 100 mL mark (≈99 mL), the volumetric flask was removed from the distillation unit. The distillates in the volumetric flask were equilibrated for a few hours in a 25°C water bath. The ethanol concentration was determined by HPLC following the method described by Wu et al (2006). Fermentation efficiencies were calculated as the actual ethanol yield divided by the theoretical ethanol yield. The theoretical ethanol yield was determined from the total starch contents in the samples, assuming 0.5672 g of ethanol from 1 g of starch (Thomas et al 1996).

TABLE II
Physical Properties of Maize Samples[y]

Irrigation Level[z]	1,000-Kernel Weight (g)	Breakage (%)	Test Weight (g/cm^3)	True Density (g/cm^3)
GS–maize				
1	329.85a	7.70a	0.805a	1.322a
2	318.85b	7.72a	0.803a	1.318b
3	228.65f	2.94d	0.790bc	1.304c
4	273.85d	6.22b	0.787c	1.300d
5	258.50e	5.96b	0.762d	1.292e
Maize–maize				
1	320.15ab	5.70bc	0.809a	1.321a
2	312.65b	6.06b	0.806a	1.318b
3	261.40e	3.47d	0.797b	1.308c
4	272.70d	5.13c	0.795b	1.307c
5	286.50c	5.20c	0.790bc	1.309c

[y] Means in the same column followed by different letters indicate significant differences ($P < 0.05$).

[z] Levels: 1 = high and 5 = low. GS = grain sorghum; see Table I for a full description of the crop rotations.

Statistical Analysis

A randomized complete block design (RCBD) was conducted with the experimental variable irrigation level (five levels) and the block variable crop rotation (two rotations). Because we were mainly interested in the variable irrigation level, the variable crop rotation was treated as the block or nuisance variable. Under this RCBD experimental plan, each factor combination was repeated twice. The statistical model for the RCBD can be written in the following model:

$$y_{ijk} = \mu + \tau_i + \beta_j + \in_{ij};\ i = 1,2,\ldots,5;\ j = 1,2;\ \text{and}\ k = 2$$

where y_{ij} is one of the response variables, τ_i is the effect of irrigation level i, β_j is the block effect of crop rotation j, $\in_{ij}$~$N(0,\sigma^2)$ is the random error term with a constant variance σ^2, and k is the two replicates of each sample. The analysis of variance was done with Minitab statistical software version 16 (Minitab, State College, PA, U.S.A.). Pearson correlation coefficients for the relationships between all response variables were also calculated with the same software.

RESULTS AND DISCUSSION

Effects on Physical Properties and Chemical Composition of Maize Samples

Weights of 1,000 kernels ranged from 228.65 to 329.85 g among GS–maize samples and from 261.40 to 320.15 g among maize–maize samples (Table II). Maize samples treated with low irrigation levels showed significantly lower 1,000-kernel weight,

