Evaluation and Strategies to Improve Fermentation Characteristics of Modified Dry-Grind Corn Processes

Ganti S. Murthy,[1] Vijay Singh,[1,2] David B. Johnston,[3] Kent D. Rausch,[1] and M. E. Tumbleson[1]

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ABSTRACT

New corn fractionation technologies that produce higher value coproducts from dry-grind processing have been developed. Wet fractionation technologies involve a short soaking of corn followed by milling to recover germ and pericarp fiber in an aqueous medium before fermentation of degermed defibered slurry. In dry fractionation technologies, a dry degerm defiber (3D) process (similar to conventional corn dry-milling) is used to separate germ and pericarp fiber before fermentation of the endosperm fraction. The effect of dry and wet fractionation technologies on the fermentation rates and ethanol yields were studied and compared with the conventional dry-grind process. The wet process had the highest fermentation rate. The endosperm fraction obtained from 3D process had lowest fermentation rate and highest residual sugars at the end of fermentation. Strategies to improve the fermentation characteristics of endosperm fraction from 3D process were evaluated using two saccharification and fermentation processes. The endosperm fraction obtained from 3D process was liquefied by enzymatic hydrolysis and fermented using either separate saccharification (SS) and fermentation or simultaneous saccharification and fermentation (SSF). Corn germ soak water and B-vitamins were added during fermentation to study the effect of micronutrient addition. Ethanol and sugar profiles were measured using HPLC. The endosperm fraction fermented using SSF produced higher ethanol yields than SS. Addition of B-vitamins and germ soak water during SSF improved fermentation of 3D process and resulted in 2.6 and 2.3% (v/v) higher ethanol concentrations and fermentation rates compared with 3D process treatment with no addition of micronutrients.

With increasing dependence on foreign oil, energy security of the United States can be achieved only by domestic alternatives for energy sources. Ethanol production from corn is an alternative that promotes local economies and reduces dependence on foreign oil. About 65% of U.S. domestic fuel ethanol production (estimated to be ≈14.74 billion L/yr) is from the dry-grind corn process industry, one of the fastest growing commodity industries (Lyons 2003). In a conventional dry-grind process, corn is ground and mixed with water to produce slurry. The slurry is cooked; starch in the slurry is liquefied, saccharified, and fermented to produce ethanol. The remaining nonfermentables in corn (germ, fiber, and protein) are recovered together at the end of the dry-grind process as an animal food coproduct called distiller dried grains with solubles (DDGS). Due to the rapid growth of the dry-grind industry, the ruminant market for DDGS is reaching saturation levels. One way to reduce the volume of the DDGS coproduct is to fractionate corn kernel to recover germ and fiber before fermentation.

Fractionation technologies have been developed for the dry-grind industry and can be broadly divided into wet and dry technologies based on the presence or absence of a corn soaking step. Wet technologies such as enzymatic dry-grind process (E-Mill process) (Singh et al 2005) (Fig. 1) involve a soaking step. Dry technologies such as dry degerm defiber process (3D process) (Murthy et al 2004; Bryan 2005) (Fig. 2) do not involve corn soaking. The E-Mill process produces germ, pericarp fiber, and endosperm fiber as coproducts, whereas the 3D process produces germ and pericarp fiber as coproducts.

These coproducts can be used to extract other valuable coproducts such as corn oil obtained from corn germ and corn fiber oil obtained from pericarp and endosperm fiber (Singh et al 1999. Corn fiber oil contains unique compounds: ferulate phytosterol esters (FPE), free phytosterols (St), and phytosterol fatty acyl esters (St:E) (Moreau et al 1996). Corn fiber oil can lower serum cholesterol levels in blood in laboratory animals (Moreau et al 1999), demonstrating nutraceutical value.

Separation of germ, fiber, and endosperm is not complete in these fractionation processes as some starch is lost with coproducts. Starch loss to coproducts reduces the value of germ and pericarp fiber and could lower ethanol yields. Germ removal in dry fractionation processes also results in reduction of nutrients in the fermentation medium. During a soaking step, water-soluble proteins and other micronutrients leach out into the soak water which is reused in the wet process. However, in the dry technologies, soluble proteins and micronutrients are removed with the germ. These constituents may be necessary for proper yeast nutrition and normal fermentation rates. Removal of these constituents could result in lower fermentation rates and final ethanol concentrations for the endosperm fraction produced from dry fractionation processes. Fermentation rate as measured by production of ethanol and final ethanol concentration are important fermentation characteristics for dry-grind corn process. The effect of dry and wet processes on these fermentation characteristics will provide an insight into the impact of these fractionation technologies on the overall economics of the dry-grind plant.

