

## Effect of the Corn Breaking Method on Oil Distribution between Stillage Phases of Dry-Grind Corn Ethanol Production

H. WANG, T. WANG,\* L. A. JOHNSON, AND A. L. POMETTO III

Department of Food Science and Human Nutrition, Center for Crops Utilization Research, Iowa State University, Ames, Iowa 50011-1061

The majority of fuel ethanol in the United States is produced by using the dry-grind corn ethanol process. The corn oil that is contained in the coproduct, distillers' dried grains with solubles (DDGS), can be recovered for use as a biodiesel feedstock. Oil removal will also improve the feed quality of DDGS. The most economical way to remove oil is considered to be at the centrifugation step for separating thin stillage (liquid) from coarse solids after distilling the ethanol. The more oil there is in the liquid, the more it can be recovered by centrifugation. Therefore, we studied the effects of corn preparation and grinding methods on oil distribution between liquid and solid phases. Grinding the corn to three different particle sizes, flaking, flaking and grinding, and flaking and extruding were used to break up the corn kernel before fermentation, and their effects on oil distribution between the liquid and solid phases were examined by simulating an industrial decanter centrifuge. Total oil contents were measured in the liquid and solids after centrifugation. Dry matter yield and oil partitioning in the thin stillage were highly positively correlated. Flaking slightly reduced bound fat. The flaked and then extruded corn meal released the highest amount of free oil, about 25% compared to 7% for the average of the other treatments. The freed oil from flaking, however, became nonextractable after the flaked corn was ground. Fine grinding alone had little effect on oil partitioning.

**KEYWORDS:** Dry grind; extrusion; flaking; fuel ethanol; grinding; oil extraction; oil partition; thin stillage

### INTRODUCTION

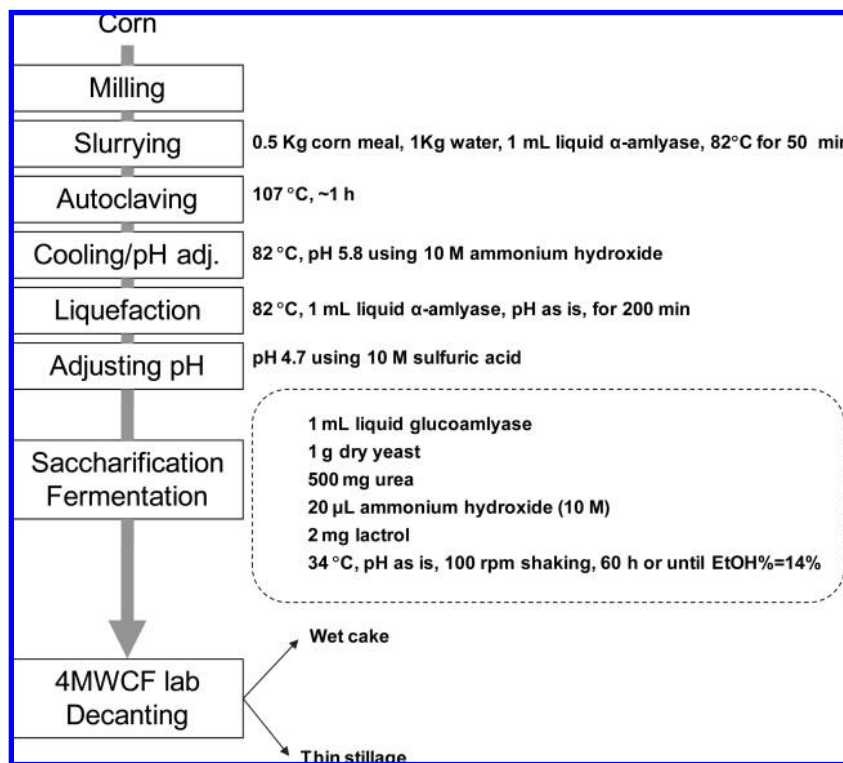
According to the Renewable Fuels Association (Washington, DC), about 27.3 billion L (7.2 billion gal) of fuel ethanol was produced in 2007, and 82% was processed by using the dry-grind corn ethanol process and the remaining using corn wet-milling (1). The typical corn for dry-grind ethanol production contains 4% oil, 72% starch, 10% protein, and 10% fiber (2). In the conventional dry-grind corn ethanol process, the kernel is ground, slurried with water at a 1:2 corn-to-water ratio, and cooked to gelatinize the starch, and the starch is thinned to dextrins by thermostable  $\alpha$ -amylase and then to fermentable glucose by glucoamylase. Yeast (*Saccharomyces cerevisiae*) converts the glucose into ethanol in a simultaneous saccharification and fermentation (SSF) process. After fermentation, the ethanol is distilled as a water–ethanol azeotrope containing 5% (v/v) of water.