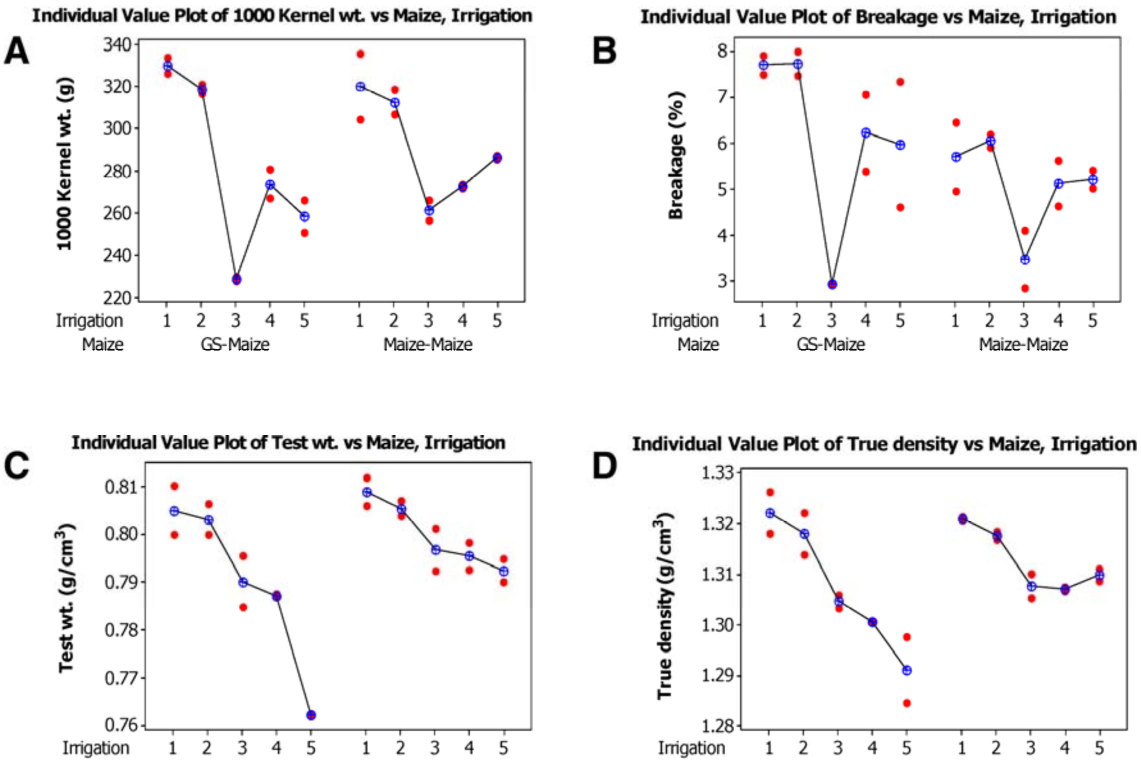

Fig. 1. Physical properties for maize grown in different rotations and irrigation levels (two-way ANOVA; $P < 0.05$). **A,** 1,000-kernel weight; **B,** breakage; **C,** test weight; and **D,** true density (● = two observations; ⊕ = mean value). Irrigation level: 1 = highest and 5 = lowest. GS = grain sorghum; see Table I for a full description of the crop rotations.

whereas the lowest 1,000-kernel weight was found in the maize treatment with the medium irrigation level (Fig. 1A). Breakage susceptibility was in the range of 2.94 to 7.72% among GS–maize samples and 3.47 to 6.06% among maize–maize samples (Table II). Maize with high irrigation level had the highest breakage susceptibility (Fig. 1B), which agreed with previous studies in maize (Bauer and Carter 1986; Kniep and Mason 1989). Kernel breakage susceptibility was based on the corneous-to-floury endosperm ratios. Typically, the ratio of corneous-to-floury endosperm is about 2 to 1 in dent maize (Wolf et al 1952). Maize with low amounts of corneous endosperm appears to be soft and easily damaged during handling (Paulsen et al 1983). Grain grown under drought conditions would have higher kernel hardness (Taylor et al 1997; Weightman et al 2008), meaning higher corneous-to-floury endosperm ratios, which tend to be more resistant to breakage. Maize's 1,000-kernel weight was significantly ($P < 0.0001$) correlated with breakage susceptibility (Table III), which is opposite the results reported by Peplinski et al (1992), that maize with low breakage susceptibility had the highest 100-kernel weight, and Pomeranz et al (1986), that little effect of kernel weight was found on breakage susceptibility. This difference is probably because the previous studies used different maize varieties. Test weight ranged from 0.762 to 0.805 g/cm^3 among GS–maize samples and from 0.790 to 0.809 g/cm^3 among maize–maize samples (Table II). All the maize test weights fell into a high-test-weight category based on the results described by Paulsen and Hill (1985) and Peplinski et al (1992), which is higher than U.S. no. 1 grade maize (0.72 g/cm^3). Maize samples treated with low irrigation levels had significantly lower test weight than those treated with high irrigation levels (Fig. 1C), which was in agreement with results reported by Griess et al (2010) for sorghum grain. True density of maize samples decreased as irrigation level decreased (Fig. 1D), which was in contrast with research on sorghum (Griess et al 2010). No relationship between kernel density and breakage susceptibility was found, which agreed with some previous studies (Bauer and Carter 1986; Kniep and Mason 1989). Crop rotation had a significant effect on maize test weight and true density (Fig. 1C and D). For maize–maize rotation, the grain test weight and true density did not keep decreasing, as was the trend of GS–maize rotation, when irrigation level decreased from level 3 to levels 4 and 5. Kernel weight ($P < 0.001$) and density ($P < 0.0001$) were positively correlated with starch contents but strongly and negatively ($P < 0.0001$) correlated with protein contents (Table III). Kernel test weight and true density were positively associated with grain yield (Kaye et al 2007). Ethanol yield was greatly affected by kernel test weight and true density ($P < 0.0001$) (Table III), which agreed with the effects of irrigation level on ethanol yield.

Starch contents of maize samples grown under a low irrigation level were approximately 3.0% lower than those under a high irrigation level (Table IV and Fig. 2A). Griess et al (2010) reported similar results for grain sorghum, in which starch concentration under irrigated conditions was significantly higher than under dryland conditions. Figure 3 shows a strong linear relationship between total starch contents and ethanol yield ($r^2 = 0.86$, $P < 0.0001$), which agrees with previous research reported by Wu et al (2008), Lacerenza et al (2008), and Yan et al (2011). Starch content was negatively correlated with fermentation efficiency, but the linear relationship was not strong ($r^2 = 0.46$). Protein contents ranged from 9.24 to 11.30% among GS–maize samples and from 9.59 to 11.02% among maize–maize samples, in which the low irrigation level resulted in the highest protein content and the high irrigation level resulted in the lowest protein content (Table IV and Fig. 2B). The grain protein content was expected to be higher in the most droughtlike conditions (Guttieri et al 2000; Weightman et al 2008). Daniel and Triboï (2002) reported that an increase in temperature as well as a drought after anthesis induced

TABLE III
Pearson Correlation Coefficients Among Evaluated Parameters for 20 Maize Samples[z]

