Comparison of Modified Dry-Grind Corn Processes for Fermentation Characteristics and DDGS Composition

Vijay Singh,[1,2] David B. Johnston,[3] Kalpana Naidu,[1] Kent D. Rausch,[1] Ronald L. Belyea,[4] and M. E. Tumbleson[1]

ABSTRACT

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Three different modified dry-grind corn processes, quick germ (QG), quick germ and quick fiber (QGQF), and enzymatic milling (E-Mill) were compared with the conventional dry-grind corn process for fermentation characteristics and distillers dried grains with solubles (DDGS) composition. Significant effects were observed on fermentation characteristics and DDGS composition with these modified dry-grind processes. The QG, QGQF, and E-Mill processes increased ethanol concentration by 8–27% relative to the conventional dry-grind process. These process modifications reduced the fiber content of DDGS from 11 to 2% and increased the protein content of DDGS from 28 to 58%.

The U.S. ethanol production capacity is projected to increase to more than 6.0 billion gallons per year by the end of 2006 (MacDonald et al 2003). Most of this increase in the ethanol capacity from a current capacity of 3.1 billion gallons will come from new dry-grind corn plants. Using the conventional process, increases in dry-grind ethanol production means the supply of distillers dried grains with solubles (DDGS) will increase proportionately. Due to its high fiber content, DDGS is sold for ruminant animal diets. To maintain demand and supply balance, there is a need to reduce the volume of DDGS and diversify its markets. If ethanol production more than doubles by 2006, the current low market value of DDGS will decrease and utilization will become more difficult.

New process modifications have been developed such as quick germ (QG) (Singh and Eckhoff 1996, 1997) and quick germ quick fiber processes (QGQF) (Singh et al 1999a; Wahjudi et al 2000). These process modifications allow removal of germ and pericarp fiber as coproducts at the beginning of the dry-grind corn process. Benefits of the QG and QGQF processes are 1) recovery of high quality germ for recovery of corn germ oil and fiber for corn fiber oil; and 2) removal of germ and fiber to increase the protein content of the residual DDGS after fermentation (Singh and Eckhoff 1997; Singh et al 1999a; Taylor et al 2001).

Another process modification called enzymatic milling (E-Mill) has been developed which is an improvement of the QG and QGQF processes and also allows recovery of endosperm fiber as a valuable coproduct. The E-Mill process involves soaking corn kernels for a short period of time (6–12 hr) followed by coarse grinding and incubating with protease and starch-degrading enzymes for 2–4 hr. After incubation, QG and QGQF processes are used to recover germ and pericarp fiber. The remaining slurry is screened on a 200-mesh sieve (0.074 mm openings) to recover endosperm fiber. The E-Mill process allows recovery of germ, pericarp fiber, and endosperm fiber as valuable coproducts at the beginning of the process before fermentation. Removal of endosperm fiber at the beginning of the dry-grind ethanol process will further increase fermentation capacity and should reduce fiber in DDGS and increase protein content of the DDGS. A reduction in fiber content and increase in protein content of DDGS could potentially allow ethanol producers to sell DDGS as nonruminant foodstuff.

Another potential benefit of these modified milling processes is additional production of ethanol per batch. In these modified dry-grind processes, nonfermentables (germ, pericarp fiber, and endosperm fiber) are removed. These nonfermentables can be replaced by a more ferementable substrate. Plants performing these modified milling processes can potentially increase the amount of corn processed and therefore produce more ethanol per batch compared with the conventional dry-grind process.

The objective of this study was to compare modified dry-grind corn processes (QG, QGQF, and E-Mill) with the conventional dry-grind corn process and determine their effects on fermentation characteristics and DDGS composition.

MATERIALS AND METHODS

Experimental Material

A yellow dent corn hybrid grown during the 2002 crop season at the Agricultural Engineering Research Farm, University of Illinois at Urbana-Champaign, was field dried to ≈15.0% 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 2000).