The ethanol-free slurry, called whole stillage, is pumped to a decanter while hot to be separated into liquid and solid fractions, i.e., thin stillage and wet cake, which typically contain 35 and 7% solids, respectively. While part of the thin stillage is usually recycled to make the starting ground corn slurry (backset), the rest is concentrated by evaporation into a thick

stillage (or syrup) with a solids content of about 30%. The wet cake and thick stillage are then combined, mixed, and dried into distillers' dried grains with solubles (DDGS), which is mainly used as cattle feed.

Because of the conversion of starch during fermentation, the oil (crude free fat) content is increased from 4% in original corn to about 14% in DDGS. Although fat increases the energy density of DDGS as livestock feed, it may interfere with normal milk production by dairy cattle, and DDGS-fed swine produce pork bellies (bacon) with an undesirable softer texture (unpublished data from Keisei et al., Japan Scientific Feeds Association, 2006). Therefore, removal of fat (oil) from DDGS will improve its feed quality. More importantly, the oil can be used to produce biodiesel and other biorenewable products. If 70% of the oil can be recovered from all dry-grind corn ethanol plants in the United States, an additional 2 billion L (440 million gal) of corn oil could be annually produced, which is nearly equivalent to the current annual biodiesel production in the U.S. (production from October 1, 2006 to September 30, 2007; data from the National Biodiesel Board) (3). Unlike front-end corn degerming (prior to fermentation), oil recovered from the dry-grind corn ethanol process is not suitable for food use, but tail-end recovery (post fermentation) is expected to require much less capital and will have lower operating costs.

\* Corresponding author. Phone: 515-294-5448. Fax: 515-294-8181. E-mail: tongwang@iastate.edu.



**Figure 1.** Schematic representation of the 1.5-L laboratory-scale corn dry-grind ethanol fermentation process. Note: 4MWCF laboratory decanting was a multiple-wash-centrifuge-filtration decanting technique developed in this study to simulate the industrial operations.

The most desirable postfermentation step to remove the oil is at the centrifugation (decanting) step. In the current industrial process, about 50% of the total oil goes to the liquid phase (thin stillage), and the remainder goes with the solids (distillers' grains) (data were obtained in our laboratory by analyzing the typical industrial whole stillage, wet cake, and thin stillage). In order to extract more oil from the dry-grind ethanol process, more oil needs to be distributed in the thin stillage. In the present study, we examined the effects of different physical methods of breaking the corn kernel (fine grinding, flaking, extrusion, and their combinations) on oil partitioning between liquid and solids phases.

Grinding tears up the corn kernel, making the starch granules more accessible to enzyme and yeast digestion. Ideally, all cells are ruptured to enable complete enzyme hydrolysis and sugar conversion. How grinding and other preparation methods affect oil partitioning has not been fully studied. Flaking produces thin flakes by forcing a conditioned or plasticized material through a small gap between a pair of counter-rotating smooth-surfaced roller mills. Roller mills are used in the soybean crushing industry to reduce oil extraction barriers by distorting or rupturing cell walls and the seed subcellular structure, thereby increasing solvent penetration and reducing the mass transfer distance during hexane extraction (4, 5). Therefore, flaking of corn may free more oil and cause it to partition into the fermentation liquid. Extrusion is another physical size reduction method in which high shear, pressure, and heat rupture cell walls and create a porous mass as is done in soybean oil extraction (5). Korn and Harper (6) found that extrusion pretreatment produced more glucose and ethanol than conventional cooking, but others reported that extrusion did not alter ethanol yield (7, 8). We expect to have more oil released into the fermentation liquid by extrusion pretreatment.

There is little information on how the physical treatments influence oil distribution in dry-grind corn ethanol fermentation. We hypothesized that more oil can be released and partitioned

from solid to liquid phase by proper physical breaking methods before fermentation. Such oil can then be efficiently recovered by centrifugation of the stillage.

## MATERIALS AND METHODS

**Corn Samples and Fermentation Materials.** No. 2 yellow dent corn from the 2007 crop year was acquired from the Heart of Iowa Cooperative (Nevada, IA). The corn was cleaned using a KICE Model 6DT4 laboratory aspirator unit (KICE Metal Products Co. Inc., Wichita, KS). Liquid  $\alpha$ -amylase SPEZYME Xtra (13642  $\alpha$ -amylase units/g) and a saccharifying enzyme G-ZYME 480 Ethanol (401 gluco-amylase units/g), both from Genencor Inc. (Cedar Rapids, IA), were used for liquefaction and saccharification, respectively. Lactrol (462 g virginiamycin/lb), an antibiotic extract, was from PhibroChem (Ridgefield Park, NJ). Dry yeast (*Saccharomyces cerevisiae*) Ethanol Red was acquired from Fermentis, a division of Lesaffre Yeast Corp. (Headland, AL). Urea was supplied by Keytrade USA Inc. (Kordova, TN). All these materials were industrial grade and are being used today in dry-grind ethanol plants in the Midwest.