Parameter	1,000-Kernel Weight	Test Weight	True Density	Breakage	Total Starch	Crude Protein	Ethanol Yield	Efficiency
1,000-kernel weight	1.000	…	…	…	…	…	…	…
Test weight	0.709***	1.000	…	…	…	…	…	…
True density	0.785***	0.959***	1.000	…	…	…	…	…
Breakage	0.719***	0.134	0.270	1.000	…	…	…	…
Total starch	0.688**	0.762***	0.820***	0.345	1.000	…	…	…
Crude protein	–0.758***	–0.725***	–0.843***	–0.441	–0.889***	1.000	…	…
Ethanol yield	0.641*	0.772***	0.845***	0.216	0.946***	–0.925***	1.000	…
Efficiency	–0.425	–0.406	–0.367	–0.341	–0.618*	0.295	–0.375	1.000

[z] *, **, and *** indicate $P < 0.05$, 0.001, and 0.0001, respectively.

TABLE IV
Chemical Composition, Ethanol Yield, and Fermentation Efficiency of Maize Samples[x]

Irrigation Level[y]	Chemical Composition (%, db)					FAN (mg/L)	Ethanol Yield (mL)[z]	Fermentation Efficiency (%)
	Total Starch	Crude Protein	Crude Fat	Crude Fiber	Ash			
GS–maize								
1	69.45a	9.24d	3.42a	1.40a	1.26a	36.30e	45.86a	91.86ab
2	70.02a	9.35d	3.49a	1.42a	1.27a	36.33e	45.89a	91.18b
3	68.03bc	10.49b	3.24a	1.45a	1.36a	38.50de	45.23bc	92.48a
4	66.93d	11.30a	3.44a	1.45a	1.43a	40.89cd	44.22d	91.91b
5	66.46d	11.20a	3.26a	1.42a	1.47a	45.72b	44.14d	92.11ab
Maize–maize								
1	69.98a	9.59c	3.34a	1.45a	1.36a	39.20cd	45.76ab	90.96bc
2	69.82a	9.76c	3.31a	1.38a	1.28a	38.56d	45.92a	91.49b
3	68.66b	10.70b	3.42a	1.33a	1.38a	40.35c	45.04c	91.26b
4	68.12c	11.02a	3.40a	1.40a	1.42a	45.12b	44.78c	91.44b
5	67.38d	10.99a	3.40a	1.49a	1.42a	47.08a	44.41d	91.68b

[x] Means in the same column followed by different letters indicate significant differences ($P < 0.05$). FAN = free amino nitrogen.
[y] Levels: 1 = high and 5 = low. GS = grain sorghum; see Table I for a full description of the crop rotations.
[z] Yield in milliliters per 100 g of maize.

an increase in the percentage of nitrogen from 1.78 to 2.6% in wheat grain. Protein content in the grain from the maize–maize rotation was not significantly different than the GS–maize rotation (Fig. 2B). Bryant et al (2009) reported that rice grain grown in continuous rice rotation had lower protein content than that in rice-soybean rotation. Protein content was negatively correlated with ethanol yield ($P < 0.0001$) (Table III). The reason could be that the grain kernels with higher protein content had lower accessibility of hydrolyzing enzymes to starch in the ground meal during mashing and fermentation processes, and some small starch granules may be embedded in the protein matrix (Wu et al 2008). Samples with high starch content and low protein content are a better choice for fuel ethanol production. Higher starch means higher ethanol yield, better processing efficiency, and less leftover residues after fermentation (Wu et al 2008). Starch content was positively correlated to grain yield (Griess et al 2010), whereas protein content was negatively correlated to grain yield (Calderón-Chinchilla et al 2008). Crude fat, fiber, and ash contents were 3.24–3.49, 1.33–1.49, and 1.26–1.47%, respectively (Table IV). No significant differences were found for ash, crude fat, and fiber among samples under different irrigation levels.

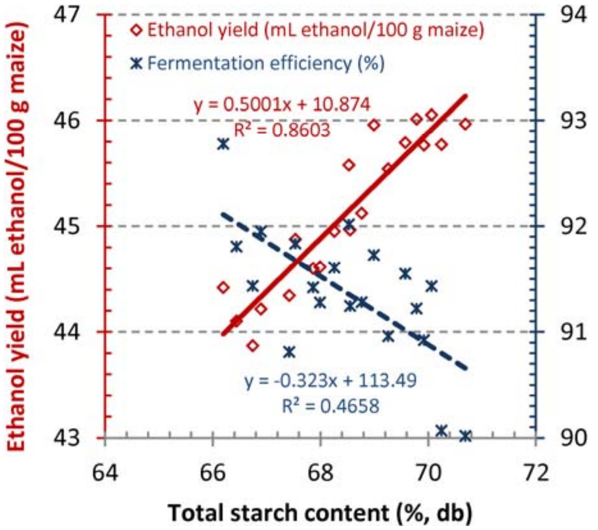

Fig. 3. Relationship between starch content of maize samples and fermentation efficiency and ethanol yield.

