Comparison of Waxy vs. Nonwaxy Wheats in Fuel Ethanol Fermentation

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ABSTRACT

Fermentation performance of eight waxy, seven nonwaxy soft, and 15 nonwaxy hard wheat cultivars was compared in a laboratory dry-grind procedure. With nitrogen supplements in the mash, the range of ethanol yields was 368–447 L/ton. Nonwaxy soft wheat had an average ethanol yield of 433 L/ton, higher than nonwaxy hard and waxy wheat. Conversion efficiencies were 91.3–96.2%. Despite having higher levels of free sugars in grain, waxy wheat had higher conversion efficiency than nonwaxy wheat. Although there was huge variation in the protein content between nonwaxy hard and soft wheat, no difference in conversion efficiency was observed. Waxy cultivars had extremely low peak viscosity during liquefaction. Novel mashing properties of waxy cultivars were related to unique pasting properties of starch granules. With nitrogen supplementation, waxy wheat had a faster fermentation rate than nonwaxy wheat. Fermentation rates for waxy cultivars without nitrogen supplementation and nonwaxy cultivars with nitrogen supplementation were comparable. Ethanol yield was highly related to both total starch and protein content, but total starch was a better predictor of ethanol yield. There were strong negative relationships between total starch content of grain and both yield and protein content of distillers dried grains with solubles (DDGS).

Use of ethanol as a fuel additive has grown over the past few years and this growth is expected to continue. In 2007, a record 6.5 billion gallons of ethanol was produced from 139 biorefineries located in 21 states across the United States. This exceeded the previous year's record by >32% (RFA 2008). Once all new biorefinery construction currently underway is complete, the national ethanol production is expected to grow to 13.4 billion gal/year (RFA 2008). Corn constitutes ≈97.5% of the feedstock for ethanol production in the United States. The other 2.5% consists primarily of grain sorghum, along with some barley, wheat, cheese whey, and potatoes. In 2007, conversion to ethanol accounted for 2.3 billion bushels of corn, ≈18% of the total U.S. corn crop production of 13.1 billion bushels (USDA 2008). The new Renewable Fuels Standard schedule in law H.R. 6, the "Energy Independence and Security Act of 2007", established a goal of increasing annual use of renewable and alternative fuels to 36 billion gallons by 2022. This is more than a fivefold increase from 2007 levels and would require much more corn than currently produced in the United States. This legislation requires that 21 billion gallons of the Standard must come from advanced biofuels, including cellulosic ethanol. Five billion gallons can come from starch sources other than corn. Likely, many sources of biomass and plant species will be selected for ecological fit as well as production and processing capability.

Because starch is the principal component of corn, other cereal grains including sorghum, wheat, millet, rice, and barley are obvious ethanol feedstocks in areas where corn production is limited. Wheat (*Triticum aestivum* L.) is the most widely cultivated cereal and a staple food for the world's population. Wheat flour is used in composition of breads, noodles, cereals, and many other food products. In Canada and Europe, wheat has been used to produce potable and fuel ethanol (Thomas and Ingledew 1990, 1992; Sosulski and Sosulski 1994; Thomas et al 1996; Loyce and Meynard 1997; Wang et al 1997; Freeze and Peters 1999; Swanston et al 2005, 2007; Agu et al 2006; Kindreda et al 2008). Because bioethanol is used world-wide as a renewable component of fuels, wheat is considered a main energy crop in Europe (Loyce et al 2002; Smith et al 2006; Rigler et al 2007). Wheat cultivars producing "feed class" grain with high starch content, and thus relatively low protein content, have been highlighted as the preferred ideotype for ethanol production (Sosulski and Sosulski 1994; Smith et al 2006; Kindreda et al 2008). Smith et al (2006) concluded that ethanol yield from the best cultivars grown under ideal United Kingdom conditions is likely to exceed 4,000 L/ha, which is comparable to corn-based biofuel production systems in the United States.

Wheat markets in the United States traditionally have been for milling (principally for the baking industry) and export. Most of the research effort with respect to wheat quality traits has been primarily targeted toward protein quantity, composition, structure, genetic basis, and functionality desirable for food utilization. Few U.S. ethanol plants currently use wheat as a feedstock (RFA 2008). In addition, existing ethanol plants that use wheat as a feedstock use a wet-grind process to produce gluten, and the isolated starch can then be used for ethanol production if desired. Thus, high starch content of the incoming wheat is not critical because ethanol is only one of a number of valuable products. The starch content of U.S. wheat cultivars has been reported as 63–72% (Lineback and Rasper 1988). The opportunity to use wheat as a feedstock affords a choice to ethanol facilities under construction in some agricultural areas outside the major corn growing regions where climatic and economic conditions are favorable for wheat production. The great advantage of, and motivation for, using wheat in the fuel ethanol industry is the opportunity to choose high-yielding, locally adapted grains, which will result in reduced transport costs and promote other local benefits (Agu et al 2006). In addition, poor quality (e.g., weather-damaged or immature) wheat grain less suitable for either human or livestock consumption may be used for ethanol production. To date, there has been little effort in breeding wheat cultivars specifically for fuel ethanol production. Compared with corn, factors affecting ethanol yield for wheat are not well understood. Little information is available on fermentation performance of wheat cultivars in a dry-grind process. Lacererenza et al (2008) recently reported that different classes of spring wheat are equally suitable for ethanol production in terms of conversion efficiency and ethanol yield and they pointed out that the traditional selection for milling and baking quality is not consistent with maximal ethanol yield per hectare.

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Waxy wheat was first developed in Japan ≈15 years ago (Nakamura et al 1995). Since then, breeders around the world have developed their own waxy wheat cultivars. Though not yet commercially available, wheat breeders in the United States have been increasingly working on the development of waxy wheat cultivars (Graybosch 1998; Morris and Konzakb 2001; Graybosch et al 2003, 2004; Morris and King 2007). Many patents have disclosed possible applications for waxy wheat (Hoshino et al 2000a,b; Wilson et al 2006; Messager and Despre 2008). Reddy and Seib (2000) used it as a substitute for waxy maize in the production of modified starches. Various laboratory-scale investigations have shown that waxy wheat may be used as a source of blending flour to improve shelf-life stability, processing quality, or palatability of baked, sheeted, and extruded wheat products. However, waxy wheat flour cannot be used alone or added excessively to normal wheat flour because of inferior functional properties (sticky, lumpy, poor machinability, open pore structure, unacceptable appearance) (Lee et al 2001; Morita et al 2002a,b; Baik and Lee 2003; Guo et al 2003a,b; Hayakawa et al 2004; Takata et al 2005; Sahlstrom et al 2006). These results make waxy wheat unattractive as a primary food ingredient. Employing waxy wheat as a feedstock for fuel ethanol production has been recommended (Smith et al 2006; Lacererenza et al 2008) but little is known about the fermentation performance of waxy wheat. Previous studies in our laboratory with existing corn hybrids with various amylose contents and corn media formulated by blending commercial corn starches with different amylose contents showed that increased amylose decreases ethanol conversion efficiency (Wu et al 2006a). Waxy and heterowaxy sorghum hybrids generally have higher conversion efficiencies than nonwaxy hybrids; amylose is likely to form amylose-lipid complexes in the caryopsis or during mashing that are resistant to enzymatic hydrolysis (Wu et al 2007).

The main objective of this study was to characterize fermentation performance (ethanol yield, conversion efficiency, mashing property, fermentation rate) of wheat grain in a laboratory dry-grind procedure with or without nitrogen food supplements in the mash. Waxy wheat was compared with nonwaxy wheat, and the influences of starch and protein content on ethanol fermentation were emphasized.