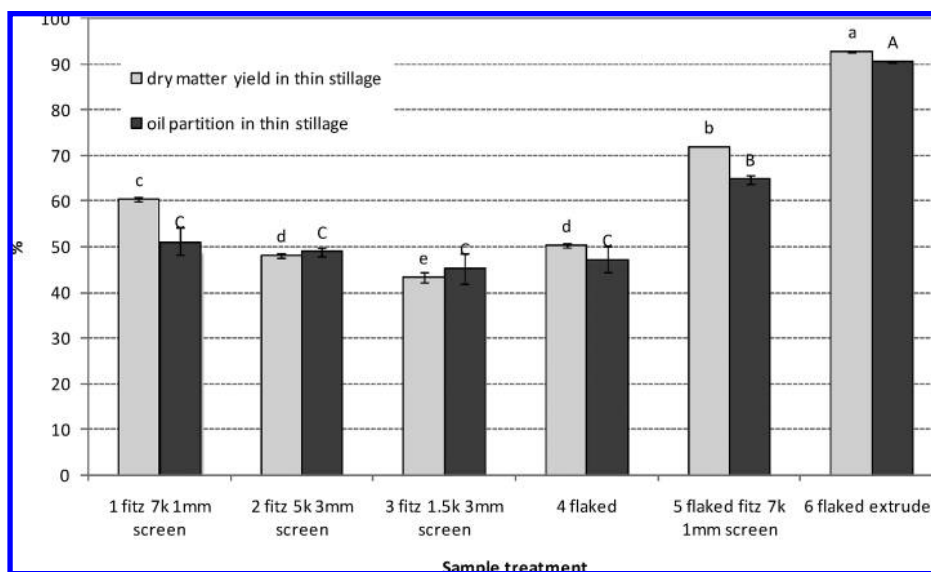
**Physical Treatments of Corn.** A total of six treatments were carried out, which were designated in the format of a sequence number followed by an abbreviated name. For ease of understanding, these treatments were categorized into two groups. The first group, three treatments, was grinding with a Fitz Mill Model DAS 06 (the Fitzpatrick Co., Elmhurst, IL) alone. Corn was ground into three different sizes using three different tangential hammer speeds and screen sizes. The corn meal with the smallest particle size was produced at 7,000 rpm with a 1-mm round-hole opening screen, the intermediate particle size meal was produced at 5,000 rpm with a 3-mm round-hole opening screen, and the coarsest was produced at 1,500 rpm with a 3-mm round-hole opening screen. Grinding with the same screen but with different rpm produced corn meal with dramatic size differences (data not shown). These three treatments/samples were labeled as 1 fitz 7k 1mm screen, 2 fitz 5k 3mm screen, and 3 fitz 1.5k 3mm screen, respectively.

The second group of samples, consisting of another 3 treatments, were corn lots being flaked first. Flaking was done by using a Roskamp Roller Mill Model K (Roskamp Mfg., Inc., Waterloo, IA) set at 0.25 mm (0.010 in.) gap between the rollers. Treatment 4 was flaked corn

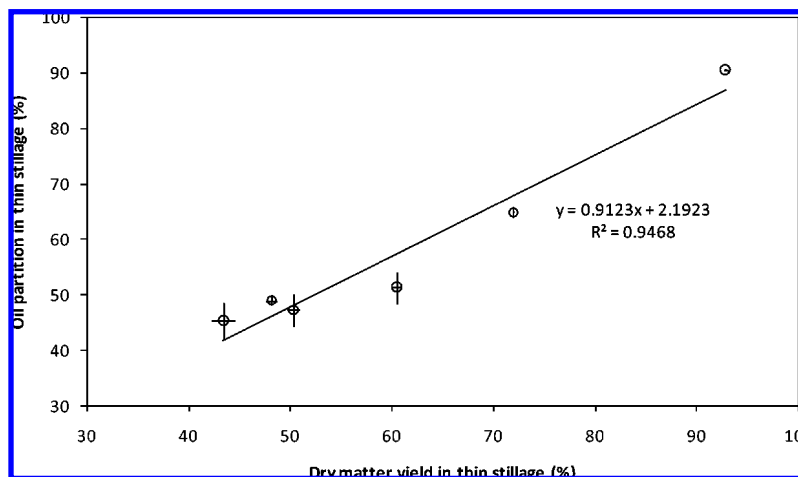
**Table 1.** Summary of the Fermentation Performance Using Different Corn-Breaking Methods<sup>a</sup>