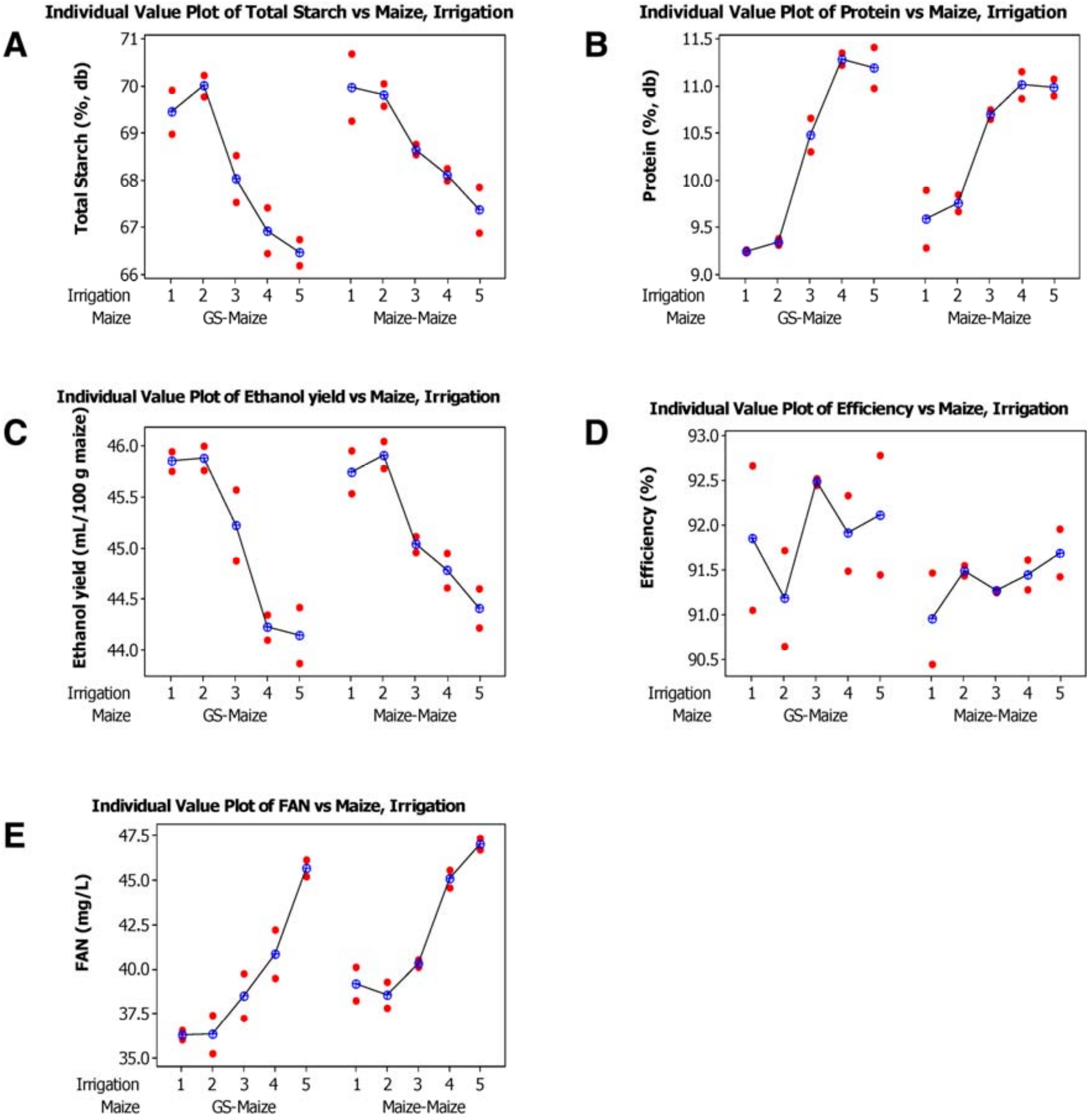

Fig. 2. Chemical composition, ethanol yield, and fermentation efficiency for maize grown in different rotations and irrigation levels (two-way ANOVA; $P < 0.05$). **A,** Total starch content; **B,** protein content; **C,** ethanol yield; **D,** fermentation efficiency; and **E,** free amino nitrogen (FAN) (● = two observations; ⊕ = mean value). Irrigation level 1 = highest and 5 = lowest. GS = grain sorghum; see Table I for a full description of the crop rotations.

The SEM images from GS–maize endosperm showed that the starch granule size of the samples with high irrigation levels was bigger than samples from low irrigation levels (Fig. 4). This result was expected, because the kernel of maize samples treated with low irrigation levels was smaller and harder than that of maize samples treated with high irrigation levels; thus, the structure of endosperm was much denser and had less space in between. The SEM image of GS–maize endosperm with irrigation level 2 (sample 217) showed more protein bodies on the surface of starch granules compared with other samples. Those protein bodies may cross-link and enmesh starch granules during mashing and block starch from exposure to hydrolyzing enzymes, which could be the reason that the fermentation efficiency of GS–maize sample 217 was significantly lower than other GS–maize samples (Table IV). Wu et al (2008) found a similar relationship in sorghum samples.