MATERIALS AND METHODS

Wheat Cultivars

Thirty wheat cultivars with a broad range of genotypes were selected for this study (Tables I and II). Wheat cultivars W01 to W08 were harvested in 2006, and cultivar W12 was harvested in 2004; all were obtained from the USDA-ARS, Grain, Forage and Bioenergy Unit (Lincoln, NE). The two commercial white wheat cultivars (W09 and W10) were harvested in 2005 and obtained from the American White Wheat Producers Association (Atchison, KS). The soft waxy cultivar (W11) was harvested in 2006 and obtained from the USDA-ARS, Western Wheat Quality Laboratory (Pullman, WA). The four commercial soft cultivars (W13–16), INW 0412, NEU, TRIBUTE, and HOPEWELL were grown in Indiana, Carolina, Virginia, and Ohio, respectively, in 2006 and obtained from the USDA-ARS, Soft Wheat Quality Laboratory (Wooster, OH). The two commercial soft wheat cultivars (W17 and W18) were planted in Sherman, TX, and Fort Worth, TX, respectively, in 2007 and obtained from Plains Grains (Stillwater, OK). An additional 12 cultivars (W19–W30 listed in Table II) were

TABLE I
Identification of 8 Waxy and 10 Nonwaxy Wheat Cultivars

Code	Cultivar	Type	Pedigree
W01	NWX03Y2459	Waxy hard red	BaiHuo/Ike//KS91H184/3*RBL//N87V106
W02	NX03Y2114	Waxy hard red	Cimarron/RioBlanco//BaiHuo4/L910145/3/Colt/Cody//Stozher/NE86582
W03	NX03Y2115	Waxy hard red	Cimarron/RioBlanco//BaiHuo4/L910145/3/Colt/Cody//Stozher/NE86582
W04	NX03Y2205	Waxy hard red	BaiHuo/Kanto107//Ike/4/KS831672/3/Rannaya12/Bez.4/2/Lancota/f9-67
W05	NX03Y2315	Waxy hard red	BaiHuoMai/Ike(97GC1015wx)//KSSB-369-7/NE88584
W06	NX03Y2489	Waxy hard red	BaiHuo/Kanto107//Ike/3/KS91H184/3*RBL//N87V106
W07	KARL92	Nonwaxy hard red	
W08	TREGO	Nonwaxy hard white	
W09	na[a]	Nonwaxy hard white	
W10	na	Nonwaxy soft white	
W11	Waxy-Pen	Waxy soft white	Penawawa *6/Wx2–2a
W12	ALWX-6	Waxy hard red	na
W13	INW 0412	Nonwaxy soft red	
W14	NEU	Nonwaxy soft red	
W15	TRIBUTE	Nonwaxy soft red	
W16	HOPEWELL	Nonwaxy soft red	
W17	na	Nonwaxy soft red	
W18	na	Nonwaxy soft red	

[a] Not available.

TABLE II
Identification of 12 Nonwaxy Wheat Cultivars Attending the 2006 Kansas Winter Wheat Performance Test

Code	Cultivar	Type	Location
W19	KS03HW6-6	Nonwaxy hard white	Harvey County, dry land
W20	Protection CL	Nonwaxy hard red	Harvey County, dry land
W21	AP Jagalene	Nonwaxy hard red	Harvey County, dry land
W22	Cutter	Nonwaxy hard red	Harvey County, dry land
W23	2145	Nonwaxy hard red	Harvey County, dry land
W24	Dominator	Nonwaxy hard red	Harvey County, dry land
W25	Danby	Nonwaxy hard white	Republic County, dry land
W26	Cutter	Nonwaxy hard red	Republic County, dry land
W27	Tarkio	Nonwaxy hard red	Riley County, dry land
W28	HV9W99-558R	Nonwaxy hard red	Finney County, irrigated
W29	OK Bullet	Nonwaxy hard red	Thomas County, dry land
W30	ACS97001	Nonwaxy hard red	Riley County, dry land

selected to extend the range of starch content, enhance even distribution of protein content, and provide extremes in ethanol fermentation using hard winter wheat.

Preparation of Samples

All samples were hand-cleaned to remove glumes, debris, and other impurities, packaged in plastic bags, and stored at 4°C until testing. Wheat kernels were ground with a mill (Udy, Fort Collins, CO) through a 1.0-mm screen for analysis of the physiochemical properties. Samples for ethanol fermentation were ground with a grain mill (model Magic Mill III Plus, Magic Mill Products & Appliances, Monsey, NY) set at level III.

Preparation of Mashes

For mashing, 30 g of ground grain (dry matter) was dispersed in a 250-mL Erlenmeyer flask with an aliquot of 100 mL of fermentation solution, which was prepared by mixing 1 L of distilled water (45–50°C) with 1.0 g of KH_2PO_4 and 200 μL of Liquozyme SC DS (240 KNU/g, 1.25 g/mL; Novozyme, Franklinton, NC), an enzyme preparation containing thermostable α-amylase. Flasks were then inserted into a water bath shaker (Amerex Instruments, Lafayette, CA) preheated to 95°C and oscillating at 100 rpm. Initially, flasks were shaken manually to prevent gel formation. This shaking process required several minutes depending on the number of flasks inserted. The water bath temperature was decreased to 82–87°C at the end of shaking, with slurries in the flasks well dispersed. The temperature was brought to 86°C and held for 90 min with continuous shaking. Flasks were then removed from the water bath, and the material on the inner surface of the flasks was scraped back into the bottom with a spatula and rinsed with 3–5 mL of deionized distilled water (DD water) using a sterilized fine-tipped polyethylene transfer pipette. After cooling to ambient temperature, liquefied mash was adjusted to pH 4.2–4.3 with 2*M* HCl.

Preparation of Inoculum

Active dry yeast (1 g) (Red Star Ethanol Red, Lesaffre, Milwaukee, WI) was dispersed in 19 mL of a preculture broth containing glucose (20 g/L), peptone (5 g/L), yeast extract (3 g/L), KH_2PO_4 (1 g/L) and $MgSO_4 \cdot 7H_2O$ (0.5 g/L) and incubated at 38°C for 30 min in an incubator shaking at 200 rpm.

Simultaneous Saccharification and Fermentation (SSF)

Nutrient broth (mL) containing yeast extract (300 g/L) or 1 mL of DD water, 1 mL of the activated yeast culture, and 100 μL of Spirizyme fuel (750 AGU/g, 1.15 g/mL, Novozyme, Franklinton, NC), an enzyme preparation containing glucoamylase, were added to each flask, which was subsequently sealed with an S-shaped airlock filled with ≈2 mL of mineral oil. Ethanol fermentation was performed in an incubator shaker (model I2400, New Brunswick Scientific, Edison, NJ) at 30°C for 72 hr with continuous shaking at 200 rpm. The fermentation process was monitored by measuring the mass loss of the mash due to CO_2 emissions during fermentation.

Distillation

At the end of fermentation, materials in each flask were transferred to a 500-mL distillation flask with 100 mL of distilled water. Beers were distilled on a distillation heating unit and the distillates were collected into a 100-mL volumetric flask that was dipped into ice water. Distillation was stopped when the collected distillates approached the 100-mL mark on the flask (≈99 mL). Collected distillates were then equilibrated to 25°C, adjusted to 100 mL, and sampled for HPLC analysis.

Preparation of Distillers Dried Grains with Solubles (DDGS)

In some instances, the residue remaining in each distillation flask was collected, frozen, and lyophilized. All the DDGS were ground with a mortar and pestle before use.

Hot-Stage Microscopic Images

Ground meal used for ethanol fermentation (0.1 g) was dispersed in distilled water (10 mL) using a vortex mixer. A small amount of high-vacuum grease (Dow Corning, Midland, MI) was spread evenly as an extremely thin layer around the edge of a square cover slip. One drop of the meal suspension was transferred onto a microscope slide and covered with the cover slip. The suspension between the slide and cover slip was heated from 30–90°C at a heating rate of 10°C/min on an STC200 hot stage. The progressive gelatinization of wheat starch was visualized with a BX51 microscope (Olympus America, Melville, NY). Images and photographs were captured using a 40× objective, a Spot Insight camera, and Spot 4.6 Windows software (Diagnostic Instrument, Sterling Heights, MI).

