Effects of Ground Corn Particle Size on Ethanol Yield and Thin Stillage Soluble Solids

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ABSTRACT

Cereal Chem. 84(1):6–9

The effects of ground corn particle size on ethanol yield and soluble solids in thin stillage was evaluated using a 2-L laboratory dry-grind procedure. The procedure was optimized for grinding, liquefaction, saccharification, and fermentation parameters. The optimized procedure was reproducible with a coefficient of variation of 3.6% in ethanol yield. Five particle size distributions of ground corn were obtained using a cross-beater mill equipped with five screens (0.5, 2, 3, 4, and 5 mm). Particle size had an effect on ethanol yield and on soluble solids concentration in thin stillage. The highest ethanol yield of 12.6 mL/100 mL of beer was achieved using a 0.5-mm screen in the cross-beater mill. Treatment using the 0.5-mm mill screen resulted in soluble solids concentration of 25.1 g/L and was higher than soluble solids concentrations obtained with other screens. No differences in soluble solid concentrations were observed in samples of thin stillage obtained from 2, 3, 4, and 5-mm screens which had a mean yield of 16.2 g/L. By optimizing particle size for maximum ethanol yield and minimum solids in thin stillage, dry-grind corn plants could realize reduced capital and operating costs.

Basic processes for making ethanol in the dry-grind corn process are grinding, cooking, liquefaction, saccharification, fermentation, distillation, and coproduct recovery. Grinding is done to reduce corn particle size. Ground corn is mixed with water to form slurry, which is cooked to break down the crystalline structure of starch granules. Enzymes are added to break down starch into sugars. Yeast ferments these sugars into ethanol during fermentation. At the end of fermentation, the resulting beer (mixture of water, ethanol, nonfermentable components of corn, and yeast) is transferred to a distillation column. Overflow from the distillation column is an ethanol and water mixture; underflow from the column is called whole stillage (nonfermentable components of corn, yeast, and water). The ethanol and water mixture is processed through molecular sieves to remove remaining water from ethanol. Whole stillage is centrifuged to produce thin stillage (water and soluble solids) and wet grains (suspended solids). Evaporator is used to concentrate thin stillage into syrup. Syrup is mixed with wet grains and dried to 12% moisture content to produce distillers dried grains with solubles (DDGS).

Fineness of grind influences amount of sugars formed due to variation in surface area of ground corn (Kelsall and Lyons 2003a). If particles are too large, starch granules are not gelatinized easily, forming fewer fermentable sugars. If particles are too small, the hammer mill needs more energy to grind corn, and it will increase soluble solids in thin stillage. Finer particles also reduce centrifuge efficiency and increase amount of soluble/insoluble solids in thin stillage. A viscous thin stillage due to higher soluble solids content places a greater load (amount of water evaporated per unit of steam supplied) on the evaporator. Thin stillage evaporation is an energy-intensive operation (Meredith 2004). It is possible to reduce capital and operating costs of a dry-grind ethanol plant by optimizing ground corn particle size for maximum ethanol yield and minimum soluble solids in thin stillage.

Elaborate laboratory studies have been conducted on fuel alcohol production from wheat, barley, oats, rye, sorghum, and triticale as feedstocks (Sosulski and Sosulski 1994; Ingledew et al 1995; Thomas and Ingledew 1995; Sosulski et al 1997; Wang et al 1997a; Zhan et al 2003), but few studies have resulted in laboratory procedures for fuel ethanol production from corn. To determine the effect of Bt corn, Dien et al (2002) used a laboratory corn dry-grind procedure using simultaneous saccharification and fermentation. Taylor (2002) used a separate saccharification and fermentation procedure to study modified dry-grind processes; however, the amount of enzyme was not optimized. Haefele et al (2004) used a procedure to determine effect of planting location on ethanol yield; however, ethanol yields were measured by CO_2 loss (indirect measure for ethanol yield). Singh and Graeber (2005) developed a 500-mL dry-grind procedure to evaluate effect of hybrid variability on ethanol yield. In small-scale (<500 mL) procedures, sample size is not large enough to evaluate thin stillage soluble solids. Therefore, to evaluate the effect of particle size on ethanol yield, sugar profiles, and soluble solids, there is a need to develop a 2-L laboratory corn dry-grind procedure using sequential saccharification and fermentation processes. The objectives were to develop a laboratory corn dry-grind procedure and use it to evaluate effects of particle size on ethanol yield and soluble solids in thin stillage.