treatment	ethanol conc. <sup>b</sup> (g/L)	acetic acid <sup>b</sup> (g/L)	lactic acid <sup>b</sup> (g/L)	solids content in finished beer (%)	ethanol yield based on mass loss <sup>c</sup> (%)
1 fitz 7k 1 mm screen	148.78 <sup>a</sup>	1.37 <sup>a</sup>	0.60 <sup>a</sup>	12.28 <sup>b</sup>	34.56 <sup>a</sup>
2 fitz 5k 3 mm screen	148.35 <sup>a</sup>	1.43 <sup>a</sup>	0.69 <sup>a</sup>	12.02 <sup>b</sup>	34.33 <sup>ab</sup>
3 fitz 1.5k 3 mm screen	147.77 <sup>a</sup>	0.61 <sup>a</sup>	0.69 <sup>a</sup>	12.89 <sup>b</sup>	33.89 <sup>bc</sup>
4 flaked	148.87 <sup>a</sup>	1.54 <sup>a</sup>	0.59 <sup>a</sup>	12.99 <sup>b</sup>	33.55 <sup>c</sup>
5 flaked fitz 7k 1 mm screen	132.06 <sup>a</sup>	1.59 <sup>a</sup>	ND	11.96 <sup>b</sup>	34.37 <sup>ab</sup>
6 flaked extruded	139.94 <sup>a</sup>	0.83 <sup>a</sup>	ND	16.25 <sup>a</sup>	34.86 <sup>a</sup>
control (industrial data) <sup>c</sup>	130–150	0.6–1.4	1.4–3.0	11.00–13.00	

<sup>a</sup>Data from 6 treatments are the means of two replicates. Means with same superscripts are not significantly different ( $P = 0.05$ ) within the same column. <sup>b</sup>Concentration in the finished beer measured by HPLC. <sup>c</sup>Ethanol yield based on mass loss was the percentage of ethanol production based on original corn meal (w/w). <sup>d</sup>Industrial data range is from the communication with industry people in the conventional dry-grind corn ethanol fermentation.



**Figure 2.** Effect of corn grinding method on thin stillage dry matter yield and oil partition (i.e., the percentage of total solids and oil distributed in thin stillage from whole beer after laboratory decanting).  $N = 2$ .



**Figure 3.** Correlation between the dry matter yield of thin stillage and oil partition in thin stillage. Note: the horizontal and vertical standard deviation bars are for oil partition ( $y$  axis) and dry matter yield ( $x$  axis), respectively.  $N = 2$ .

only. Treatment 5 was flaking the corn first followed by grinding using the Fitz Mill at 7,000 rpm with a 1-mm round-hole opening screen. Treatment 6 was flaking the corn followed by extruding. Extrusion was carried out using a Leistritz ZSE 27 twin-screw extruder (American Leistritz Extruder Corp., Somerville, NJ) with water cooling to control the temperature. The twin-screw configuration with corotating screws at rpm 100 was used. The temperature profile was 100 °C for the feeding zone, 120 °C for all extrusion zones, and 120 °C for the rod-shaped die. The moisture of the corn flakes was as-is (~14%). These three treatments/samples were designated as 4 flaked, 5 flaked fitz 7k 1mm screen, and 6 flaked extruded, respectively.

**Fermentation.** A 1.5-L laboratory-scale fermentation procedure, designed in our laboratory based on industrial parameters in conventional dry-grind corn ethanol fermentation, was employed. Two-liter flasks were used for all fermentation runs. The flasks were covered with aluminum foil during incubation. **Figure 1** details the fermentation protocol. The equipment used to carry out the fermentation included an incubator, LAB-LINE Incubator-Shaker, model 3525 (Lab-line Instruments Inc., Melrose Park, IL); an autoclave, Tomy Autoclave SS-325E (Tomy Seiko Co., Ltd., Tokyo, Japan); a water bath, Isotemp 220 (Fisher Scientific, Pittsburgh, PA); a pH meter IQ 150 (IQ Scientific Instruments Inc., Carlsbad, CA); and two stir motors (Fisher Scientific,

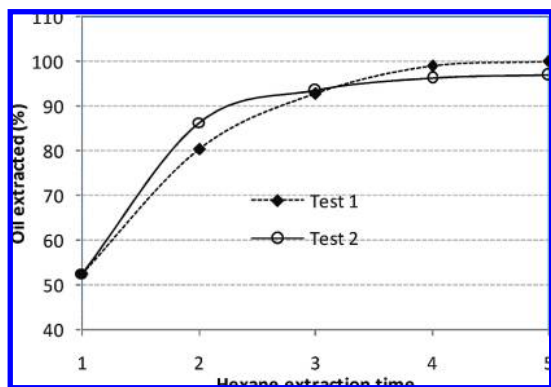


Figure 4. Effect of 2-mL hexane transfer time on the yield of centrifugable oil.