Mashing Properties

Mashing properties of ground grain were measured using a Rapid Visco-Analyser (RVA) (model RVA-4, Newport Scientific, Warriewood, Australia) and a 10-min liquefaction procedure developed by Zhao et al (2008). The temperature profile was set to maintain a constant block temperature of 95°C for 10 min. Before initiating a sample measurement, a plastic paddle was attached to the stirring head of the RVA and zeroed at 160 rpm against air. Ground grain samples (8.00 g, 14% wb) were dispersed in 20.0 mL (14% wb) of distilled water and 1.0 mL of an enzyme solution containing thermostable α-amylase (2.3 mL/L of Liquozyme SC DC) in sample canisters. After pouring the sample into the liquid, a plastic paddle was inserted into the sample canister, rotated, and jogged up and down by hand for 45–60 sec to remove lumps. The slurries were premixed for 10 sec at 960 rpm; thereafter, a speed of 160 rpm was applied. Rheological measurement data were recorded at 4-sec intervals and stored by RVA dedicated software. Peak viscosity, peak time, and final viscosity were measured.

Extraction of Fermentable Sugars

Ground meal (1 g) was dispersed in 20 mL of 5 m*M* $HgCl_2$ in a 50-mL disposable centrifuge tube. The tube was sealed and then horizontally placed in an incubator shaker at 30°C for 2 hr with continuous shaking at 200 rpm. The suspension was centrifuged at 2,460 × *g* for 5 min, and the supernatant was sampled and filtered through a 0.20-μm Millipore membrane before HPLC analysis for fermentable sugars including glucose, fructose, sucrose, and maltose.

Analytical Methods

Moisture content was measured by Approved Method 44-15A (AACC International 2000). Average kernel weight, diameter and hardness were scored with a single kernel characterization system (model SKCS-4100, Perten Instruments) according to AACC Approved Method 55-31. Falling number (FN) was measured using an FN device (model 1900, Perten Instruments) according to AACC Approved Method 56-81B. The nitrogen content was analyzed by using AACC Approved Method 46-30 with a nitrogen determinator (model FP-528, Leco, St. Joseph, MI). Nitrogen values were multiplied by 5.7 to convert to protein values. Total starch content was determined using a Megazyme total starch kit according to AACC Approved Method 76-13. Method B was used, which involves pretreatment with dimethyl sulfoxide at 100°C. Amylose content of starch was analyzed following the method of Gibson et al (1997) using an amylose-amylopectin assay kit (Megazyme International Ireland, Wicklow, Ireland). For glycerol measurement, 1 g of DDGS samples was well dispersed in 100 mL of DD water. The suspension was sampled and filtered through a 0.20-μm Millipore membrane before HPLC analysis. Ethanol in distillate samples, glycerol in diluted DDGS samples, fructose, glucose, and sum of sucrose and maltose in sugar extracts were determined using an HPLC system (Shimadzu Scientific Instruments, Columbia, MD) equipped with a Rezex RCM 7.8 × 300 mm column and a security

guard column (Phenomenex, Torrance, CA). The mobile phase was DD water at a flow rate of 0.6 mL/min. The column temperature was 80°C with a 20-μL injection volume. Maltose in sugar extracts was analyzed using the same Shimadzu HPLC system but using Phenomenex Rezex ROA 7.8 × 300 mm column and a security guard column. The mobile phase was 5 m*M* H_2SO_4 at a flow rate of 0.6 mL/min, and the column was maintained at 65°C with a 20-μL injection volume. All components were analyzed using a refractive index detector (model RID-10A, Shimadzu) with the detection cell maintained at 40°C. HPLC data were processed using Shimadzu EZStart 7.4 software. Ethanol yields were quoted as liters of ethanol per ton of dry grain (L/ton). Sucrose content was obtained by deducting maltose analyzed by the ROA column from the sum of maltose and sucrose analyzed by the RCM column. Conversion efficiencies were calculated as a ratio of the experimentally determined ethanol yield to the theoretical ethanol yield based on the total starch content in a sample. Adjusted conversion efficiencies were calculated in consideration of not only the total starch but also fructose and sucrose contents of the sample. DDGS yields were expressed as a ratio of the amount of DDGS to the amount of ground grain used in fermentation (dry matter). Yields of the by-product (glycerol) were reported as liters of glycerol per ton of dry grain (L/ton).

Statistical Analyses

All experiments were performed at least in duplicate. The tabular results presented are the mean values of repeated experiments. The viscosity curves represent one-sample measurements. Analysis of variance (ANOVA), least significant difference (LSD), and linear regression were performed using SAS software (v.9.1, SAS Institute, Cary, NC).

RESULTS

Physicochemical Properties

The 18 wheat cultivars in Table I were subjected to measurements of physicochemical properties including single kernel characteristics, amylose (Table III), total starch and protein contents (Table IV). For the 12 hard winter wheat cultivars in Table II, only total starch and protein contents were analyzed.

Partial waxy wheats typically produce starches with amylose content of ≤15% (Graybosch 1998). Starches are defined as waxy when the ratio of amylose to total starch is <15%; normal when amylose is ≈16–35%; and high-amylose when amylose is >36% (Tester et al 2004). As expected, the eight waxy wheat cultivars contained very low levels of amylose (2.7–4.1%, mean 3.2%) and there was no significant difference in amylose content among any of the tested samples (Table III). Except W09, nonwaxy wheat cultivars (hard and soft) had amylose contents of 26–28%, which was consistent with the values reported for normal wheat (Hung et al 2006).

Significant variation in grain hardness occurred among the wheat cultivars (Table III). As expected, nonwaxy hard and soft cultivars had hardness scores of 58–66 (mean 63) and 1–21 (mean 11), respectively. Grain (kernel) hardness is independent of starch amylose levels (Morris and Konzakb 2001; Graybosch et al 2003). Waxy cultivar W11 had a hardness score of 10, indicative of typical soft wheat endosperm; whereas the other seven waxy cultivars had a mean value of 61 (range 51–70), conforming to the classification as hard wheats. Waxy cultivars except W12 had lower kernel weight as well as smaller kernel size than nonwaxy counterparts (Table III). Most soft cultivars had higher kernel weight as well as larger kernel size than hard cultivars, with the exception of cultivar W18, for which average kernel weight and diameter were 31.6 mg and 2.24 mm, respectively.

The FN test has been widely employed to indirectly detect the extent of sprout damage within a wheat sample, and an FN device measures the time (seconds) for a stirrer to fall to the bottom of a glass tube filled with a heated paste of ground meal or flour and water. Generally, an FN value of ≥350 sec indicates low enzyme activity and very sound wheat, and values <200 sec indicate serious sprout damage (Sologuk and Sorenson 2005). Average FN for the eight waxy wheat cultivars was only 68 sec (Table III), which was in agreement with the results of Graybosch et al (2000). With

TABLE III
Amylose Contents, Falling Numbers, RVA Peak Viscosities, and Single Kernel Characteristics of 18 Wheat Cultivars

Code	Type	Amylose (%)	Falling Number (sec)	RVA Peak Viscosity (cP)	Single Kernel Characteristics		
					Hardness	Weight (mg)	Diameter (mm)
W01	Waxy	3.0	67	2453	59	30.0	2.25
W02	Waxy	3.3	69	2430	61	29.8	2.30
W03	Waxy	2.7	70	2341	56	29.9	2.30
W04	Waxy	2.9	69	2367	67	25.9	2.11
W05	Waxy	3.2	69	2146	63	31.7	2.29
W06	Waxy	2.9	62	2302	70	27.6	2.10
W11	Waxy	3.1	69	1901	10	33.3	2.52
W12	Waxy	4.1	67	2711	51	37.1	2.51
W10	Soft	26.7	312	7968	14	37.6	2.50
W13	Soft	27.9	469	10578	21	42.1	2.64
W14	Soft	27.2	403	8711	9	37.8	2.61
W15	Soft	26.6	237	8139	10	38.5	2.54
W16	Soft	27.5	373	8661	-1	42.9	2.59
W17	Soft	27.0	176	6815	10	37.4	2.49
W18	Soft	26.4	217	8114	11	31.6	2.24
W07	Hard	27.5	576	7468	58	33.7	2.39
W08	Hard	26.3	525	7559	66	31.5	2.30
W09	Hard	22.6	779	6620	66	35.9	2.50
Replicates		2	2	2	300	300	300
Pooled standard error		1.3	4	385	1	0.5	0.03
$LSD_{0.05}$		3.8	12	1143	3	1.3	0.07
Type averages							
	Waxy	3.2c[a]	68c	2331c	61a[b]	30.7b	2.30b
	Soft	27.0a	312b	8427a	11b	38.3a	2.52a
	Hard	25.5b	627a	7816b	63a	33.7a,b	2.40a,b

[a] Values followed by different letters in the same column are significantly different ($P < 0.05$).
[b] Waxy soft cultivar, W11, was excluded from the waxy group.

the exception of W06, there was little difference in FN among the other waxy wheat cultivars. In contrast, the three nonwaxy hard cultivars had much higher FN values than nonwaxy soft counterparts. The soft cultivar, W17, looked sound but had an FN value of 176, suggesting the presence of minor sprout damage. Pooled standard error of FN for the eight waxy cultivars was only 0.6 sec and much less than that for all 18 samples (4 sec, Table III).