MATERIALS AND METHODS

Experimental Material

Samples (2 kg) of a yellow dent corn hybrid (33A14 Pioneer Hi-Bred International, Johnston, IA) were grown during the 2002 crop season at the Agricultural Engineering Research Farm, University of Illinois at Urbana-Champaign, field-dried to 15% moisture content, and combine-harvested. Corn samples were hand-cleaned to remove broken corn and foreign material, packaged in plastic bags, and stored at 4°C until processing. Whole kernel moisture content was measured using a 103°C convection oven method (Approved Method 44-15A, AACC International 2000).

α-Amylase (α-amylase solution *Bacillus licheniformis*, type XII-A saline solution 500 to 1,000 U/mg of protein, 1,4-α-D-glucan-glucanohydrolase, 9000-85-5) and glucoamylase (amyloglucosidase from *Aspergillus niger*, glucoamylase, 1,4-α-D-glucan glucohydrolase, exo-1,4-α-glucosidase, 9032-08-0), were obtained from Sigma-Aldrich (St. Louis, MO). Active dry yeast (Fleischmann's Yeast, Fenton, MO), ammonium sulfate for fermentation, and 1*N* sulfuric acid were obtained locally.

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DOI: 10.1094/CCHEM-84-1-0006

Preparation of Mash

Corn samples were milled in a laboratory cross-beater mill (model Retsch SK 100, Glen Mills, Clifton, NJ) equipped with a 2.0-mm sieve. An electromagnetic vibratory feeder (model F-To, FMC Corporation, Homer City, PA) maintained a feed rate of 2.43 ± 0.11 kg/min to the grinding chamber of the mill. Moisture content of ground corn was measured using a two-stage convection oven method (Approved Method 44-18, AACC International 2000). Ground corn (500 g, db) and tap water (35°C) were combined to obtain 25% solids mash. Each run was made in a 3-L flask equipped with an overhead drive with paddle type agitator (model DHOD-182, Bellco Glass, Vineland, NJ).

Dry-Grind Process

A range of liquefaction temperatures, α-amylase levels, saccharification temperatures, and glucoamylase levels were tested to determine operating parameters (Table I). Samples were liquefied by increasing the temperature of the slurry and adding α-amylase. Slurry was held during liquefaction for 90 min with continuous agitation (150 rpm). The temperature was lowered to saccharification temperature and slurry was adjusted to pH 4.1 ± 0.5 with 1*N* sulfuric acid solution. Glucoamylase was added and the slurry was held for 2 hr with constant agitation (150 rpm). Saccharified slurry was cooled to 30°C. Ammonium sulfate (1.4 g/L) was added as a yeast nutrient to provide supplemental nitrogen concentration of 300 ppm in the mash. Yeast inoculum was prepared (using the procedure of Wang et al 1997b) by dispersing 11 g of active dry yeast in 99 mL of distilled water and agitated at 38°C for 20 min. It had a viable cell count of $\approx 2 \times 10^8$. Fermentation was conducted for 72 hr at 30°C with continuous agitation (50 rpm).