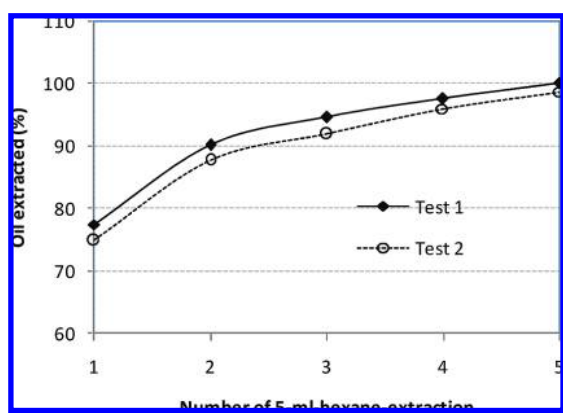


Figure 5. Effect of number of 5-mL hexane extraction on yield of the hexane extractable oil.

Dubuque, IA). After fermentation, the finished beer was heated to 70 °C in a water bath for 20 min to inactivate the yeast. The flasks were tightly stoppered during heating to prevent ethanol loss.

The ethanol concentration, along with acetic and lactic acid levels, was quantified by high performance liquid chromatography (HPLC) (9) after removing solid particles from the whole beer. For the sample cleanup, about 20 g of beer was centrifuged at 10,000g at ambient temperature for 10 min. The supernatant was then passed through a syringe filter with 0.20- $\mu$ m pores (Corning Inc., Corning, NY). Acetic and lactic acid concentrations were used as indicators of microbial contamination during fermentation. After sampling to quantify for ethanol production, 100 ppm sodium azide (Sigma Chemical Co., St. Louis, MO) was added to the finished beer to prevent microbial spoilage during storage. The beer samples were kept at 5 °C for further experiments.

The mass loss during fermentation was also recorded as the weight difference between the slurry at the beginning of yeast inoculation and the finished beer after the yeast was destroyed, and it was assumed that the mass loss was solely due to CO<sub>2</sub> production. This assumption was confirmed by multiple control tests to evaluate the mass loss caused by water and ethanol evaporation. About 1500 g of 16% aqueous ethanol was put in the same size flask, under the same fermentation conditions. The mass loss from the evaporation of water and ethanol was 0.2 g/1500 g ethanol solution on average or 0.013% over 60 h at 34 °C. Thus, the loss due to water and ethanol evaporation during fermentation was ignored.

Because yeast anaerobic respiration involves C<sub>6</sub>H<sub>12</sub>O<sub>6</sub> (glucose) → 2CO<sub>2</sub> + 2 C<sub>2</sub>H<sub>6</sub>O, then, ethanol production (based on mass loss, % of the original corn mass) = 46 × (CO<sub>2</sub> production)/44 = 46 × (mass loss)/44.

Two fermentation replicates were carried out for each treatment. For all the subsampling and measurements, two replicates were used for each sample.

**Effect of the Corn Breaking Method on Dry Matter Yield and Oil Partitioning into Thin Stillage.** Laboratory decanting to separate

the corn beer into thin stillage and wet cake was carried out following a multiple-wash-centrifuge-filtration (MWCF) procedure using a device designed in our laboratory and shown to simulate well the industrial decanting operation (10). Four washings were carried to make the benchtop thin stillage. Centrifuging was carried out at 3000g and ambient temperature (25 °C) for 2 min. Between the two centrifugations, manual shaking was used to remix the cake with the liquid. The shaking time was 1 min per wash. The wet and dry matter yields and oil contents of the resulting thin stillage and wet cake were quantified. The oil partition in the thin stillage was calculated from the difference between the oil mass in the original whole beer and the oil mass in the wet cake, considering that oil measurement in the thin stillage is difficult because of its low oil content. After the whole beer and wet cake were dried at 80 °C, the oil contents were quantified by using an acid-hydrolysis method based on AOAC Official Method 922.06 (11). The dry matter yield of thin stillage and the oil partitioning in thin stillage were defined as the percentages of total dry matter and oil in the thin stillage on the basis of the whole beer from which the thin stillage was produced. It should be noted that laboratory decanting was carried out on whole beer, that is, without distilling the ethanol.

**Effect of the Corn Breaking Method on Oil Extractability.** In order to differentiate treatment effects on oil extractability, we also quantified oils under different extraction conditions. Two oil measurement methods were used, which were referred to as centrifugable oil and hexane-extractable oil.

The centrifugable oil was the oil floating on top of the supernatant after centrifuging 40 g of whole beer in a 50-mL centrifuge tube at 3000g and ambient temperature for 10 min using an IEC Centra MP4 centrifuge with a fixed-angle Rotor 854 (International Equipment Co., Needham Heights, MA). The oil was then recovered by five consecutive 2-mL hexane transfers. All five hexane extracts were combined, the solvent was evaporated, and the total lipid was further dried at 80 °C for 3 h in an aluminum pan and quantified gravimetrically.