As shown in Table IV, protein and total starch content varied considerably among the 30 wheat cultivars used in this study. The ranges for all cultivars were 9.9–18.0% and 55.6–66.7% for protein and total starch content, respectively, which are in line with previous reports for normal spring wheat (Sosulski and Sosulski 1994; Wang et al 1997; Lacerenza et al 2008). Nonwaxy soft wheat had overall lower protein and higher total starch content than nonwaxy hard and waxy wheat. According to linear regression analysis on data in Table IV, total starch was negatively correlated with protein ($R^2 = 0.80$, $P < 0.0001$), confirming the inverse relationship (Smith et al 2006).

Hot-Stage Microscopic Images

Changes in morphology of starch granules from wheat grain were continuously recorded with temperature, and representative microscopic images are displayed in Fig. 1. For measurements, a 1% aqueous suspension of whole meal ground for ethanol fermentation was used rather than pure starch. Starch granules were clearly seen at 30°C under polarized light (Fig. 1A) and started swelling at ≈60–62°C with sizes increased slightly (Fig. 1B). Most of the swelling occurred at 60–75°C. An important finding in the present study is that waxy wheat starch granules swelled greatly at 65–70°C, then ruptured into many small fragments and melted away, leading to a complete disruption/dissolution at 70–80°C. No clear outline of swollen granules was observed at the end of heating. Comparatively, nonwaxy granules rapidly swelled at a relatively higher temperature, and the outline of the fully swollen granules remained intact throughout the heating process on the hot stage. As shown in Fig. 1C for the waxy cultivar W11, most of the swollen granules ruptured and melted away at 71.3°C, and no swollen granules were observed as the temperature was continuously increased to 75°C; for the nonwaxy cultivar W16, outlines of the fully swollen granules were clearly seen even at 91.1°C.

Mashing Properties

The 10-min liquefaction test was programmed to mimic the liquefaction process during mash preparation. Solids level in the pasting slurry was ≈24% (w/w), similar to that in mash for fermentation. The enzyme dosage was calculated based on 10 μL of heat-stable α-amylase/30 g of dry solids, which was half the dosage currently used in this study for fermentation. An RVA canister with the slurry was placed into the heating sink, similar to inserting a flask into a hot water bath for the fermentation test. Representative viscosity curves of waxy and nonwaxy wheat cultivars are shown in Fig. 2, and RVA peak viscosities for the 18 cultivars

TABLE IV
Protein and Total Starch Contents, Ethanol Yields, and Conversion Efficiencies of 30 Wheat Cultivars

Code	Type	Protein (%, db)	Total Starch (%, db)	Ethanol Yield (L/ton)		Conversion Efficiency (%)	
				With Nitrogen	Without Nitrogen	With Nitrogen	Without Nitrogen
W01	Waxy	15.78	59.5	400	398	92.8	92.5
W02	Waxy	16.17	59.4	400	398	93.0	92.7
W03	Waxy	15.41	59.4	399	397	92.8	92.3
W04	Waxy	17.50	56.3	382	380	93.9	93.3
W05	Waxy	15.36	58.6	397	394	93.5	93.0
W06	Waxy	14.29	59.4	403	401	93.8	93.3
W11	Waxy	13.18	60.8	419	418	95.4	95.0
W12	Waxy	12.73	59.4	414	412	96.2	95.8
W10	Soft	9.91	66.5	444	435	92.4	90.5
W13	Soft	13.12	63.7	423	413	91.8	89.6
W14	Soft	9.99	67.1	447	431	92.0	88.6
W15	Soft	9.77	67.7	447	439	91.4	89.8
W16	Soft	9.58	66.2	443	424	92.5	88.6
W17	Soft	13.51	63.6	420	416	91.3	90.4
W18	Soft	14.70	61.3	409	408	92.3	92.1
W07	Hard	16.97	60.0	399	395	91.8	90.9
W08	Hard	14.28	62.9	418	413	91.8	90.7
W09	Hard	14.08	63.2	423	416	92.5	90.9
W19	Hard	10.46	64.6	432	420	92.6	89.8
W20	Hard	11.07	63.7	427	417	92.7	90.7
W21	Hard	11.24	63.2	428	418	93.5	91.5
W22	Hard	11.64	62.7	418	411	92.2	90.7
W23	Hard	12.19	62.3	413	409	91.7	90.8
W24	Hard	12.77	61.3	409	401	92.2	90.5
W25	Hard	13.34	62.8	417	413	91.7	90.9
W26	Hard	13.87	61.5	412	405	92.6	91.0
W27	Hard	14.85	57.2	380	375	91.8	90.5
W28	Hard	15.85	56.9	381	377	92.4	91.5
W29	Hard	16.83	57.9	388	384	92.8	91.8
W30	Hard	17.99	55.6	368	365	91.5	90.6
Replicates		2	2	2	2	2	2
Pooled standard error		0.04	0.22	0.9	0.9	0.21	0.19
$LSD_{0.05}$		0.11	0.63	2.6	2.5	0.60	0.55
Type averages							
	Waxy	15.05a	59.1b[a]	402bA[b]	400bB	93.9aA	93.5aB
	Soft	11.51b	65.2a	433aA	424aB	92.0bA	89.9cB
	Hard	13.83a	61.1b	408bA	401bB	92.3bA	90.9bB

[a] Values followed by different lowercase letters in the same column are significantly different ($P < 0.05$);
[b] Values followed by uppercase letters in the last three rows for ethanol yield or conversion efficiency are significantly different ($P < 0.01$) between the two treatments (with or without nitrogen food supplements in the mash).

are listed in Table III. Significant differences in peak viscosity were observed for all cultivars. The eight waxy cultivars had an average peak viscosity of 2331 cP (1901–2711 cP), which was much lower than that obtained from nonwaxy counterparts. With the exception of W17 (with low FN value), the nonwaxy soft wheat tended to have larger peak viscosity than hard wheat. Again, the pooled standard error of peak viscosity for the eight waxy cultivars was only 35 cP, much less than that for all 18 samples (385 cP, Table III).

Rates of Fermentation

Theoretically, yeast can convert 1 mole of glucose to 2 moles of CO_2 and ethanol and, in practice, the mass losses caused by the emission of CO_2 are proportional to the amounts of ethanol produced during ethanol fermentation (Wu et al 2006b). As shown in Fig. 3, the accumulated mass losses were plotted over time and fermentation processes in the Erlenmeyer flasks were monitored. For tests with nitrogen food supplements in the mash (Fig. 3), fermentation ended at 24–40 hr after inoculation, suggesting the advantageous fermentation rate of wheat is comparable to that of pearl millet (Wu et al 2006b) but faster than corn and sorghum (unpublished data). Waxy wheat cultivars had faster rates of fermentation and came to a stationary phase in the curves of mass losses 6–10 hr earlier than nonwaxy counterparts. As reported by Thomas and Ingledew (1990), fermentation became sluggish and protracted in the mashes without nitrogen supplementation. It is obvious in Fig. 3 that the two nonwaxy cultivars (W08 and W13) required an extra 30 hr to complete fermentation after the mashes