Conventional Dry-Grind Laboratory Process

Conventional dry-grind processing used a 500-g laboratory procedure. Corn samples were ground at 500 rpm in a laboratory hammer mill (model MHM4, Glen Mills, Clifton, NJ) equipped with a 2.0-mm sieve. Ground corn weighing 500 g (as is) was mixed with tap water at 35°C to obtain mash with 25% solids (db). Initial pH of the mash was 5.7 ± 0.1. All experiments were performed in 3L flasks with overhead drives (model DHOD-182, Bellco Glass, Vineland, NJ) for agitation. Samples were liquefied by increasing the temperature of the slurry to 85 ± 1°C and adding 1 mL of α-amylase obtained from Sigma-Aldrich (α-amylase solution *Bacillus licheniformis*, type XII-A saline solution 500–1,000 units/mg of protein, 1,4-α-D-glucan-glucanohydrolase, 9000-85-5). Slurry was held at 85°C for 90 min with continuous agitation at 150 rpm. After 90 min, slurry temperature was decreased to 60°C. Slurry was adjusted to pH 4.1–4.2 with 1*N* sulfuric acid solution. Saccharification of samples was done by adding 1 mL of glucoamylase obtained from Sigma-Aldrich (amyloglucosidase from *Aspergillus niger*, glucoamylase, 1,4-α-D-glucan glucohydrolase, exo-1,4-α-glucosidase, 9032-08-0) and holding the slurry at 60°C for 2 hr with constant agitation at 150 rpm. For fermentation, the saccharified mash was cooled to 30°C. Yeast (Fleischmann's Yeast,

[1] Department of Agricultural and Biological Engineering, University of Illinois, 360G, AESB, 1304 West Pennsylvania Avenue, Urbana, IL 61801.
[2] Corresponding author. Phone: 217-333-9510. Fax: 217-244-0323. E-mail: vsingh@uiuc.edu
[3] U.S. Department of Agriculture, Eastern Regional Research Center, Agricultural Research Service, 600 E. Mermaid Lane, Wyndmoor, PA 19038. Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by the USDA implies no approval of the product to the exclusion of others that may also be suitable.
[4] Animal Science Department, University of Missouri, Columbia, MO 65211.

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Fenton, MO) inoculum was prepared using the procedure by Wang et al (1997) by dispersing active dry yeast (11 g) in 99 mL of distilled water and agitating at 38°C for 20 min. It had a viable cell count of ≈2 × 10^8. Yeast innoculum (2 mL) was added to the saccharified mash. Ammonium nitrogen (300 ppm) was provided by adding $(NH_4)_2SO_4$ as yeast nutrient.

Fermentation was conducted for 72 hr at 30°C with continuous agitation at 50 rpm. Fermentation was monitored by taking 5 mL samples every 12 hr and measuring ethanol concentrations using HPLC. From each 5-mL sample, clear supernatant liquid was obtained by centrifuging the sample at 2,500 rpm (model Durafuge 100, Precision, Winchester, VA). Supernatant was passed through a 0.2-µm syringe filter into 1-mL vials. Filtered 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 from the column with HPLC-grade water containing 5 m*M* sulfuric acid. The elution rate was 0.6 mL/min. Separated components were detected with a refractive index detector (model 2414, Waters Corporation, Milford, MA). Data was processed using HPLC software (Waters Corporation).

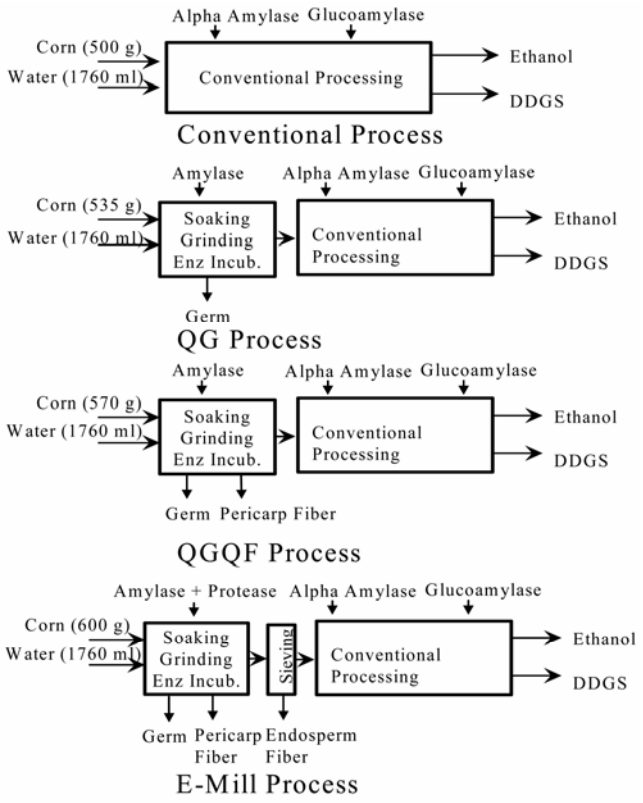

Fig. 1. Schematic of conventional, quick germ (QG), quick germ quick fiber processes (QGQF), and enzymatic milling (E-Mill) dry-grind corn processes.