The objectives of this study were to 1) evaluate fermentation characteristics of a dry (3D process) and a wet fractionation processes (E-Mill process) by comparing ethanol yields and rates of fermentation of the endosperm fraction; 2) investigate the effect of addition of B-vitamins and germ soak water on improving the fermentation characteristics of endosperm fraction obtained from 3D processing.

MATERIALS AND METHODS

Materials

Yellow dent corn grown during the 2003 crop season at the Agricultural and Biological Engineering Research Farm, University of Illinois at Urbana-Champaign was used for the study. Samples were hand-cleaned and moisture content was determined using a standard two-stage convection oven method (AACC International 2000). Corn proximate analysis as determined by near-infrared spectrometer (model OmegaAnalyserG, Dickey John, Springfield, IL) was 15.4% moisture, 7.7% protein, 5.1% oil, and 71.9% starch.

The α-amylase (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, Sigma-Aldrich, St. Louis,

[1] Agricultural and Biological Engineering Department, University of Illinois at Urbana-Champaign, University of Illinois, Urbana, IL 61801.
[2] Corresponding author. Phone: 217-333-9510. Fax: 217-244-0323. E-mail: vsingh @uiuc.edu
[3] Eastern Regional Research Center, ARS, USDA, Wyndmoor, PA. Mention of brand or firm names does not constitute an endorsement by University of Illinois or USDA above others of similar nature not mentioned.

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MO) and glucoamylase (amyloglucosidase from *Aspergillus niger*, glucoamylase, 1,4-α-D-glucan glucohydrolase, exo-1,4-α-glucosidase, 9032-08-0, Sigma-Aldrich) with activity of 21,390 and 300 units/mL were used for liquefaction and saccharification, respectively.

Two experiments were conducted in this study. In the first experiment, fermentation characteristics of the E-Mill process (a wet technology) and 3D process (a dry technology) were compared with the conventional dry-grind (CDG) process. Different amounts of corn were used for the CDG, E-Mill, and 3D processes to account for the removal of germ and pericarp fiber before fermentation. The endosperm fiber recovery step of the E-Mill process was eliminated to allow a comparison of 3D and E-Mill processes. The fermenter solids level was kept constant at 25% (wet basis)