Effects on Starch Thermal and Pasting Properties

The transition temperatures (T_o, T_p, and T_c) and enthalpies of gelatinization (ΔH_{gel}) of the maize samples were determined by DSC. T_o, T_p, and T_c of GS–maize samples were 70.6–73.1, 76.8–

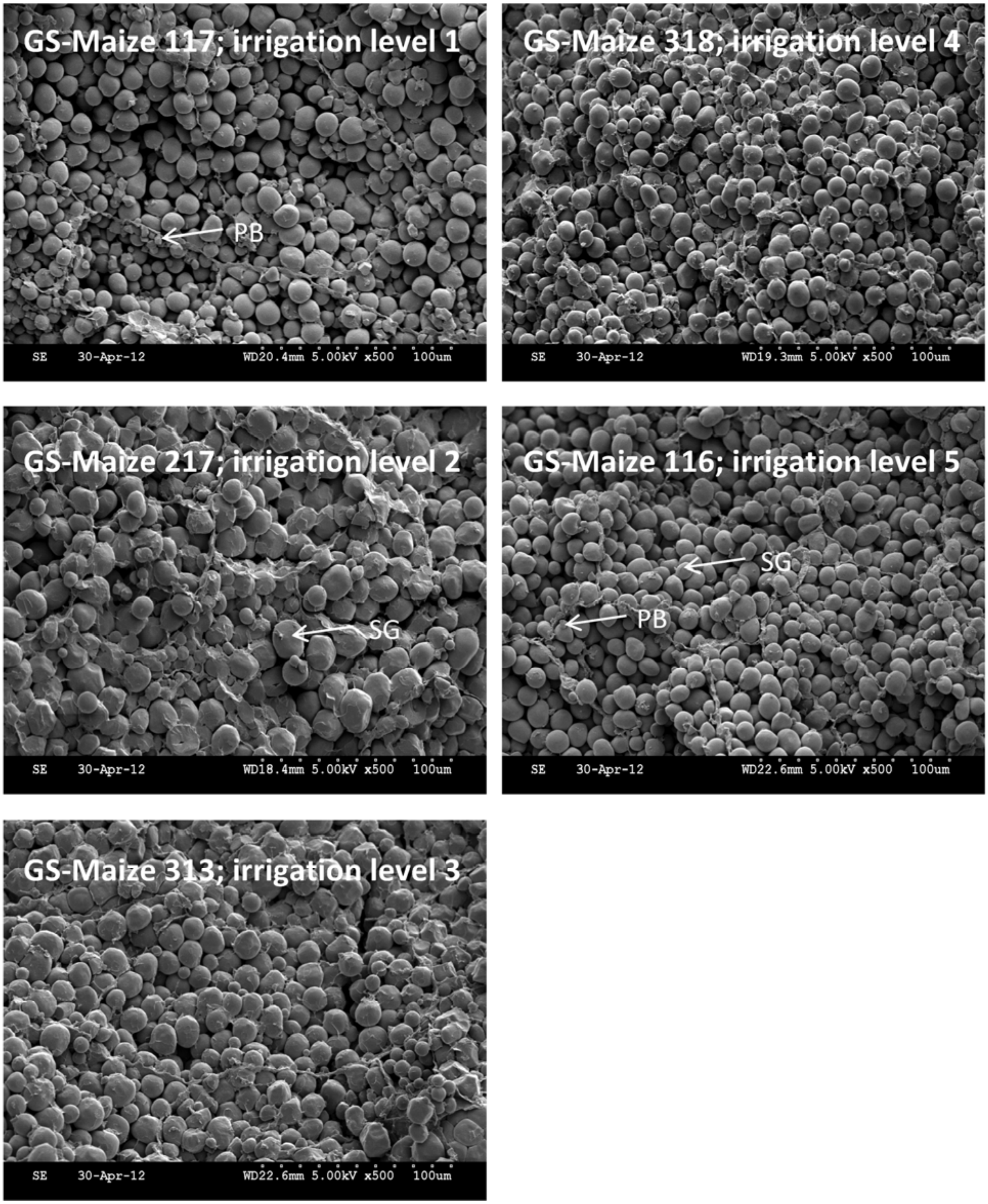

Fig. 4. Scanning electron microscopy images of starch granules and protein matrix in maize endosperm from grain sorghum (GS)–maize crop rotation kernels (irrigation level: 1 = highest and 5 = lowest). SG = starch granule, and PB = protein body.