Parameter Variation for Process Optimization

Procedure was optimized for six parameters (Table I). For enzymes, liquefaction and saccharification temperatures were recommended by the enzyme companies. Recommended values for yeast inoculum and dry solids were from Wang et al (1997b). After 72 hr of fermentation, ethanol concentration was measured using HPLC. For yeast inoculum variation, ethanol concentrations were measured every hour for the first 8 hr and then every 12 hr for 72 hr of fermentation. Fermentation rates were calculated from the linear portion of the curve for the first 8 hr. Each treatment was repeated three times. A full-factorial randomized complete block design was used to optimize process parameters. Analysis of variance (ANOVA) and least significant difference (LSD) procedures were used to compare mean ethanol yields (SAS Institute, Cary, NC).

HPLC Analyses

Mash samples collected during fermentation and estimates of sugars, organic acids, and alcohols were determined using HPLC. Supernatant liquids were obtained with a centrifuge (model Durafuge 100, Precision, Winchester, VA) using a 50-mL tube, 5,000 rpm, and 2,306 × *g* force. Supernatant was filtered with a 0.2-μm syringe filter into 1-mL vials. This liquid was injected into an ion-exclusion column (Aminex HPX-87H, Bio-Rad, Hercules, CA) maintained at 50°C. Sugars, organic acids, and alcohols were eluted at a rate of 0.6 mL/min from the column with HPLC-grade water (deionized water at pH 7.0) containing 5 m*M* sulfuric aid. Separated components were detected with a refractive index detector (model 2414, Waters Corporation, Milford, MA). To determine ethanol concentration, HPLC data were processed using Breeze 3.2 software (Waters).

Reproducibility of Procedure

Using optimal parameters determined in this study (Table I), procedural standard deviation of measurement was found for the same hybrid used in the previous study. Ten samples (500 g each) of corn hybrid 33A14 (Pioneer Hi-Bred International, Johnston, IA) were processed using the 2-L dry-grind procedure, and ethanol concentrations were measured. Procedure coefficient of variation was determined.

Effects of Particle Size on Ethanol Yield and Thin Stillage Soluble Solids

Five samples (2 kg) of corn hybrid 33A14 (Pioneer Hi-Bred International, Johnston, IA) were ground using 0.5, 2, 3, 4, and 5-mm screens and a hammer mill as described above. Each sample was divided into triplicates of 500 g each. Each sample was processed for ethanol yield using optimal parameters (Table I) of the dry-grind procedure.

Particle Size Analysis

Particle size analysis of ground corn samples was conducted using a sonic sifter (model L3P, ATM Advan Tech Manufacturing, New Berlin, WI) equipped with standard sieves with openings of 841 (US No 20), 595 (US No 30), 420 (US No 40), 250 (US No 60), 180 (US No 80), and 125 (US No 120) μm (ASAE Standards 2002). Each sample was sifted in triplicate and means were reported. The Suhm (1969) method was used to analyze particle size. The ASAE method (S319.3) was used to express ground corn particle size. Commercial ground corn samples were obtained from two Midwestern dry-grind corn plants (IL and MN) using two different sieve sizes (2.3 and 3.5 mm). Particle size distributions of commercial ground corn samples were measured using the procedures described above.

Soluble Solids Measurement

Fermented slurry (beer) was heated to 80°C and held for 2 hr to evaporate ethanol. The remaining portion (whole stillage) was filtered through a US No. 200 screen (74 μm) to obtain a liquid fraction (thin stillage) and a semisolid fraction (wet distillers grains). Subsamples (20 mL each) of thin stillage were filtered through a standard glass fiber filter (1 μm pore size, Pall Corporation, East Hills, NY) and dried to constant weight at 180°C.