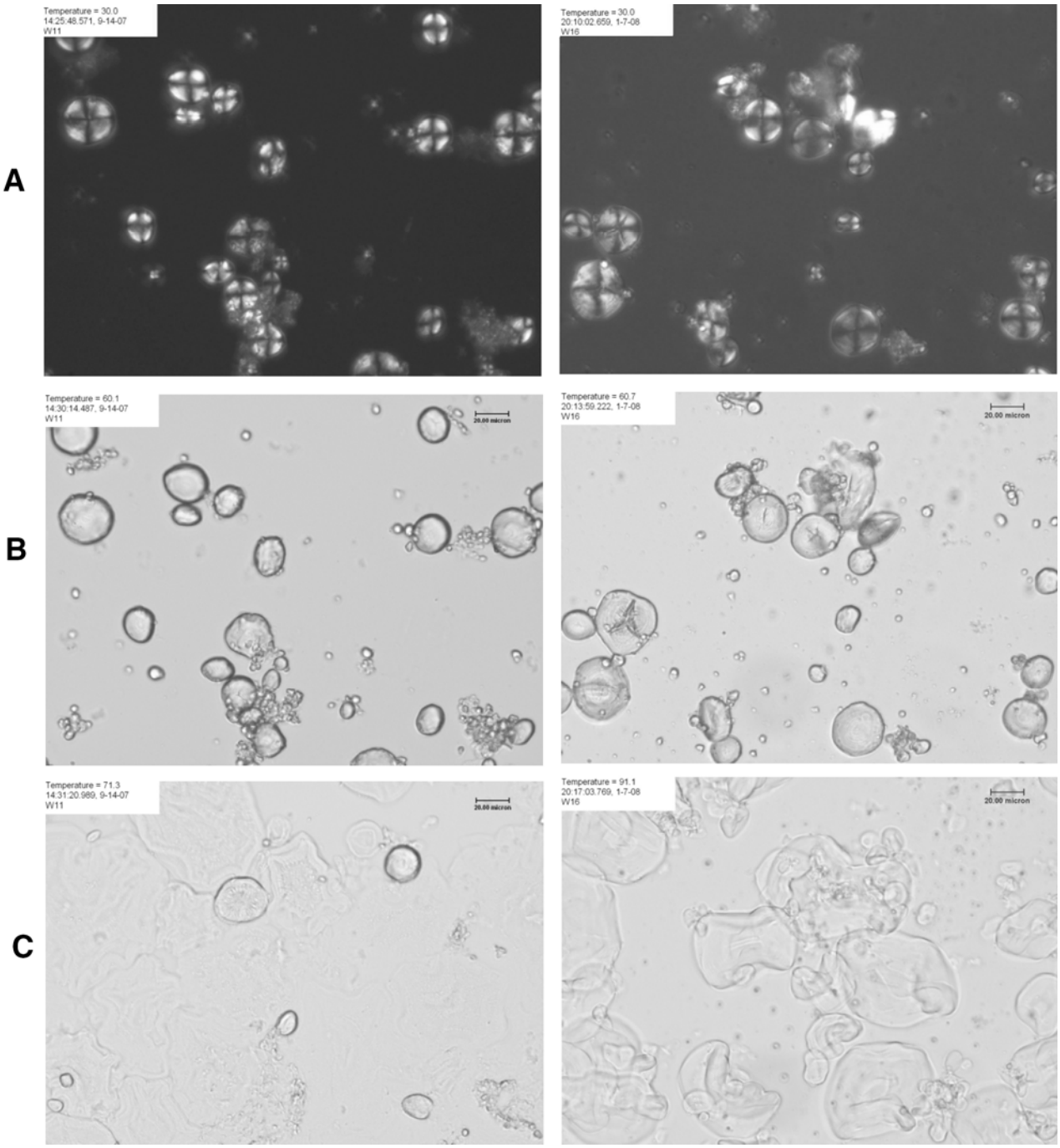

Fig. 1. Microscopic images of starch granules in slurries of two cultivars, waxy soft wheat W11 (left) and nonwaxy soft wheat W16 (right): **A,** at the beginning of heating (30°C) on a hot-stage microscope viewed under polarized light; **B,** in the middle of heating (≈60°C) on a hot-stagemicroscope viewed under normal light; **C,** at 71.3 and 91.1°C for W11 and W16, respectively, on a hot-stage microscope viewed under normal light.

were deprived of exogenous nitrogen food. However, without nitrogen supplementation, the waxy cultivar (W11) was fermented to near completion only 10 hr later than its control. The two curves of mass losses, one from W13 with nitrogen food and the other from W11 without nitrogen food, nearly overlapped each other, indicating that the fermentation rates for waxy cultivars without nitrogen food are comparable to those for nonwaxy cultivars with nitrogen food. The effect of exogenous nitrogen food on fermentation rate of waxy wheat was less significant than for nonwaxy wheat, suggesting that waxy cultivars may contain more assimilable nitrogen in mash than nonwaxy counterparts.

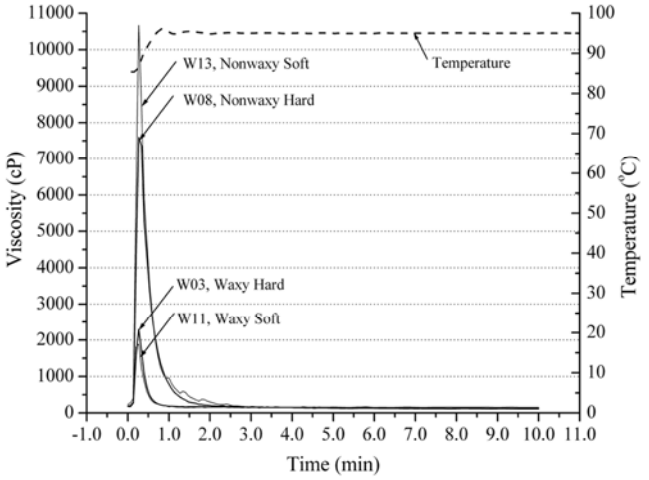

Fig. 2. Comparison of viscosity curves of waxy and nonwaxy wheat cultivars measured by 10-min temperature RVA profile with 10 μL of heat-stable α-amylase/30 g of dry solids in the slurries.

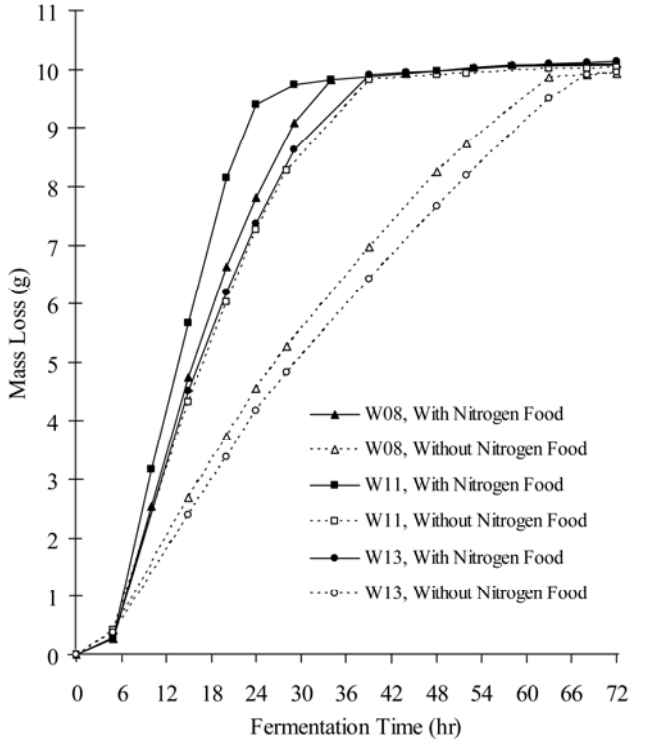

Fig. 3. Comparison of curves of mass losses from CO_2 evolution in a laboratory dry-grind procedure with or without nitrogen food supplement. W08, W11, and W13 were nonwaxy hard, waxy, and nonwaxy soft wheat cultivars, respectively. Each experiment was duplicated (two flasks) with repeated measurements taken over time. Standard error for mean values of mass losses was 0.04.