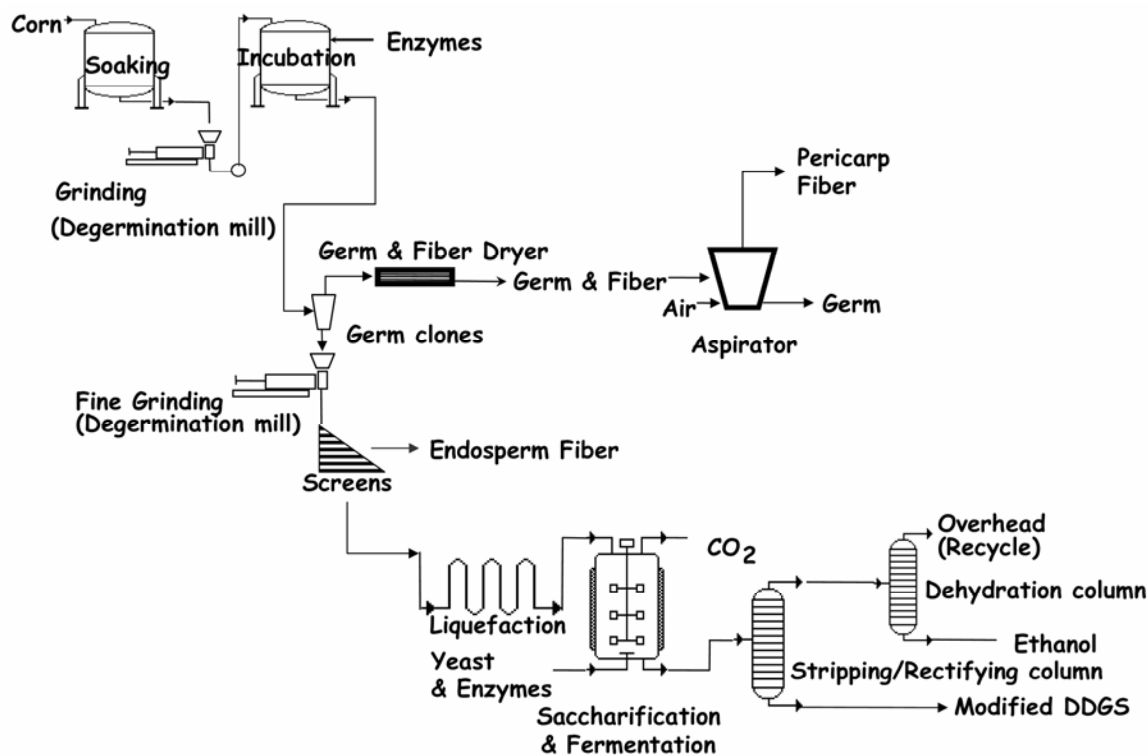

Fig 1. Enzymatic dry grind (E-Mill) process (Singh et al 2005).

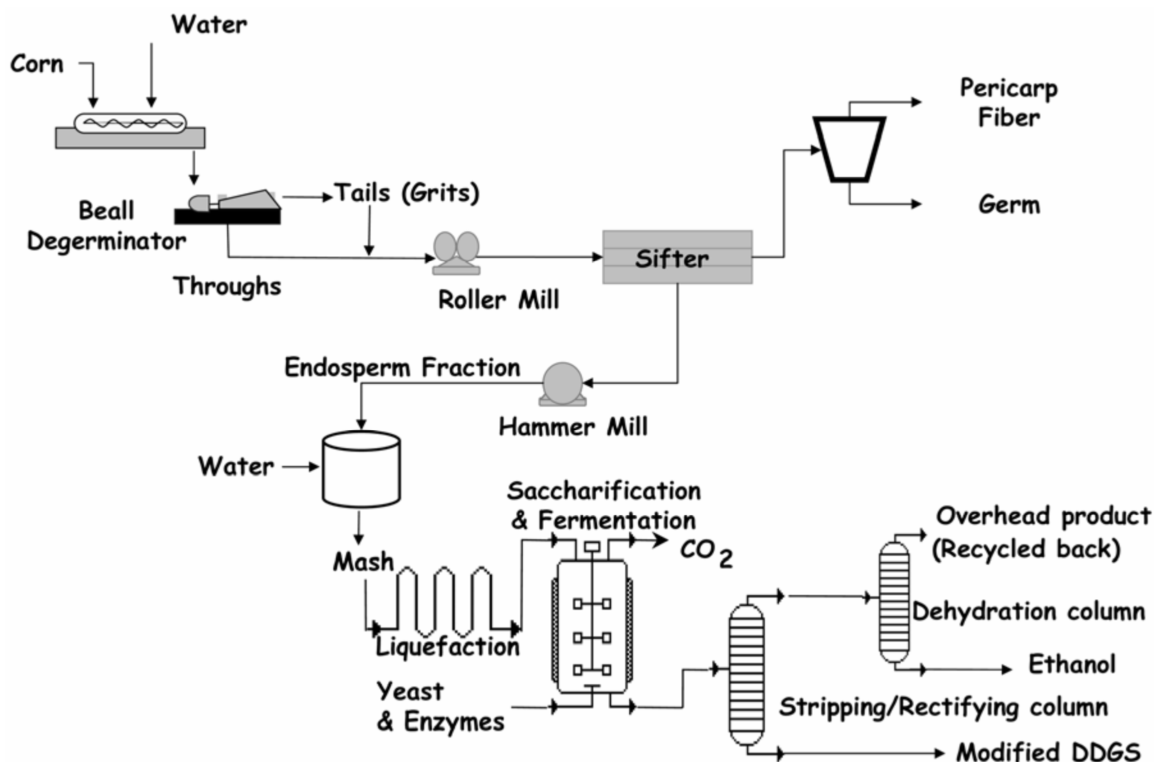

Fig. 2. Dry degerm defiber (3D) process (Murthy et al 2004).

for each treatment. In the second experiment, the effect of addition of germ soak water and B-vitamins on fermentation characteristics of the endosperm fraction obtained from the 3D process was studied using separate saccharification (SS) and simultaneous saccharification and fermentation (SSF) processes.