The hexane-extractable oil was the oil that can be extracted by mixing 5 mL of hexane with 40 g of whole beer, shaking vigorously for 2 min, and then centrifuging under the same conditions as those in the centrifugable oil measurement. After the hexane extract was removed, four consecutive 2-mL hexane transfers were used to recover all of the oil on the top of the supernatant. The hexane extracts were then combined, dried, and weighed. Theoretically, the hexane-extractable oil should include the centrifugable oil and the additional oil that can be solubilized by hexane. From these two measurements, oil in the finished beer could be categorized into three types with different extractabilities: (1) free oil (FO), the centrifugable oil; (2) trapped oil (TO), the oil that cannot be extracted by centrifugation but can be extracted by hexane; and (3) bound oil (BO), the oil that cannot be extracted by hexane.

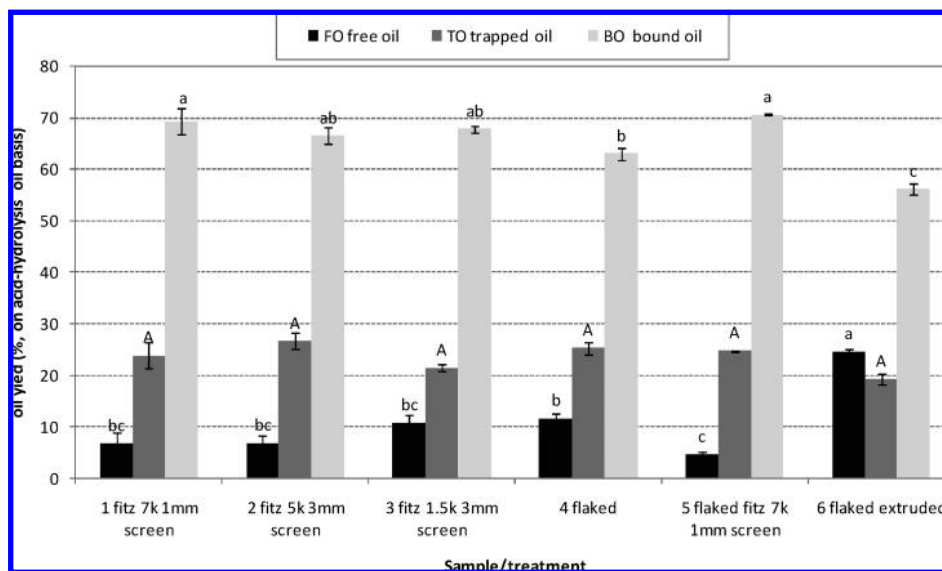
All of the oil contents were expressed as percentages of total oil, which was quantified by using the acid-hydrolysis method (11) with the whole finished beer. The relationships of the three types of oils are FO + TO + BO = 100; where TO = hexane-extractable oil - FO, and BO = 100 - hexane-extractable oil.

**Experimental Design and Data Analysis.** A randomized complete block design with two blocks and six treatments was used for this experiment. Statistical analysis was performed using General Linear Model procedures of SAS 9.1 (12).

## RESULTS AND DISCUSSION

**Effect of the Corn Breaking Method on Fermentation Performance.** Generally speaking, the ethanol concentrations as measured by HPLC were not significantly different among different treatments, and they were comparable to industrial practice (Table 1). The acetic and lactic acid concentrations in the finished beer were no more than 1.6 and 0.7 g/L, which indicate that ethanol fermentations were successful and that the microbial contamination was well controlled for all of the samples with different treatments.

Considering that the weights and solids contents of the final beer were slightly different for different treatments, we also



**Figure 6.** Effect of corn breaking method on the extractability of oil from fermentation beer.  $N = 2$ . Statistical comparisons should be made within each type of oil.

calculated the ethanol yields based on the original corn using the mass loss data. Using  $\text{CO}_2$  production or weight loss as a measure of fermentation was also reported by other researchers (13).

The calculated ethanol yields were 33.6–34.6% of the original corn meal. Statistically, there were no differences in calculated ethanol yields between treatments 1 and 2, and 2 and 3, but the difference was significant between treatments 1 and 3. Ethanol yield from treatment 4 was significantly lower than those from treatments 5 and 6. Within the Fitz Mill grinding treatments (treatments 1–3, from the finest to the coarsest), the finer grinding resulted in higher ethanol yield on the basis of mass loss calculation, although the difference was less than 0.5%. A similar trend was also found for the flaking treatment group (treatments 4–6, from coarsest to finest), even though the difference was relatively small.

Mass loss seemed to be a convenient quantification method for ethanol quantification, and it is an easy and fast method for laboratory-scale batch fermentation runs. For large-scale industrial fermentation,  $\text{CO}_2$  headspace concentration and airflow can be measured continuously. This real-time monitoring of  $\text{CO}_2$  and thus ethanol production may be used as a dynamic control to optimize the fermentation process.