Ethanol Yields and Conversion Efficiencies

Similar to protein and total starch content, ethanol yields varied significantly among the 30 wheat cultivars (Table IV). For fermentation with nitrogen supplementation, the range for all cultivars was 368–447 L/ton (mean 412), which conforms to previous observations for normal wheat (Sosulski and Sosulski 1994; Wang et al 1997; Swanston et al 2005, 2007; Agu et al 2006; Kindreda et al 2008). Consistent with total starch content, nonwaxy soft wheat had an average ethanol yield of 433 L/ton (409–447), larger than those of nonwaxy hard and waxy wheats. Conversion efficiencies for all the cultivars were 91.3–96.2% (mean 92.6%). Waxy cultivars, especially W11 and W12, for which conversion efficiencies were 95.4 and 96.2%, respectively, had overall higher conversion efficiencies than nonwaxy counterparts. Despite the huge variation in protein content among cultivars, there was little difference in the conversion efficiency among nonwaxy hard and soft cultivars, with the exception of W21.

Without any nitrogen supplementation, not only was the rate of fermentation reduced, as discussed previously, but both ethanol yield and conversion efficiency decreased significantly ($P < 0.01$), irrespective of wheat cultivar (Table IV). The average losses in ethanol yield and conversion efficiency were 6 L/ton and 1.3%, respectively, for exogenous nitrogen food excluded from mash. Again, the effect of nitrogen food on ethanol yield and conversion efficiency in waxy wheat was not as significant as that in nonwaxy wheat. As shown in Fig. 4, loss of ethanol yield for waxy cultivars had little to do with protein content ($P = 0.50$); however, the negative correlation between protein content and loss of ethanol yield for all nonwaxy cultivars was significant ($R^2 = 0.55$, $P < 0.0001$). Lower protein content (<12%) tended to result in larger loss of ethanol yield, indicating the necessity for a nitrogen food supplement to the mash of these lower protein wheat cultivars.

According to linear regression analyses (Table IV), there were significant correlations between the ethanol yield and both total starch and protein content, regardless whether nitrogen food was supplemented (Table V). Generally, total starch was a better predictor of ethanol yield than was protein, especially for the 15 hard winter cultivars. For the 22 nonwaxy wheat cultivars, the coeffi-

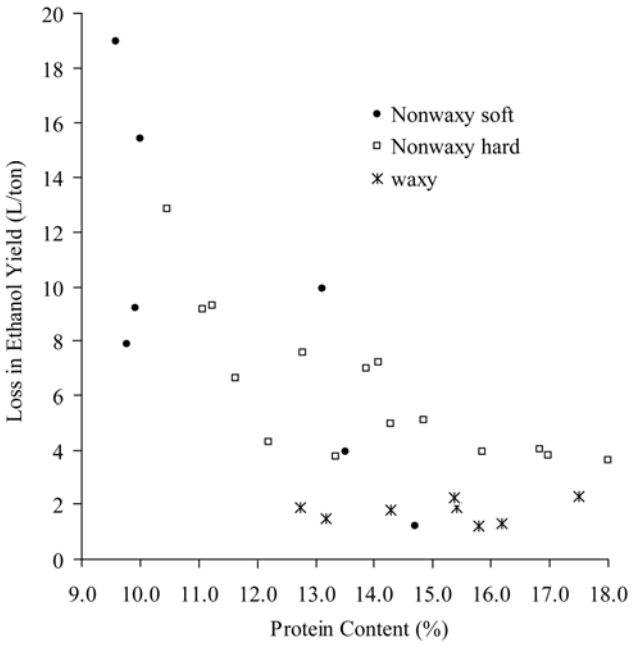

Fig. 4. Effect of protein content on loss of ethanol yield in exogenous nitrogen food excluded from the mash of wheat cultivars. Loss of ethanol yield (L/ton) was calculated as the difference between ethanol yield with nitrogen food supplement and without. Samples for seven nonwaxy soft, 15 nonwaxy hard, and eight waxy cultivars.

cients of determination were $R^2 = 0.99$ for total starch and ethanol yield and $R^2 = 0.82$ for protein and ethanol yield (data not shown). Because of the strong negative relationship between total starch and protein, the results of multiple linear regression showed that the role of total starch was dominant ($P < 0.0001$) when starch was combined with protein to predict overall ethanol yield (data not shown). With nitrogen food supplements in the mash, conversion efficiency was not related to either total starch or protein. Interestingly, the relationship between conversion efficiency and both starch and protein became significant when the mash was deprived of exogenous nitrogen food (Table V).

Fermentable Sugars in Wheat Grains and Adjusted Conversion Efficiencies

Several factors, including incomplete starch hydrolysis during mashing, glucose consumption for yeast growth, and inevitable production of by-products during ethanol fermentation, influence conversion efficiency, which is usually 90–95% (Thomas and Ingledew 1990). As mentioned previously, the two waxy cultivars (W11 and W12) had exceptional conversion efficiencies (>95%). Yeast fermented not only the glucose hydrolyzed from starch but also other fermentable sugars, including those existing in grains before mashing and those probably released from nonstarch polysaccharides during mashing. For the total starch assay in this study, we did not measure free sugars separately by extracting a sample in ethanol solution, and some fermentable sugars such as maltose and glucose would contribute to total starch content. However, other fermentable sugars such as sucrose and fructose could not be converted to glucose by heat-stable α-amylase during liquefaction or amyloglucosidase during saccharification and would not be assessed as contributors to total starch content. For simplicity, only the main fermentable sugars including glucose, fructose, sucrose, and maltose (Russell 2003) were assayed, and fructose and sucrose were expected to give rise to high conversion efficiencies in waxy wheat cultivars. These sugars were extracted by a 5 m*M* $HgCl_2$ solution, which was used to inhibit the activity of invertase (Lin et al 1999) in the grain and improve the repeatability of sugar analysis.

Significant variations in sugar levels were observed among the 18 wheat cultivars (Table VI). The sum of the four sugars was 0.83–2.82%, less than the previous results of 2–3% for alcohol-soluble sugars (Lineback and Rasper 1988). Other free sugars such as glucofructosans, glucodifructose, trisaccharides, and raffinose might exist but were not measured in this study. Compared with maltose and sucrose, the amounts of glucose and fructose were low and considered insignificant. The quantity of sucrose

TABLE V
Coefficients of Determination (R^2) for Total Starch and Protein Contents of 30 Wheat Cultivars and Fermentation Parameters in a Laboratory Dry-Grind Procedure[a]

	With Nitrogen Food Supplements in the Mash					Without Nitrogen Food Supplements in the Mash	
	Ethanol Yield				Conversion Efficiency	Ethanol Yield	Conversion Efficiency
	Waxy (8)	Soft (7)	Hard (15)	Pooled (30)	Pooled (30)	Pooled (30)	Pooled (30)
Total starch content	0.79**	0.98***	0.99***	0.95***	0.09ns	0.89***	0.34***
Protein content	0.88***	0.98***	0.73***	0.84***	0.003ns	0.76***	0.19*

[a] Values in parentheses are numbers of wheat cultivars in each type; ns indicates not significant ($P > 0.05$); *, significant ($P < 0.05$); **, significant ($P < 0.01$); and ***, significant ($P < 0.001$).