Experiment 1: Comparison of Dry and Wet Fractionation Technologies: Conventional Dry Grind

Corn (1,000 g) was milled at 500 rpm in a cross-beater mill (model MHM4, Glen Mills, Clifton, NJ) equipped with a 0.5-mm round-hole sieve. Moisture content of milled corn was analyzed using a two-stage conventional oven method (AACC International 2000) and milled corn (500 g, db) was mixed with water at 60°C to form 25% solids mash. The mash was liquefied using 2.8 mL of α-amylase for 90 min in a water bath maintained at 90°C. After adjusting the mash to pH 4.2 using 1.0*N* sulfuric acid, the mash was subjected to SSF.

Enzymatic Dry Grind (E-Mill)

Corn (1500 g) was processed using the E-Mill process as outlined by (Singh et al 2005) with one modification. In the E-Mill process, corn is soaked in water for a short period of time, followed by coarse grinding and recovery of germ, pericarp, and endosperm fiber before fermentation (Singh et al 2005). Endosperm fiber recovery of the E-Mill process was not done to allow comparison of E-Mill process with the 3D process and protease was not used in the incubation step. Water (soak water plus additional water) used was adjusted to maintain 25% solids in the mash before fermentation.

Dry Degerm Defiber (3D)

Corn (3,000 g) was tempered to a moisture content of 22.5% (wb) for 18 min at 25°C. Corn was passed through a horizontal drum degermination mill (model SPL56CC17 F20 51EP, Marathon Electric, Wausau, WI) and dried at 49°C for 2 hr to ≈15% (db) moisture. After four passes through a roller mill (fluted rollers, 150 mm diameter, 240 mm width, 155 rpm) driven by an electric motor (2 hp; model EVGD, US Electrical Motors, Los Angeles, CA), corn was sieved over a 2.03-mm (10-mesh) sieve for 5 min. Germ and fiber fractions retained on the sieve were separated by aspiration (6DT4, Kice Metal Products, Wichita, KA). The remaining endosperm fraction was milled in a cross-beater mill (model MHM4, Glen Mills, Clifton, NJ) at 500 rpm using a 0.5-mm round-hole sieve. Milled endosperm fraction was analyzed for moisture (AACC International 2000). Milled endosperm fraction (500 g, db) was mixed with water at 60°C to form 25% solids mash. The slurry was liquefied using 2.8 mL of α-amylase for 90 min in a water bath maintained at 90°C. After adjusting slurry to pH 4.2 using 1.0*N* sulfuric acid, mash was subjected to SSF.

Experiment 2: Endosperm Fraction Treatments from 3D Process

The hypothesis of micronutrient deficiency in dry-milled corn flour was tested by supplementing the endosperm fraction obtained from the 3D process with B-vitamin complex and germ soak water in the fermenter. In the first treatment, 200-g (db) corn samples were inoculated with B-vitamins (B1, B2, B6, Niacinamide, Panthothenic acid, Para amino benzoic acid, Inositol @ 5,000 ppm; Folic acid, B12 @ 5 ppm, Vitamin B-complex) (KLG Corp. South El Monte, CA). The second treatment used germ soak water in preparation of the slurry. The germ fraction, obtained from the 3D process, proportional to 200 g (db) of endosperm fraction was soaked in 200 mL of water for 30°C for 2 hr. After germ soaking, water remaining was added to additional water used to make 25% solid (wb) slurry. The third treatment (200 g, db) was a control with no added B-vitamin or germ soak water. Each treatment used 200 g of endosperm fraction mixed with water at 60°C to form 25% solids (wb) slurry. The slurry samples were liquefied using 2.8 mL of α-amylase for 90 min in a water bath maintained at 90°C. Each of the samples was subsequently fermented using SSF and SS processes.