The typical solid contents in the finished beer were about 12% (the solid contents in the starting slurries before fermentation were about 33%), with the only exceptions of the flaked and extruded samples, which were 15, and 17% for the first and second fermentation replicates. Flaking and extruding broke the corn more extensively compared to other treatments. It may have fully ruptured the cellular structures, released more oil, partially gelatinized the starch, and produced a much finer corn meal. The heat during extrusion also dehydrated the extruded meal, which had 4% moisture content, compared to corn meals from other treatments that contained about 14% moisture. It is worth noting that organic acid production in the flaked and extruded beer was much lower than that for other treatments, which we attributed to the corn meal having less microbial contamination after extrusion sterilization compared to that in other treatments.

We observed that corn meal produced by different treatments had different physical properties. When slurried with water, the corn samples prepared by all treatments mixed well except for the flaked-extruded meal, which formed a thick nonflowable paste. The viscosities of all samples increased with heating at 82 °C. With the addition of  $\alpha$ -amylase, the viscosity decreased because of the

enzymatic hydrolysis of the gelatinized starch. At the end of liquefaction, the flaked and coarsely ground samples had higher viscosities than the finely ground and flaked-extruded corn samples, which was detected by observing mixing speed. The power supply for the stir motor was set at mark 60 of 140 voltage of a variable autotransformer, type 2PF1010 (Staco Energy Products Co., Dayton, OH). However, the stirring speed varied greatly from sample to sample because of the different viscosity properties of the slurry and oftentimes even for the same slurry because the heating and  $\alpha$ -amylase hydrolysis changed the viscosity. Generally speaking, the stirring speeds were <300 rpm.

**Effect of the Corn Breaking Method on Dry Matter Yield and Oil Partitioning into Thin Stillage after Laboratory Decanting.** Dry matter yield of the thin stillage decreased with increasing particle size (group 1 with Fitz Mill grinding only, treatments 1–3; and group 2 with flaking first, treatments 4–6) (Figure 2), which was reasonable because finer particles partition more completely to the thin stillage after decanting. The flaked-ground and flaked-extruded samples had excessive fine particles, which led about 70 to 90% of the total solids in the beer partitioning to thin stillage. In commercial operations, about 48% of the total solids in the whole stillage partitions to the thin stillage (data were obtained in our laboratory by analyzing typical industrial whole stillage, wet cake, and thin stillage), and the same whole beer when separated by our laboratory decanting method also gave about 50% dry matter yield in the thin stillage. This indicated that flaking and then further grinding or extruding may not be practical without changing the decanting operating parameters or changing how further grinding or extrusion is done. The reproducibility of the dry matter yield was good. The effectiveness of the laboratory decanting method specifically designed for this study was excellent (10) because of the similar dry matter yield produced in the laboratory and industry using the same beer.

Oil partitioning into the thin stillage was similar to dry matter yield in the thin stillage (Figure 2), which suggested that the majority of the oil may be attached to or contained within solid particles. On the basis of one batch of industrial decanting, the dry matter yield and oil partitioning in the industrial thin stillage were 48 and 53%, respectively, which were within the range of our treatments 1–4. Treatments 5 and 6, which were flaked-ground and flaked-extruded, gave much higher oil partitioning into the thin stillage with a corresponding increase in suspended

solids. **Figure 3** shows the correlation between dry matter yield and oil partitioning in the thin stillage.

Dry matter yield and oil partition in the thin stillage, however, were only generally correlated, which may mean that oil in some samples was freer than in others (the oil did not attach to nor was contained in the solids in the same manner after different treatments). Therefore, an oil extraction experiment was conducted to test oil extractability. **Figure 4** shows the effectiveness of multiple 2-mL hexane transfers during centrifugable oil measurement. After four transfers, the oil on top of the supernatant was effectively extracted. Five transfers were selected for this study to ensure complete oil recovery. **Figure 5** shows the effect of the number of 5-mL hexane extractions on the yield of hexane-extractable oil. For 40 g of finished beer, about 0.3–0.4 g of oil was extracted by one 5-mL of hexane mixing (the total oil content in 40 g of finished beer is 0.9 g), which was far from saturating the solvent. Although one 5-mL hexane extraction may not be an exhaustive extraction, it removed nearly 80% of the oil that was extracted by five 5-mL hexane extractions, and the results were highly reproducible. Therefore, one 5-mL hexane extraction was selected to represent the hexane-extractable oil. Higher centrifugal speed (10,000g for 10 min vs 3,000g for 10 min) was also tested. The oil yield increased by 10%, but the difference in oil yield among different treatments was only 3%. The 3,000g centrifugal force was selected because it was similar to the industrial decanting force.

**Figure 6** shows the oil extractability after different corn breaking treatments. Overall, 60–70% of the total oil in the beer was bound oil, about 25% was trapped oil, and only about 7% was the free oil. Flaking tended to reduce the amount of bound oil by slightly increasing the free oil and trapped oil compared to that in Fitz Mill grinding alone (treatments 1–3). Flaking probably crushed the corn germ more and freed more oil than grinding alone. Flaked and then extruded corn meal released the highest amount of free oil (25%), compared to the average of 7% for all the other treatments. The increase in free oil corresponded to the decrease in bound oil, which were all significantly different. Extrusion likely ruptured the cellular structure, fully destroyed the oil bodies, and therefore released more oil.