After 72 hr of fermentation, mash was heated to 85°C for 2 hr to evaporate ethanol. Stillage (mash left after boiling) was poured into 2L flat-bottom, open aluminum pans and dried in a convective oven for 24 hr at 59°C. Dried stillage was called distillers dried grains with solubles (DDGS) and analyzed for crude protein (Method 990.03, AOAC 2003), crude fat (Method 920.39, AOAC 2003), ash (Method 942.05, AOAC 2003), and acid detergent fiber (Method 973.18, AOAC 2003). The moisture content of the DDGS was determined using the two-stage convection oven method (Approved Method 44-18, AACC 2000).

QG and QGQF Laboratory Processes

The QG process was performed as described by Singh and Eckhoff (1996) and the QGQF process was done as described in Singh et al (1999a). Both QG and QGQF procedures were modified slightly to maintain the specific gravity of slurry for recovery of germ and fiber. The modification included addition of 3 mL of enzyme (α-amylase, *Bacillus Amyloliquefaciens*, 1,4-α-D-glucan glucanohydrolase, 9000-85-5, MFCD00081319), incubation of the slurry for 4 hr after soaking, and coarse grinding of the corn kernels. Water used in the QG and QGQF process was the same amount as used in the conventional dry-grind process. Because the QG process recovers 7% germ (7% of original corn solids) (Singh and Eckhoff 1996), the amount of corn used in this study for the QG treatment was increased by 7% compared with the conventional process. For the QGQF process, an additional 14% corn was used because of removal of pericarp fiber in addition to germ. For QG and QGQF treatments, 535 and 570 g of corn were used, respectively. After germ and coarse fiber recovery, samples were liquefied, saccharified, and fermented using the conventional dry-grind procedure described above. Dried material recovered at the end of the QG and QGQF processes was called DDGS.

E-Mill Laboratory Process

For the E-Mill process, the amount of corn was increased by 20% compared with the amount used for the conventional process because of removal of germ, pericarp fiber, and endosperm fiber. The E-Mill procedure began by soaking 600-g samples for 12 hr. After soaking, samples were ground coarsely and incubated with starch-degrading enzymes for 2 hr (55°C and pH 5.0) and proteolytic enzymes (GC106, Genencor International, Palo Alto, CA) for 2 hr (45°C and pH 5.0) with intermittent gentle mixing of the sample every 30 min. After the enzyme incubation step, germ and pericarp fiber were recovered using the same procedure as used in QGQF process. The slurry was screened through a standard 200-mesh sieve (0.074 mm openings) and washed with 100 mL of distilled water to recover endosperm fiber. Amount of water in the E-Mill process was controlled to maintain the same amount of water as used in the conventional dry-grind process. The remaining material was liquefied, saccharified, and fermented using the conventional dry-grind procedure described above. Dried material recovered at the end of the E-Mill process was called DDGS.

Schematics of conventional and modified dry-grind processes (QG, QGQF, and E-Mill) are shown in Fig. 1. Process parameters

TABLE I
Process Parameters for Conventional and Modified Dry-Grind Ethanol Technologies

	Conventional Process	Quick Germ Process	Quick Germ and Quick Fiber Process	Enzymatic Milling Process
Corn (g)	500	535	570	600
Soak time (hr)	na[a]	12	12	12
Enzyme incubation time (hr)	na	4	4	4
Enzymes used for incubation	na	α-Amylase	α-Amylase	α-Amylase + Protease
Specific gravity of slurry before incubation (Baume)	na	1.0462 (6.4)	1.0484 (6.7)	1.0469 (6.5)
Specific gravity of slurry after incubation (Baume)	na	1.062 (8.5)	1.066 (9.1)	1.075 (10.2)

[a] Not applicable.

of conventional and modified processes are given in Table I. All treatments (conventional dry-grind, QG, QGQF, and E-Mill) were conducted with two replicates. Fermentation samples from both replicates were analyzed using HPLC with at least two determinations. HPLC analyses for each replicate were averaged. Fermentation profiles were generated and rate of fermentation was calculated for the linear portion of the fermentation profiles. Ethanol yields were calculated based on the final average ethanol concentrations and the amount of initial corn processed. Analysis of variance (ANOVA) and Duncan's multiple range test (SAS Institute, Cary, NC) were used to compare means of crude protein, crude fat, ash, and acid detergent fiber in the DDGS. The level selected to show statistical significance was 5% ($P < 0.05$).