Separate Saccharification and Fermentation (SS)

Liquefied mash was cooled to 60°C and adjusted to pH 4.2 using 1.0*N* sulfuric acid. Mash was saccharified with addition of 2.8 mL of glucoamylase and held at 60°C for 2 hr with constant agitation at 150 rpm. Mash was cooled to 30°C and inoculated with 0.022g/g of dry solids active dry yeast (Fleischmann's Yeast, Fenton, MO). The $(NH_4)_2SO_4$ was added to provide 500 ppm of free amino nitrogen for yeast growth. Fermentation was performed at 30°C for 60 hr with continuous agitation at 50 rpm. Fermentation was monitored by withdrawing 10 mL of fermentation broth at 12-hr intervals and measuring sugar and ethanol concentrations using HPLC methods.

Simultaneous Saccharification and Fermentation (SSF)

Liquefied mash was cooled to 30°C and adjusted to pH 4.2 using 1.0*N* sulfuric acid. The mash was saccharified by adding 2.8 mL of glucoamylase. Simultaneously, mash was inoculated with 0.022g/g of dry solids active dry yeast (Fleischmann's). The $(NH_4)_2SO_4$ was added to provide 500 ppm of free amino nitrogen. SSF was performed at 30°C for 60 hr with continuous agitation at 50 rpm. SSF was monitored by withdrawing 10 mL of fermentation broth at 12-hr intervals and measuring sugar and ethanol concentrations using HPLC methods.

HPLC Analyses

Samples (10 mL) drawn from fermentation vessels were centrifuged (model Durafuge 100, Precision, Winchester, VA) at 1,476 × *g* for 5 min and supernatant was filtered through a 0.2-μm filter. Filtered supernatant liquid (5 μL) was injected into an ion-exclusion column (Aminex HPX-87H, Bio-Rad, Hercules, CA) maintained at 50°C. Sugars (glucose, fructose, maltose, and maltotriose), organic acids (lactic, succinic, and acetic acid) and alcohols (ethanol, methanol, and glycerol) were eluted from the column with HPLC-grade water containing 5 m*M* sulfuric acid. Separated components were detected with a refractive index detector (model 2414, Waters Corp., Milford, MA). Elution rate was 0.6 mL/min; a calibration standard was used before each batch of samples. Data were processed using HPLC software (v. 3.01, Waters).

Statistical Analyses

A complete randomized block design was used. For the first experiment, three replicates were conducted for conventional dry-grind, E-Mill, and 3D processes. For the second experiment, two

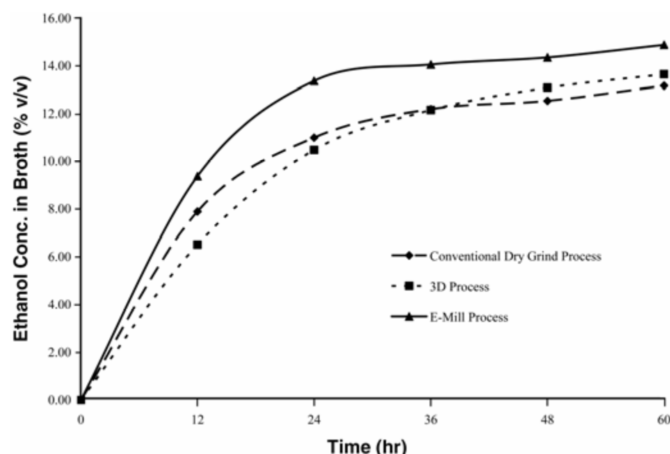

Fig. 3. Ethanol profiles (ethanol concentration vs. fermentation time) for conventional dry grind (CDG), dry degerm defiber (3D), and enzymatic dry grind (E-Mill) processes.

replicate fermentations, using SS or SSF process, were conducted for control sample, treatment with B-vitamins, and germ soak water. Fermentation samples from all experiments were analyzed using a mean of two values from HPLC analyses.