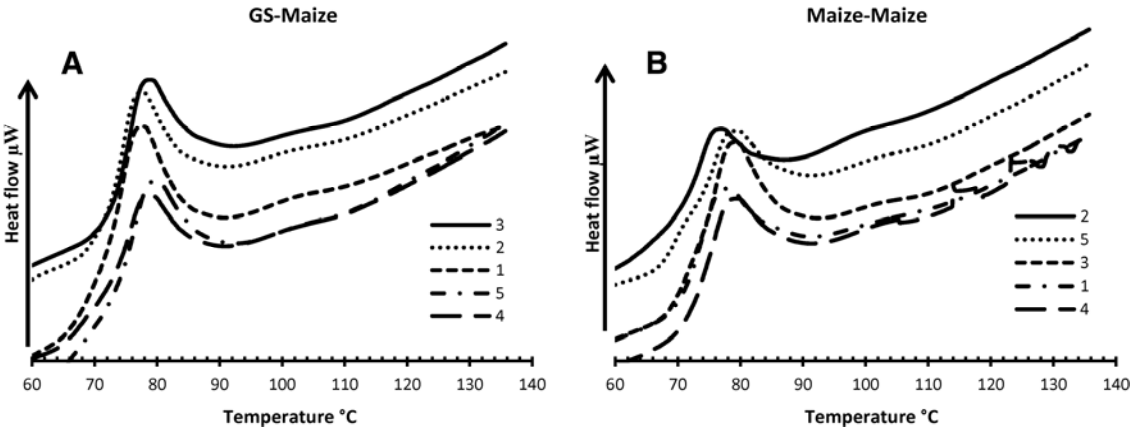

Fig. 5. A, DSC curve for grain sorghum (GS)–maize crop rotation samples from five different irrigation levels. **B,** DSC curve for maize–maize crop rotation samples from five different irrigation levels. Irrigation level: 1 = highest and 5 = lowest.

TABLE V
Starch Pasting Properties of Maize Samples[y]

		Torque (BU)					
Irrigation Level[z]	**BOG (°C)**	**Peak**	**Start of Holding**	**Start of Cooling**	**End of Cooling**	**Breakdown**	**Setback**
GS–maize							
1	73.3e	162c	160b	158b	417a	4.5a	256.0b
2	73.2e	175a	170a	170a	443a	4.5a	272.5a
3	76.0b	127d	119c	125c	326b	2.0c	201.0e
4	76.0b	111f	102d	110d	287c	1.0d	177.0f
5	76.5a	112f	108d	110d	287c	1.5cd	176.5f
Maize–maize							
1	73.2e	169b	164b	165a	426a	4.0a	266.0ab
2	72.8e	159c	152b	156b	396a	3.0a	240.0c
3	75.4c	133d	124c	132c	348b	1.0d	215.5d
4	75.9b	122e	115c	120cd	322b	1.5cd	202.0e
5	75.1d	125e	120c	123cd	327b	2.0c	203.5e

[y] Means in the same column followed by different letters indicate significant differences ($P < 0.05$). BOG = temperature at which beginning of gelatinization occurs; GS = grain sorghum; see Table I for a full description of the crop rotations.

[z] Levels: 1 = high and 5 = low.

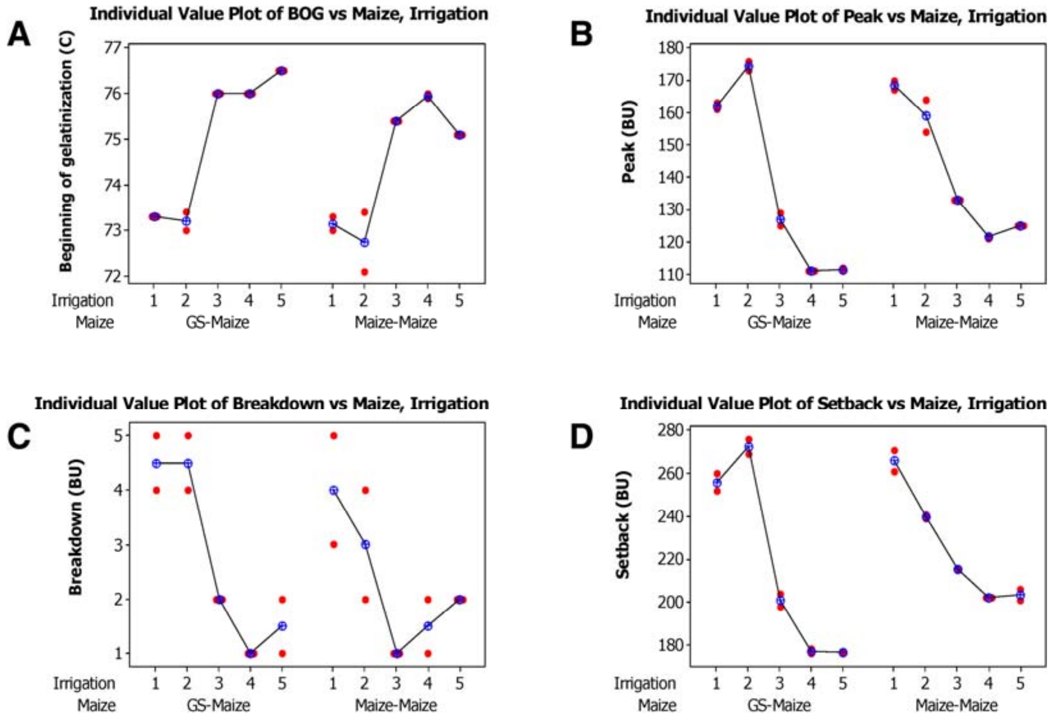

Fig. 6. Starch pasting properties for maize grown in different rotations and irrigation levels (two-way ANOVA; $P < 0.05$). **A,** Beginning of gelatinization (BOG); **B,** peak viscosity; **C,** breakdown viscosity; and **D,** setback viscosity (● = two observations; ⊕ = mean value). Irrigation level: 1 = highest and 5 = lowest. GS = grain sorghum; see Table I for a full description of the crop rotations.