TABLE VI
Fermentable Sugars and Adjusted Conversion Efficiencies[a] of 18 Wheat Cultivars

		Fermentable Sugar Content (%, db)					Adj Conversion Efficiency (%)
Code	Type	Maltose	Sucrose	Glucose	Fructose	Sum	
W01	Waxy	0.77	0.76	0.16	0.15	1.84	91.5
W02	Waxy	0.64	0.98	0.15	0.15	1.92	91.4
W03	Waxy	0.61	1.14	0.19	0.20	2.14	90.8
W04	Waxy	0.66	0.60	0.24	0.21	1.71	92.7
W05	Waxy	0.64	0.62	0.19	0.16	1.62	92.3
W06	Waxy	0.69	0.92	0.24	0.21	2.06	92.1
W11	Waxy	0.38	1.26	0.15	0.18	1.98	93.3
W12	Waxy	0.60	1.78	0.20	0.24	2.82	93.2
W10	Soft	0.21	0.47	0.14	0.16	0.98	91.6
W13	Soft	0.32	0.67	0.14	0.13	1.27	90.7
W14	Soft	0.22	0.73	0.14	0.11	1.20	90.9
W15	Soft	0.23	0.80	0.19	0.13	1.35	90.2
W16	Soft	0.26	0.78	0.13	0.12	1.29	91.4
W17	Soft	0.25	0.82	0.17	0.14	1.38	90.0
W18	Soft	0.24	0.84	0.20	0.17	1.44	90.9
W07	Hard	0.38	0.67	0.14	0.13	1.32	90.7
W08	Hard	0.42	0.55	0.12	0.11	1.21	90.9
W09	Hard	0.24	0.29	0.14	0.16	0.83	91.9
Replicates		2	2	2	2	2	–
Pooled standard error		0.01	0.01	0.01	0.01	0.02	–
$LSD_{0.05}$		0.02	0.04	0.02	0.02	0.06	–
Type averages							
	Waxy	0.62a[b]	1.01a	0.19a	0.19a	2.01a	92.2a
	Soft	0.25b	0.73a,b	0.16a,b	0.14b,c	1.27b,c	90.8b
	Hard	0.35b	0.50b	0.13b	0.13c	1.12c	91.2a,b

[a] Calculated using the average contents of total starch, sucrose, and fructose in samples.
[b] Values followed by different letters in the same column are significantly different ($P < 0.05$).

ranked the highest among all sugars. Waxy cultivars had overall higher sugar levels than nonwaxy counterparts. The two waxy cultivars (W11 and W12) contained the most sucrose (1.26 and 1.78%, respectively), which partially explained abnormal conversion efficiencies. Adjusted conversion efficiency was calculated as a ratio of the experimentally determined ethanol yield to the adjusted theoretical ethanol yield, which was obtained by adding the theoretical ethanol yield contributed by sucrose and fructose to the theoretical ethanol yield of starch. After adjustment, conversion efficiencies decreased 0.6–3.0% (mean 1.4%) but were still high (90.0–93.3%, mean 91.5%).

Properties of DDGS

As shown in Table VII, distinctive variations in DDGS yield and composition also occurred among the 18 wheat cultivars. Average values for yield, starch, protein, and glycerol content of DDGS were 40.2% (35.6–44.9%), 0.72% (0.30–1.57%), 35.7% (28.2–41.0%), and 8.5% (7.4–9.9%), respectively. Nonwaxy soft cultivars produced overall lower yields of DDGS than nonwaxy hard and waxy cultivars. In general, DDGS from nonwaxy soft cultivars contained less protein than those from nonwaxy hard and waxy cultivars. The data in Tables IV and VII indicate 99.1–99.8% of the total starch in the grain had been broken down and very little starch remained in the DDGS. All DDGS from waxy cultivars had less amounts of residual starch than those from nonwaxy cultivars.

For nonwaxy cultivars, DDGS contained 0.7–1.6% of starch, which is in agreement with previous work (Sosulski and Sosulski 1994; Smith et al 2006). DDGS from nonwaxy soft cultivars also contained a higher level of glycerol, a major by-product of ethanol fermentation (Russell 2003). Glycerol yields were calculated using DDGS yield and glycerol content. Interestingly, glycerol yield was independent of ethanol yield. Glycerol (≈1 g) was produced in each fermentation flask starting with 30 g of dry grains. However, slightly less glycerol was produced by the waxy cultivars.

Linear regression analysis revealed the strong negative relationships between total starch content of grain and both yield and protein content of DDGS (Table VIII). Protein content of grain was positively correlated to both yield and protein content of DDGS. Obviously, grain protein was a better predictor of DDGS protein than grain starch. Again, because of the strong negative relationship between grain starch and protein, combining these two variables together in multiple linear regression showed significant ($P < 0.001$) effects on both DDGS yield and protein content, explaining 98% of the variations in yield and protein content of DDGS (data not shown).

DISCUSSION

Considering that yeast growth normally accounts for ≈8% of the sugars available for fermentation, Smith et al (2006) predicted a conversion efficiency nearer to 92% for U.K. wheat. To mimic fuel ethanol production in the dry-grind industry, the SSF procedure was used in this research. Because amyloglucosidase and yeast were added simultaneously, a concentrated glucose solution was avoided, and the initial osmotic stress of yeast was then lowered (Bothast and Schlicher 2005), which could be a reason why conversion efficiency (91.3–93.5%, mean 92.2%) for nonwaxy wheat cultivars (Table IV) was higher than the previous reports

TABLE VIII
Coefficients of Determination (R^2) for Total Starch and Protein Contents of Grain and Yield and Protein Content of DDGS for 18 Wheat Cultivars[a]

	DDGS	
	Yield	Protein Content
Total starch content	0.93***	0.65***
Protein content	0.93***	0.96***

[a] *** Significant ($P < 0.0001$).

TABLE VII
Yields and Chemical Compositions of DDGS Prepared from 18 Wheat Cultivars Using a Laboratory Dry-Grind Procedure with Nitrogen Food Supplements in the Mash[a]

			DDGS Composition (%, db)			Glycerol Yield
Code	Type	DDGS Yield (%, db)	Starch	Protein	Glycerol	(L/ton)[b]
W01	Waxy	42.3	0.49	38.9	8.0	26.9
W02	Waxy	42.6	0.30	39.8	7.9	26.7
W03	Waxy	42.4	0.42	38.2	8.0	26.7
W04	Waxy	44.9	0.50	40.5	7.4	26.2
W05	Waxy	43.1	0.33	37.9	7.8	26.5
W06	Waxy	42.2	0.44	36.1	7.9	26.3
W11	Waxy	39.6	0.46	34.7	8.6	26.6
W12	Waxy	40.5	0.46	33.0	8.4	27.7
W10	Soft	36.5	1.11	29.2	9.7	26.8
W13	Soft	39.4	0.93	34.5	8.5	28.2
W14	Soft	35.7	0.99	30.5	9.9	27.0
W15	Soft	35.6	0.89	30.3	9.9	27.0
W16	Soft	36.6	1.57	28.2	9.4	26.6
W17	Soft	39.7	0.85	35.9	9.0	27.9
W18	Soft	41.0	0.71	37.6	8.3	28.0
W07	Hard	42.8	1.08	41.0	7.8	27.2
W08	Hard	39.9	0.70	38.3	8.8	28.1
W09	Hard	39.7	0.78	37.2	8.5	26.8
Replicates		2	2	2	2	–
Pooled standard error		0.46	0.11	0.09	0.10	–
$LSD_{0.05}$		1.36	0.34	0.26	0.29	–
Type averages						
	Waxy	42.2a	0.43b	37.4a	7.99b	26.7b
	Soft	37.8b	1.01a	32.3b	9.23a	27.4a
	Hard	40.8a	0.85a	38.8a	8.38b	27.4a

[a] Values followed by different letters in the same column are significantly different ($P < 0.05$).
[b] Calculated using the average DDGS yields and glycerol contents in DDGS.

(Sosulski and Sosulski 1994; Wang et al 1997; Kindreda et al 2008; Lacerenza et al 2008). In a recent study, Zhao et al (2009) observed a relative increase of 3.0% (on average) in conversion efficiencies for 18 sorghum hybrids when the fermentation method was changed from a traditional dry-grind procedure to SSF. DDGS of nonwaxy wheat cultivars contained only 0.7–1.6% unconverted starch (Table VII), which is indicative of a high conversion rate of starch. In contrast, there was 5–6% residual starch in commercial corn (Kim et al 2008) and sorghum (Corredor et al 2006) DDGS. In conjunction with fermentation tests conducted by our group, a conclusion can be inferred that conversion efficiency of wheat is generally superior to corn and sorghum and comparable to pearl millet (unpublished data). There was no difference between hard and soft wheat in terms of conversion efficiency (i.e., no variation in starch quality related to ethanol fermentation was observed).