RESULTS AND DISCUSSION

Experiment 1: Comparison of Dry and Wet Fractionation Technologies

The fermentation rate, inferred by the rate of increase of ethanol concentration in the fermenter, was highest for slurry obtained from E-Mill process (Fig. 3). Fermentation rate of 3D process was slowest initially and increased after 36 hr as compared to CDG. Initially slow rates of 3D process could be due to the loss of micronutrients with germ during degermination process. Yeast are able to synthesize all the chemicals needed for its growth from simple carbon and nitrogen sources. However, the time required to synthesize these nutrients could result in slow rates of fermentation as observed in the 3D process.

Average ethanol concentrations were different ($P < 0.001$) at the end of the fermentation (13.2, 13.7, and 14.9%, v/v, for CDG, 3D, and E-Mill processes, respectively). Higher concentrations were expected because removal of germ and pericarp fiber in 3D and E-Mill processes resulted in more fermentable material in the fermenter. The lower final ethanol concentration for 3D (dry fractionation) compared with E-Mill (wet fractionation) suggests that there is more starch loss in the 3D process. CDG process had the highest yield of ethanol (0.43 ± 0.02 L/kg of whole corn, 2.9 ± 0.11gal/bu) followed by E-Mill process (0.35± 0.01 L/kg of whole corn, 2.4 ± 0.07 gal/bu) and 3D process (0.33 ± 0.03 L/kg, 2.2 ± 0.22 gal/bu). In the 3D process, separation of germ and pericarp fiber from endosperm is not complete and endosperm pieces are still attached to germ and pericarp fiber. There is 70% more starch and 22.3% less protein in germ obtained from the dry milling (dry fractionation) process compared with germ from the quick germ (wet fractionation) process (Johnston et al 2005). Use of proteolytic enzymes such as GC106 (Genencor International, Rochester, NY), during wet fractionation helps in breakdown of protein matrix surrounding starch particles and lower starch loss in fiber fraction (Johnston and Singh 2004). In the E-Mill process, water hydration and addition of protease (GC106) before milling result in cleaner separation of germ, pericarp fiber and endosperm.

Concentrations of lactic acid >0.2–0.8% (w/v) stress yeast and result in lower growth rates and ethanol production rates (Narendranath et al 2001). Glycerol, an indicator of yeast stress, is produced in small amounts (≈1.2–1.5%) in all dry-grind ethanol fermentations (Russel 2003). Glycerol concentration were low among all samples (<1.4%, v/v). Lactic acid, an indicator of infection by Lactobacillus sp., was consistently low among all samples (<0.5%, w/v). Consistently low levels of lactic acid and glycerol show that the yeast were not stressed and there were no major bacterial infection problems.

Experiment 2: Treatments to Endosperm Fraction from the 3D Process

Fermentation characteristics of 3D process endosperm fraction were poor as compared with CDG and E-Mill process (Experiment 1). To improve the fermentation characteristics, effect of addition of B-vitamins and germ soak water was studied. 3D process endosperm fraction without any added nutrients was used as control for the second experiment.

Final ethanol concentrations, obtained by fermentation of endosperm fraction from 3D process, were higher and significantly different for all treatments using SSF compared with SS (Table I). SSF resulted in more complete fermentation when compared with SS for all treatments as indicated by lower final glucose concentrations (Table II). There were no differences among the replicates.

Fermentation rates and final ethanol concentrations increased when B-vitamins or germ soak water were added to the endosperm fraction from 3D process for SSF and SS processes (Table I). The final ethanol concentration was higher ($P < 0.001$) for the germ soak water treated sample (13.1%, v/v) compared with control

TABLE I
Effect of B-Vitamin and Germ Soak Water Addition on Ethanol Concentration (% v/v) During Separation Saccharification (SS) and Fermentation and Simultaneous Saccharification and Fermentation (SSF) for Dry Degerm Defiber (3D) Process

	Time (hr)				
Treatment	12	24	36	48	60
SS Process					
B-Vitamins	4.1	6.7	9.4	10.8	12.4
Germ soak water	4.7	7.7	9.3	11.4	13.1
Control	4.3	6.2	8.9	11.0	11.8
SSF Process					
B-Vitamins	4.7	7.0	9.2	13.9	15.0
Germ soak water	5.5	7.8	9.9	13.2	14.7
Control	4.8	7.6	8.6	12.2	12.3

[a] Reported values are mean values of two replicate fermentations.