TABLE I
Range of Process Parameters Evaluated, Recommended, and Selected for Dry-Grind Ethanol Procedure

Process Parameter	Range				Recommended Value	Selected Value
	A	B	C	D		
Enzyme conc. (U/g of corn, db)						
α-amylase	4.3	8.6	21.4	42.8	21.4	42.8
Glucoamylase	0.15	0.3	0.6	0.9	0.6	0.6
Temperature (°C)						
Liquefaction	80	85	90	95	90	85
Saccharification	50	55	60	65	60	55
Dry solids (%)	20	25	30	35	25	25
Amount of yeast inoculum (g of yeast/g of corn, db)	0.001	0.002	0.003	0.004	0.002	0.004

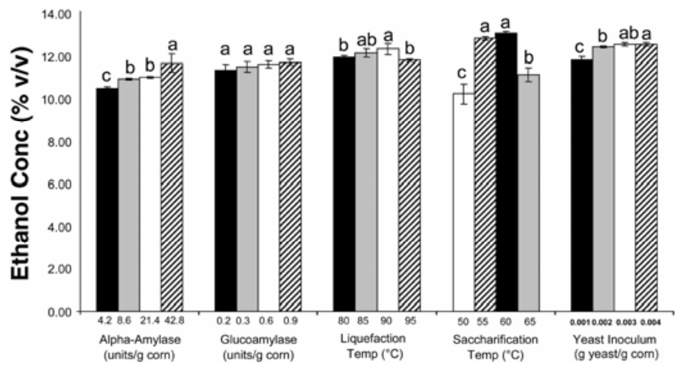

Fig. 1. Final ethanol concentration and variation in dry-grind procedure parameters. Mean concentrations with same letter are not different ($P < 0.05$). Error bars are ± 1 SD.

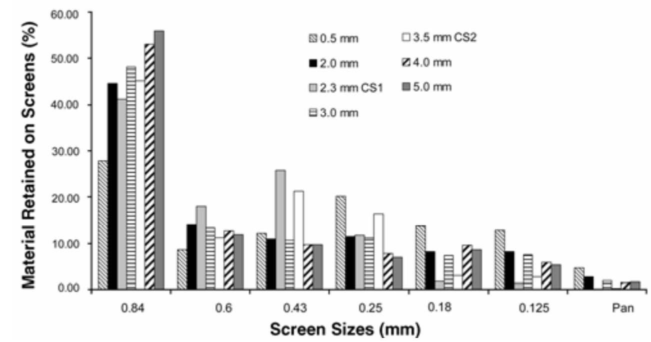

Fig. 3. Particle size distributions of ground corn from different size laboratory hammer-mill screens. CS1 and CS2 were ground corn samples obtained from dry-grind corn plants using 2.3- and 3.5-mm hammer-mill screens, respectively.

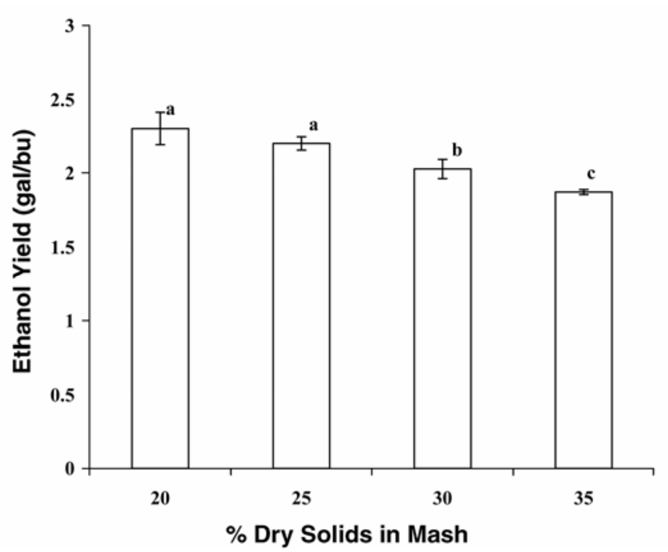

Fig. 2. Ethanol yield (gal/bu) and variation in mash solids. Mean ethanol yields with same letter are not different ($P < 0.05$). Error bars are ± 1 SD.

The increase in dish weight represented the total soluble solids (Method 2540C, APHA 1998). Soluble solids content for each sample of thin stillage was determined in triplicate, and mean values were reported.