78.7, and 84.8–88.2°C, respectively, and for maize–maize samples they were 70.8–72.4, 75.9–78.3, and 83.8–87.5°C, respectively. The DSC results showed that starch gelatinization onset, peak, and conclusion temperatures of the maize samples treated with low irrigation levels were significantly higher than in samples treated with high irrigation levels (Fig. 5). High transition temperatures resulted from a high degree of crystallinity, which made the starch granules more resistant to starch gelatinization and required more energy to initiate it (Barichello et al 1990). Lower gelatinization temperature means easier enzymatic hydrolysis and higher fermentation efficiency (Wu et al 2008). Amylases are easily inactivated by heat; if the temperature increases, the enzyme may be inactivated, which leaves the starch hydrolysis process incomplete. Singh et al (2008) reported the relationship between starch granule sizes and transition temperatures in wheat grain. More research is needed to investigate the effect of maize starch granule sizes on the transition temperatures. ΔH_{gel} of GS–maize samples ranged from 8.11 to 11.32 J/g, and maize–maize samples ranged from 6.03 to 8.29 J/g. ΔH_{gel} from all maize samples increased as irrigation level decreased. ΔH_{gel} reflected the loss of double-helical order (Cooke and Gidley 1992), and the variations in ΔH_{gel} represented differences in bonding forces between the double helices that form the amylopectin crystallites (McPherson and Jane 1999). ΔH_{gel} was observed to be positively correlated with T_o, T_p, and T_c, which agreed with results reported by Sandhu and Singh (2007).

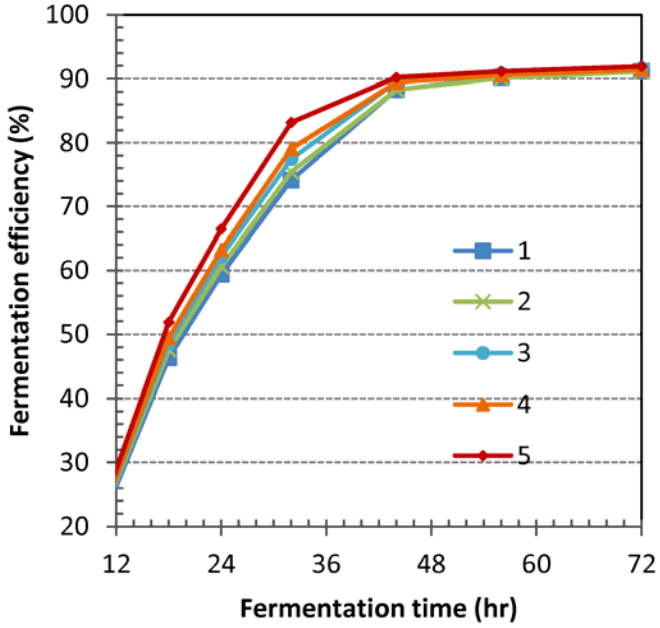

Fig. 7. Relationship between fermentation efficiency and fermentation time among 20 maize samples from five different irrigation levels (1 = highest and 5 = lowest).

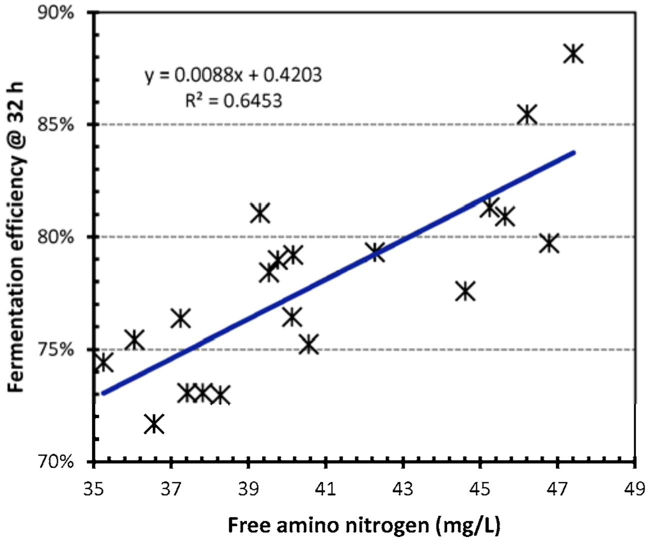

Fig. 8. Linear correlation between free amino nitrogen content (mg/L) in 20 original maize samples and fermentation efficiency after 32 h of fermentation.

Micro Visco-Amylo-Graph-U starch pasting profiles of maize samples treated with low irrigation levels showed a higher pasting temperature, lower peak viscosity, and lower setback viscosity than maize samples treated with high irrigation levels (Table V and Fig. 6). Taylor et al (1997) reported similar results that sorghums grown under supplementary irrigation had higher peak pasting viscosity and setback viscosity than those produced under rainfed conditions. The beginning of pasting temperature is defined by the initial increase in viscosity and is higher than the gelatinization onset temperature, meaning the starch particles are gelatinized before the viscosity begins to increase (Liang and King 2003). Setback is a process that occurs during cooling in which the starch molecules start to reorder and subsequently form a gel structure. Lower setback values are indicative of slower rates of starch retrogradation (Varavinit et al 2003). Starch peak viscosity and setback viscosity were negatively ($P < 0.001$) correlated with fermentation efficiency.