TABLE II
Effect of B-Vitamin and Germ Soak Water Addition on Glucose Concentration (% w/v) During Separation Saccharification (SS) and Fermentation and Simultaneous Saccharification and Fermentation (SSF) for Dry Degerm Defiber (3D) Process

	Time (hr)				
Treatment	12	24	36	48	60
SS Process					
B-vitamins	13.8	10.9	5.6	3.7	1.3
Germ soak water	14.2	9.5	5.3	2.5	0.7
Control	15.3	10.5	7.4	5.1	2.8
SSF Process					
B-vitamins	12.3	8.6	5.5	2.3	0.3
Germ soak water	13.0	8.8	4.6	2.8	0.4
Control	14.5	12.2	6.9	4.8	2.3

[a] Reported values are mean values of two replicate fermentations.

(11.8%, v/v) when using SS. With SSF, mean final ethanol concentration of the B-vitamin supplemented samples was 15.0% and was 2.6 %, v/v, more than the control. Enhancing carbohydrate metabolism can improve the supply of metabolic precursors essential for growth. Availability of metabolic precursors determines the growth rates of organisms. Hence supplementing the fermentation medium with B-vitamin complex which consists of essential coenzymes in carbohydrate metabolism (Jansen 1972) can promote growth of yeast. Riboflavin (Vitamin B2) is essential for lipid synthesis in microorganisms (Horwitt 1972). Vitamin B6 is essential for nitrogen metabolism (Sauberlich 1972). Abundant supply of B-vitamins could remove the restrictions on the growth of yeast due to unavailability of the metabolic precursors and can therefore improve fermentation rates of yeast.

Germ has high concentration of vital nutrients for growth of the corn seedling and has many water-soluble proteins that play a vital role in seedling growth. It also has enzymes necessary for supply of nutrients to the germinating seedling from the nutritional resources in the kernel (Ashton 1976). Soaking germ in water, as done in E-Mill, leaches out these important proteins and the addition of soak water to fermentations therefore improves metabolism of yeast by supplying essential micronutrients.

In SS and SSF, residual glucose levels were much lower (0.7 and 0.3%, w/v, respectively) for than the treatments with no added soak water or B-vitamins (2.8 and 2.3 %, w/v, respectively), indicating complete fermentations. Consistently low levels of lactic acid (<0.5%, v/v) and glycerol (<1.4%, v/v) indicate that results were not due to any contamination during fermentation.

Addition of germ soak water has a process advantage; it recovers additional starch removed from the endosperm fraction during the sieving step of the 3D process. Use of germ soak water in the process has other advantages since the germ is already a coproduct from the 3D process. Soaking the germ fraction obtained from the 3D process leaches out the soluble solids and should increase the oil content of germ. Value of corn germ is determined by its oil content (Johnston et al 2005), therefore soaking of germ will increase its value. The germ value would likely be greater than germ from wet-milling, as it is not exposed to SO_2, which degrades the oil quality.

Dry-grind plants using a dry degermination step could consider using germ soak water to improve fermentation rates and achieve lower residual sugars in DDGS. Lower residual levels of glucose would reduce the Maillard reactions during the DDGS drying step and could potentially lead to a lighter colored DDGS with improved flowability. Lower residual sugars would also cause fewer problems in the thin stillage evaporation and could lead to reduced evaporator fouling. Potential benefits such as increased final ethanol concentrations through better utilization of sugars, increased fermentation rates, increased germ oil content, decreased residual sugars in DDGS, and benefits of germ soak water addition would likely justify the minimal modifications required in dry-grind plants.

CONCLUSIONS

Wet (E-Mill) and dry (3D) fractionation technologies provide valuable coproducts such as germ and pericarp fiber. These fractionation technologies also produced larger amounts of ethanol per fermenter but lower ethanol yields per ton corn than the conventional dry-grind process due to some loss of starch in coproducts. Among the two fractionation processes evaluated, the E-Mill process resulted in higher ethanol yields than the 3D process.

Using SSF, 3D processing of endosperm fraction resulted in more complete utilization of sugars and produced higher ethanol concentrations than the SS process. During 3D processing, supplementation with B-vitamins and germ soak water improved fermentation rate and increased glucose utilization, resulting in higher ethanol yields compared with no addition of micronutrients.

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