RESULTS AND DISCUSSION

Dry-Grind Laboratory Procedure

Dry-grind laboratory procedure parameters were optimized (Table I). Recommended conditions or (if available) already optimized parameters were used for each experiment as stated in Table I. α-Amylase activity affected final ethanol concentrations (Fig. 1). Final ethanol concentration increased from 10.5 to 11.6% (v/v) as α-amylase concentration increased. An α-amylase concentration of 42.8 U/g of corn was selected for the dry-grind laboratory procedure. Glucoamylase activity did not affect ethanol concentrations (Fig. 1). Glucoamylase concentration of 0.6 U/g of corn (as recommend by the enzyme manufacturer) was selected for the procedure. Lower ethanol concentrations were observed for 80 and 95°C liquefaction temperatures compared with 90°C. No differences were observed between 85 and 90°C liquefaction temperatures. Because there were lower evaporative losses at 85°C than with 90°C, a lower liquefaction temperature of 85°C was selected for further experimentation. No differences were observed between 55 and 60°C saccharification temperature. Saccharification at 55°C was selected for further experimentation.

Differences in final ethanol concentrations were observed when yeast inoculum was increased from 0.002 (recommended value) to 0.004 g of yeast/g of corn (Fig. 1). Initial rates of fermentation (first 8 hr) for different yeast inoculum were different and increased as the amount of inoculum increased from 0.001 to 0.004 g of yeast/g of corn. The highest initial rate of fermentation was observed at a yeast inoculum of 0.004 g of yeast/g of corn; therefore it was selected for further experimentation. As mash solids increased, ethanol yields decreased (Fig. 2). Higher ethanol yields were observed for 20 and 25% solids concentration compared with 30% solids concentration or higher. The recommended value of 25% (Wang et al 1997b) was selected for mash solids.

The reproducibility study of the procedure used the selected parameters (Table I). Ten replicates gave a coefficient of variation of 3.6%, which suggested the procedure was reproducible and could be used to observe the effect of particle size on ethanol concentration.

Effect of Particle Size on Ethanol Yield and Soluble Solids in Thin Stillage

Similar trends in ground corn particle size distributions were observed from laboratory and commercial hammer mills (Fig. 3). Some differences were observed for the material retained on sieve (0.425 mm). More ground corn material was retained on sieve 0.425 mm for commercial samples than for laboratory ground corn samples. Overall, the particle size distribution of laboratory samples was similar to commercial samples.

Final ethanol concentrations increased as ground corn particle size decreased (Fig. 4). Ethanol concentrations increased from 10.4 to 11.2% (v/v) for treatments using a 3-mm screen compared with a 5-mm screen. An ethanol concentration of 12.6% (v/v) beer was produced from the 0.5-mm mill screen and was higher than ethanol concentrations for other treatments. Overall, as the cross-beater mill screen size decreased from 5 to 0.5 mm, ethanol concentration increased from 10.4 to 12.6% (v/v) beer, which represents a 22% increase in ethanol concentration. This increase in ethanol concentration due to smaller particle size is important for dry-grind operations. It is estimated that a 0.5% (v/v) increase in ethanol concentration could result in $0.5 M/yr additional revenue for a 40 million gal/yr plant (Kelsall and Lyons 2003b).

No effect of particle size on thin stillage soluble solids was observed using most screens (Fig. 5). However, higher soluble solids were observed when using the 0.5-mm screen, compared with other treatments. Soluble solids concentration in thin stillage of 25.1 g/L was observed for a 0.5-mm screen. The mean soluble solids concentration in thin stillage for 2, 3, 4, and 5-mm screens was 16.2 g/L. Soluble solids concentration of thin stillage in dry-grind corn plants varies from 24 to 45 g/L (Ingledew 2003).