RESULTS AND DISCUSSION

Fermentation Results

Significant differences were observed in fermentation profiles of modified dry-grind technologies (QG, QGQF, and E-Mill) compared with the conventional process (Fig. 2). As more nonfermentables (germ, pericarp fiber, and endosperm fiber) were removed from the mash and replaced with fermentable substrate (corn), higher ethanol concentrations were achieved. Higher ethanol concentrations (8–27%) were obtained with modified dry-grind ethanol processes compared with the conventional process. Rates of fermentation for modified dry-grind processes increased from 1.41 to 3.77 g/L/hr as more nonfermentables were removed (Fig. 2). The likely reason for this increase was better mixing of mash due to removal of suspended solids and more rapid heat transfer leading to potentially higher rates of reaction (Taylor et al 2001). For E-Mill and QGQF processes, fermentation reached a peak at 36–48 hr compared with the QG process and conventional processes, which peaked at 60–72 hr. The highest rate of fermentation was achieved for the E-Mill process (3.77 g/L/hr) because of a lower amount of suspended solids and due to addition of protease GC106, which is known to help the rate of fermentation by hydrolyzing protein into free amino nitrogen (Lantero and Fish 1993). GC106 enzyme also provides better separation between starch and protein by breaking down proteins that surround starch particles (Johnston and Singh 2001; Johnston et al 2003).

Final ethanol concentration was higher for all modified dry-grind processes compared with the conventional dry-grind process. However, ethanol yields for all modified dry-grind processes were lower compared with the conventional dry-grind process (Fig. 2). Ethanol yields were lower because there is loss of starch during recovery of coproducts. Ethanol yields for the QG and QGQF processes were ≈6% lower compared with the conventional dry-grind process. Ethanol yield of the E-Mill process was higher than QG and QGQF processes but lower than the conventional dry-grind process. Higher ethanol yield of the E-Mill process compared with the QG and QGQF processes was probably due to addition of the protease GC106, which previously has been shown to reduce the loss of starch in fiber (Johnston and Singh 2001; Singh and Johnston 2004) and increases the ethanol yield in the dry-grind process (Lantero and Fish 1993).

Theoretically, removal of germ in modified dry-grind processes could cause foaming of process material. In the conventional dry-grind process, oil (in germ) floats on the top of fermentation broth and reduces air contact with proteins, which could lead to foaming. However, no foaming was observed during any modified dry-grind fermentation processes. These results are in agreement with the previous work done by Taylor et al (2001) on the QG process. The reason no foaming is observed is that the germ was not recovered completely in modified dry-grind processes; some remained in the mash, resulting in small amounts of oil in the fermentation broth. Removal of germ (oil) will reduce the fouling of heat transfer surfaces in the dry-grind process as previously reported by Singh et al (1999b) and hypothesized by Taylor et al (2001).

DDGS Composition

Effect of all three modified milling processes on DDGS composition was significant (Table II). Protein content of DDGS material was 28, 36, 49, and 58% for conventional, QG, QGQF, and E-Mill processes, respectively. Protein content of DDGS for the E-Mill process (58%) was higher than protein content of other high protein foodstuffs such as soybean meal (54%) (Table II).

Depending upon the process modification, fat content of DDGS decreased compared with fat content of the conventional DDGS. The fat content of DDGS material was 12.7, 4.8, 3.8, and 4.5% for conventional, QG, QGQF, and E-Mill processes, respectively. No differences were observed in ash contents of DDGS. Due to process modifications, acid detergent fiber (ADF) content of DDGS was reduced. Compared with conventional DDGS, the ADF content was reduced by 24% for the QG process, 37% for the QGQF process, and 81% for the E-Mill process (Table II).

Higher protein and lower fiber content can diversify DDGS as a more valuable foodstuff for nonruminant animals. This is important

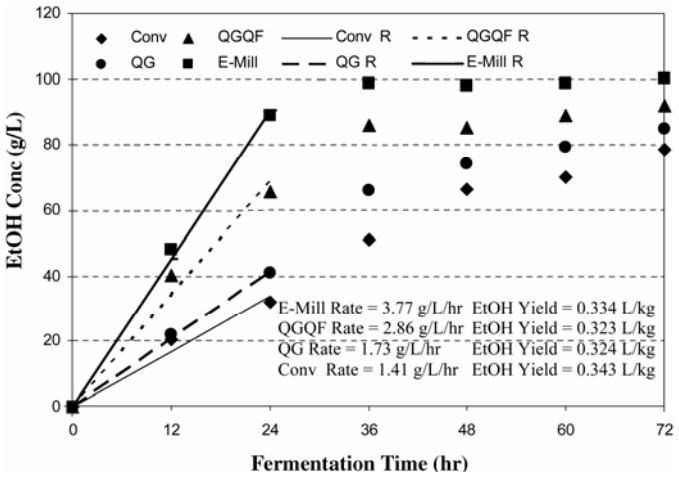

Fig. 2. Ethanol concentrations vs. fermentation times for conventional (Conv), quick germ (QG), quick germ quick fiber (QGQF), and enzymatic milling (E-Mill) dry-grind ethanol processes. Rate of fermentation for conventional (Conv R), quick germ (QG R), quick germ quick fiber (QGQF R), and enzymatic milling (E-Mill R).