Because starch is converted to ethanol in a dry-grind process, it seems logical to assume that the amount of starch would be related to ethanol yield. However, even with the same laboratory protocol tailored to simulate a commercial production process, controversial observations about the relationship between starch content and alcohol yield exist. Swanston et al (2007) reported that starch content did not significantly correlate with the ethanol yield; Kindreda et al (2008) found a positive relationship between alcohol yield and starch concentration, but starch could explain only 37% of the variance in alcohol yield; and in a report reviewed by Smith et al (2006), there was a much better correlation between starch and alcohol yield (R^2 = 0.78). Recently, using a dry-grind procedure, Lacerenza et al (2008) reported that starch content in spring wheat was highly correlated to ethanol yield (R^2 = 0.60). The inconsistent results were due to the inherent variability and difficulty in the starch measurements (Smith et al 2006; Kindreda et al 2008). Another explanation may be related to fermentation procedures. Using sorghum grain, Zhao et al (2009) found a positive correlation between total starch and ethanol yield (R^2 = 0.86) in SSF was stronger than that in traditional fermentation (R^2 = 0.78). Protein content was negatively correlated with ethanol yield and gives better precision in predicting ethanol yield than starch content (Swanston et al 2005, 2007; Smith et al 2006; Kindreda et al 2008). Notably, our current results showed highly significant correlations between ethanol yield and both total starch and protein content. For all 30 wheat cultivars with a broad range of genotypes, up to 95% of variation in ethanol yield could be explained by total starch, and 84% by protein (Table V). Ethanol yield, perhaps the most important fermentation performance criterion for the fuel ethanol industry, proved a starch-related property of wheat.

According to the estimation by Schultze et al (2005), feedstocks represent 55–70% of bioethanol processing costs, which leaves little doubt to feedstock quality affecting the profit margins of ethanol producers. Using wheat as an example, there were variations in fermentation performance of feedstock in terms of ethanol yield, conversion efficiency, ease of mashing, fermentation rate, and the yield and quality of DDGS. For nonwaxy wheat, the selection of wheat cultivars as a feedstock for production of fuel ethanol would become simpler than selection of wheat for breadmaking because grain hardness did not influence the extent of starch conversion, and starch and protein content were the most significant factors in determining ethanol yield, the yield and quality of DDGS. Obviously, a wheat cultivar with higher starch content in its grain is desirable because it will provide more ethanol per ton of grain and produce smaller amounts of DDGS, resulting in less residual material left over and a greater energy saving during DDGS drying. The most effective way to increase ethanol yield from wheat is to increase the amounts of starch and sugar in the grain (Simth et al 2006). Soft wheat was superior to hard wheat in fermentation performance due to its higher starch content. Soft wheat generally yields far more than hard wheat (Economic Research Service/USDA 2008; Lacerenza et al 2008).

At present, the challenge for the U.S. fuel ethanol industry is that wheat has a low agronomic yield compared with corn. Wheat for biofuel production should give higher grain yields, making it more financially viable for growers. For U.K. wheat, Kindreda et al (2008) predicted a grain yield of 9.6 ton/ha at the economically optimum fertilizer nitrogen rate. Thus, soft wheat is, potentially, a technically and economically attractive crop for fuel ethanol production.

Wheat is rich in protein but only a small amount of free amino nitrogen (FAN) is liberated during the mashing, and this amount is not sufficient to support the fermentation at the fastest rate, especially when high concentrations of sugars are to be fermented (Thomas and Ingledew 1990). Exogenous assimilable nitrogen such as urea and ammonium is often supplemented into the mash (Russell 2003). Wheat proteins can be hydrolyzed by commercial proteases to substitute for the exogenous nitrogen sources (Thomas and Ingledew 1990; Jones and Ingledew 1994; Lee et al 2000). In the absence of nutrient supplements, wheat mashes containing very little FAN can be fermented to near completion, though the fermentation rate is low (Thomas and Ingledew 1990). Our current results show that wheat has a faster rate of fermentation than corn and sorghum when the mashes are supplemented with yeast extract as a nitrogen food for yeast growth. We anticipated that protein, one of the major components in wheat, could play a role and that a difference in protein quality among wheat cultivars related to ethanol fermentation could exist. However, protein content did not affect conversion efficiency (Table V). The effect of wheat protein on ethanol fermentation may be masked by the addition of 0.3 g of yeast extract, equivalent to 18 mg of FAN/100 mL of mash, in each flask. Thus, fermentation tests without nitrogen food supplemented into the mash were conducted to provide insight into the role of protein. Deprivation of exogenous nitrogen food resulted in a significant decrease in the rate of fermentation (Fig. 3) and losses in both ethanol yield and conversion efficiency (Table IV). There seemed to be protein compositional traits related to fermentation, as seen by the significant variation in loss of ethanol yield observed among wheat cultivars with very similar protein content. For example, the four soft cultivars (W10, W14, W15, and W16) had protein content of ≈10%, but losses in ethanol yield varied tremendously at 8–19 L/ton (Fig. 4). On the other hand, solely increasing the quantity of protein content in high-protein wheat cultivars did not reduce losses in ethanol yield (Fig. 4). For example, the four hard cultivars (W27 to W30) had almost identical values of loss of ethanol yield (4 L/ton), but protein content was 15–18%. We are further investigating some protein-related properties of wheat including FAN and protein composition, which might be related to fermentation.

Waxy wheat starches, flours, or ground meals have been characterized as having lower peak temperatures (i.e., taking less time to reach maximum viscosities) and lower final viscosities (Hayakawa et al 1997, 2004; Graybosch et al 2000, 2003; Sasaki et al 2000; Abdel-Aal et al 2002; Takata et al 2005; Sahlstrom et al 2006). Graybosch et al (2000) initially reported waxy wheat flours had extremely low FN values (mean 71.2 sec for 40 samples) independent of α-amylase activity. By comparing RVA pasting curves of waxy and nonwaxy wheat lines, these researchers attributed the low FN of waxy wheats to unique flour-starch pasting properties being more susceptible to breakdown under high temperature and mechanical shear conditions than those of nonwaxy counterparts. Hot-stage microscopic images (Fig. 1) gave visual evidence that waxy starch granules swelled more rapidly, ruptured more extensively even without mechanical shear, and dispersed more readily in solution than nonwaxy counterparts. As shown in Fig. 2, starch slurry gelatinized almost immediately in a block preheated to 95°C, and the viscosity of the slurries increased dramatically. Meanwhile, heat-stable α-amylase tended to reduce viscosity by liquefying the gelatinized starch. There was a

balance between gelatinization and liquefaction, which led to peak viscosity. When gelatinization dominated, viscosity increased until reaching a peak value. Viscosity decreased gradually after peak value when the slurries were stirred continuously and the block temperature was maintained at a constant 95°C. For waxy wheat cultivars, gelatinized starch granules were more susceptible to breakdown under liquefaction conditions; thus, starch molecules were more extensively exposed and more accessible to heat-stable α-amylase, so lower peak viscosities were obtained. Peak viscosities appeared at the initiation of liquefaction (peak times <20 sec for all cultivars in Fig. 2). No difference ($P > 0.05$) was observed in final viscosities (at 10 min, Fig. 2) among waxy and nonwaxy cultivars, indicating all gelatinized starch had been enzymatically liquefied. The novel mashing properties of waxy cultivars (Fig. 2 and Table III) reflect the unique pasting properties of the starch granules. Due to the low peak viscosity for waxy wheat during liquefaction, the dry-grind industry could thus increase the solids content in a mash, lower α-amylase dosages, or decrease energy requirements for stirring systems when waxy wheat is used as a feedstock.

Other performance advantages for waxy wheat fermentation were rate of fermentation, reduced nitrogen food requirement, and conversion efficiency. With nitrogen food supplements in the mash, waxy cultivars had faster rates of fermentation than nonwaxy counterparts. Shorter batch fermentation time would result in greater ethanol output, more savings in facility energy consumption, and less risk of bad production that must be recycled. Without nitrogen food supplements in the mash, the fermentation rates for waxy cultivars were comparable to those for nonwaxy cultivars with nitrogen food supplements. This should be very beneficial to ethanol producers because the cost of exogenous nitrogen food could be avoided without loss of production rate. Regardless of higher levels of free sugars in grain, waxy cultivars had an overall higher conversion efficiency than nonwaxy cultivars. Results of this study afforded an approach for utilization of waxy wheat.

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LITERATURE CITED

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