Effects on Fermentation Efficiency and Ethanol Yield

Deficit irrigation had a negative impact on ethanol yield (Fig. 2C). The maize with low irrigation yielded about 4.0% less ethanol (44.14 mL/100 g of maize) than the maize with higher irrigation (45.92 mL/100 g of maize) (Table IV). Current technologies allow for 44.88 mL/100 g of maize (2.8 gallons/bushel) of ethanol from maize by the dry-grind process (Bothast and Schlicher 2005). The final fermentation efficiency (after a 72 h process) of GS–maize samples ranged from 91.18 to 92.48%, and maize–maize samples ranged from 90.96 to 91.68% (Table IV). No significant differences were found for fermentation efficiency among different samples (Fig. 2D). By monitoring the changes in conversion efficiency through the whole 72 h fermentation process, the dynamics in the process of reaching their final efficiencies were quite different (Fig. 7). Maize samples from the low irrigation level (low starch contents) had obviously higher conversion efficiency (maximum of 10% higher) than samples from the high

TABLE VI
Chemical Composition of Distillers Dried Grain with Solubles from Maize Samples (%, db)[y]

Irrigation Level[z]	Total Starch	Crude Protein	Crude Fat	Crude Fiber	Ash
	Chemical Composition (%, db)				
GS–maize					
1	0.96a	30.36d	9.73a	3.94a	5.16a
2	0.94a	30.44d	10.09a	4.40a	5.01a
3	1.01a	32.86b	9.69a	4.78a	4.93a
4	0.81a	33.78a	9.32a	3.91a	4.70a
5	0.80a	32.98b	9.10a	4.40a	5.27a
Maize–maize					
1	0.95a	31.02cd	9.88a	4.76a	4.96a
2	0.92a	31.20c	9.62a	3.90a	4.80a
3	1.02a	33.08b	9.48a	3.90a	4.62a
4	0.88a	33.80a	9.48a	3.92a	4.89a
5	0.85a	32.74b	9.18a	4.16a	5.00a

[y] Means in the same column followed by different letters indicate significant differences ($P < 0.05$).

[z] Levels: 1 = high and 5 = low. GS = grain sorghum; see Table I for a full description of the crop rotations.

irrigation level (high starch contents), which we observed during the first 36 h (Fig. 7). Samples with lower starch contents would have higher conversion efficiency if the same amount of yeast were put into the fermentation broths and the inoculated yeast converted sugar to ethanol at a similar rate. Another important factor that may affect the fermentation efficiency is FAN content. This research determined that initial FAN content was significantly affected by irrigation level; it increased as irrigation level decreased (Fig. 2E). A positive linear relationship ($r^2 = 0.645$) was found between the initial FAN contents and fermentation efficiency after 32 h of fermentation (Fig. 8), whereas no linear relationship was found between the initial FAN contents and fermentation efficiency after 72 h. Initial FAN content of samples is a crucial nutrient to yeast cell growth at the early stage of the fermentation process. The higher FAN contents resulted in a faster fermentation process, which was similar to several previous studies on sorghum samples (Yan et al 2009, 2010, 2011; Wu et al 2010) and wheat samples (Casey et al 1984). Sufficient yeast nutrients were put in the tested samples that almost all the sugars were converted into ethanol; therefore, the final fermentation efficiency among samples was close.

Chemical Composition of Distillers Dried Grains with Solubles

Distillers dried grains with solubles (DDGS) is a by-product of the ethanol production process and is a high-nutrient feed for the livestock industry. Protein, fat, and fiber are the main remaining nutrients used for livestock feed. The nutritional composition is critical to farmers because it determines the sale price of DDGS. Table VI shows the major components of maize DDGS. Residual starch contents were similar between high-irrigation-level and low-irrigation-level maize samples. As discussed earlier, it was because sufficient yeast nutrients had been put in the tested samples and almost all the sugars were converted to ethanol; therefore, the final conversion efficiency was not significantly different. DDGS with low irrigation levels had higher crude protein content, which means better quality for livestock feed uses. There were no differences in crude fat, fiber, and ash contents among all DDGS samples.

CONCLUSIONS

Deficit irrigation had significant effects on grain physical properties, chemical composition, and ethanol yield. Maize kernel weight, density, and breakage susceptibility decreased as irrigation level decreased. Starch contents in maize samples at the low irrigation level were lower than those at the high irrigation level and gave the lowest ethanol yield. The FAN content increased as irrigation level decreased and greatly affected fermentation efficiency at the early stage (the first 36 h), which had a positive linear correlation with 32 h fermentation efficiency. The starch granule size was affected by irrigation level, and the starch–protein matrix in the grain may affect fermentation efficiency. Crop rotation had significant effects on grain test weight and true density. More research needs to be done on the effect of crop rotation on grain qualities in the future.

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