TABLE II
Distillers Dried Grains with Solubles (DDGS) Composition (db) for Conventional, Quick Germ, Quick Germ and Quick Fiber, and Enzymatic Milling Dry-Grind Ethanol Processes[a]

	Conventional Process	Quick Germ Process	Quick Germ and Quick Fiber Process	Enzymatic Milling Process	Corn Gluten Meal	Soybean Meal
Crude protein (%)	28.50d	35.91c	49.31b	58.50a	66.70	53.90
Crude fat (%)	12.70a	4.83b	3.85b	4.53b	2.77	1.11
Ash (%)	3.61ab	4.05a	4.13a	3.24b	–	–
Acid detergent fiber (%)	10.8a	8.22b	6.80c	2.03d	6.88	5.95

[a] Values followed by the same letter in the same row are not significantly different ($P < 0.01$).

TABLE III
Coproduct Yields[a] from Quick Germ, Quick Germ and Quick Fiber, and Enzymatic Milling Dry-Grind Ethanol Technologies

Coproducts	Quick Germ Process	Quick Germ and Quick Fiber Process	Enzymatic Milling Process
Germ (%)	6.1 ± 0.17	7.7 ± 0.24	8.1 ± 0.23
Pericarp fiber (%)	–	9.1 ± 0.16	10.2 ± 1.10
Endosperm fiber (%)	–	–	4.6 ± 0.56

[a] Mean ± standard deviation.

because the predicted growth in the ethanol industry by the California Energy Commission (MacDonald et al 2003) could lead to the overproduction of conventional DDGS and limited market demand as ruminant foodstuff.

Coproducts from Modified Dry-Grind Processes

In addition to DDGS, several coproducts can be produced from modifying the conventional dry-grind process. Germ can be recovered from the QG process; germ and pericarp fiber can be recovered from the QGQF process; germ, pericarp fiber, and endosperm fiber can be recovered from the E-Mill process (Table III). Pericarp fiber from QGQF or E-Mill processes can be used as feedstock for other valuable coproducts (Moreau et al 1999; Singh et al 1999a; Hicks and Moreau 2001). Germ recovery was 6.1, 7.7, and 8.1% from QG, QGQF, and E-Mill processes, respectively. Higher germ recovery for the QGQF and E-Mill processes compared with the QF process was due to a higher amount of corn processed and to germ and pericarp fiber being recovered together, which increased yields of germ and fiber. A higher germ recovery for E-Mill process was due to a higher amount of corn processed, as well as improved specific gravity of the slurry caused by protease addition (Table I). Pericarp fiber recovery also was higher for E-Mill compared with QGQF due to a higher amount of corn and higher specific gravity achieved in the E-Mill process.

E-Mill process allows recovery of pericarp fiber and endosperm fiber separately. Researchers have shown that there are potentially valuable gums in corn fiber valued at $2.20 to 4.40/kg ($1 to 2/lb) (Doner and Hicks 1997; Doner et al 1998); compositions and properties of these gums derived from pericarp fiber and endosperm fiber fractions (Singh et al 2000) are different. Therefore, E-Mill allows selective extraction of unique fiber fractions for gum manufacture. Recovery of valuable coproducts is important for the dry-grind ethanol industry. These coproducts can reduce the cost of ethanol production.

CONCLUSIONS

Significant effects of modified dry-grind ethanol processes were observed on fermentation characteristics and DDGS composition. Higher rates of fermentation and 8–27% higher ethanol concentrations were obtained with modified dry-grind ethanol processes compared with the conventional process. Depending on the modification, protein contents of DDGS increased from 28 to 58% and acid detergent fiber content was reduced from 11 to 2%, relative to conventional dry-grind process. High protein and low fiber DDGS recovered from the E-Mill process could be used as a nonruminant foodstuff. This is particularly important because current growth in ethanol industry could lead to overproduction of conventional DDGS and limited market utilization as ruminant foodstuff.

These process modifications increase final ethanol concentration of conventional dry-grind ethanol plants, allow recovery of valuable coproducts, and improve nutritional characteristics of DDGS. Together, these advantages could increase profitability of ethanol production.

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