There is considerable variation in the screen size used in hammer mills in U.S. dry-grind corn plants. Screen sizes varied from 2 to

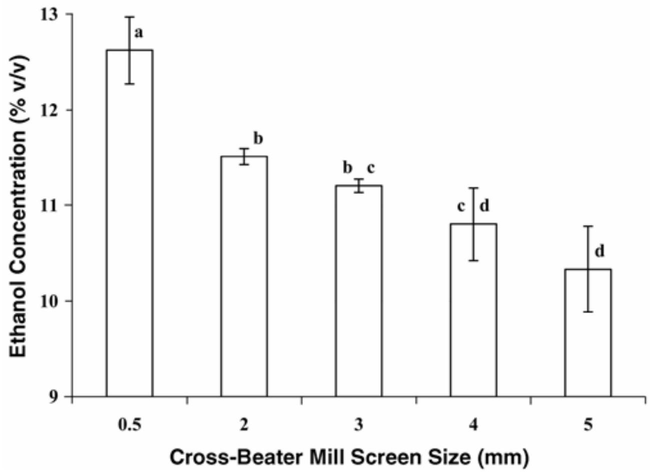

Fig. 4. Effect of grinding treatment on ethanol yield. Mean concentrations with the same letters are not different ($P < 0.05$).

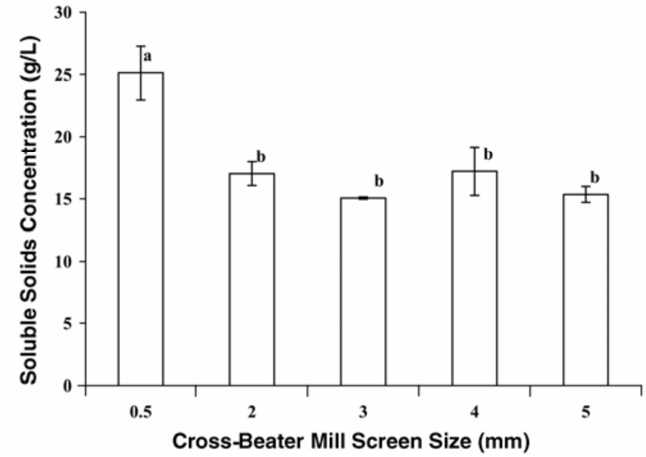

Fig. 5. Effect of grinding treatment on soluble solids concentration in thin stillage. Mean yields with the same letters are not different ($P < 0.05$).

5 mm for dry-grind corn plants in IL, MN, MO, and SD. Plants using larger screens (≥3 mm) will benefit by replacing their screens with 2-mm screen size. Changing screens will increase ethanol yield and not affect thin stillage soluble solids. Use of screens <2 mm could increase ethanol yields; however, it will affect thin-stillage soluble solids concentration and might affect evaporator economy.

CONCLUSIONS

Ethanol yield was affected by variation in α-amylase enzyme activity, saccharification, and liquefaction temperatures, mash solids concentration, and yeast inoculum. Using selected parameters that gave high ethanol yields, a 2-L dry-grind laboratory procedure had CV 3.6%. Grinding treatment had an effect on ethanol yield and on soluble solids concentration in thin stillage. Final ethanol concentration increased as ground corn particle size decreased. A 22% increase in ethanol concentration was observed as the grinding screen size decreased from 5 to 0.5 mm. Higher soluble solids concentration (25.1 g/L) in thin stillage was observed for treatment using 0.5-mm screen compared with soluble solids concentration (16.2 g/L) for 2, 3, 4, and 5-mm screens. Dry-grind plants can increase final ethanol concentrations by using a 2-mm screen without affecting thin stillage soluble solids concentrations.

ACKNOWLEDGMENTS

Supported in part by Specific Cooperative Research Agreement No. 1935-41000-059-01S with the Eastern Regional Research Center, Agricultural Research Service, U.S. Department of Agriculture.

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[Received January 30, 2006. Accepted June 23, 2006.]