Fitz Mill grinding with different particle sizes had little effect on oil extractability. No significant difference was found in free, trapped, and bound oils among the three grinding treatments (treatments 1–3) (**Figure 6**). Thus, grinding may have two opposite effects on oil release. One effect is that when the oil-bearing corn germ was broken into finer particles, the oil became more extractable; however, fine-grinding may have also produced an opposite effect, that is, it may have more thoroughly mixed the oil-rich germ particles (33.4% oil) with the oil-poor endosperm pieces (0.8% oil) (2). Since the endosperm pieces contain highly hydrophobic proteins and fibers, the oil released from the finely ground germ may be absorbed by the endosperm particles, thus becoming nonextractable. This possible effect was observed for flaked-ground beer (treatment 5, **Figure 6**), which had the lowest free oil yield. After fine grinding, the free oil released by flaking (treatment 4) became nonextractable again.

In summary, the laboratory simulation of industrial dry-grind corn ethanol fermentation showed that the oil partition in thin stillage positively correlates to dry matter yield of the thin stillage. Grinding alone has limited effect on oil partition. Flaking made more oil extractable. Whereas, flaking-extrusion released the highest amount of free oil among all physical size reduction treatments tested. Oil released by flaking was less

extractable after fine grinding, probably due to the mixing of the free oil with the hydrophobic components in the endosperm particles. In order to maximize the oil partitioning in liquid phase during corn ethanol fermentation, two aspects have to be considered: (1) enhancing the release of oil from the corn germ particles and (2) preventing released oil from being absorbed by the endosperm or corn meal. Extrusion showed promise to be an effective way for corn pretreatment to maximize oil recovery; however, the meal fines in the liquid must be greatly reduced. These findings will help design future experiments to find a means of recovering oil in high yields postfermentation to improve profitability of dry-grind ethanol plants.

#### ABBREVIATIONS USED

BO, bound oil; DDGS, distillers' dried grains with solubles; FO, free oil; HPLC, high-pressure liquid chromatography; MWCF, multiple-wash–centrifuge–filtration; TO, trapped oil; TS, thin stillage.

#### LITERATURE CITED

- (1) <http://www.ethanolrfa.org/>, accessed May 2008.
- (2) Watson, S. A. Chapter 3, Description, Development, Structure, and Composition of the Corn Kernel. In *Corn: Chemistry and Technology*, 2nd ed.; White, P. J., Johnson, L. A., Eds; American Association of Cereal Chemists: St. Paul, MN, 2003; pp 71.
- (3) <http://www.biodiesel.org/>, accessed in May 2008.
- (4) Singh, P. P.; Maier, D. E.; Okos, M. R.; Cattanach, E.; Trumble, K. P. Effects of physical properties and operating parameters on soybean flaking. *J. Am. Oil Chem. Soc.* **1999**, *76*, 981–987.
- (5) Johnson, L. A. Chapter 11, Oil Recovery from Soybeans. *Soybeans: Chemistry, Production, Processing and Utilization*; Johnson, L. A., White, P. J., Galloway, R., Eds.; AOCS Press: Champaign, IL, 2008; pp 331.
- (6) Korn, S. R.; Harper, J. M. Extrusion of corn for ethanol fermentation. *Biotechnol. Lett.* **1982**, *4*, 417–422.
- (7) Park, Y. K.; Sato, H. H.; Martin, M., E. S.; Ciacco, C. F. Production of ethanol from extruded corn starch by a nonconventional fermentation method. *Biotechnol. Lett.* **1987**, *9*, 143–146.
- (8) Grafelman, D. D.; Meagher, M. M. Liquefaction of starch by a single-screw extruder and post-extrusion static-mixer reactor. *J. Food Eng.* **1995**, *24*, 529–542.
- (9) Prachand, S.; Rasmussen, M.; Khanal, S. K.; Pometto, A. L., III.; van Leeuwen, J. Solid-substrate fermentation of corn fiber by *Phanerochaete chrysosporium* and subsequent fermentation of hydrolysate into ethanol. *J. Agric. Food Chem.* **2008**, *56*, 3918–3924.
- (10) Wang, H.; Wang, T.; Pometto, A. L., III.; Johnson, L. A. Establishing a laboratory decanting procedure to simulate the industrial corn dry grind fuel ethanol process. *J. Am. Oil Chem. Soc.*, submitted for publication.
- (11) *Official Method 996.06, Official Methods of Analysis of AOAC International*, 17th ed.; AOAC International: Gaithersburg, MD, 2000.
- (12) *Statistical Analysis System*; SAS Institute: Cary, NC, 2002–2003.
- (13) Pandiella, S. S.; Garcia, L. A.; Diaz, M.; Daoud, I. S. Monitoring the production of carbon dioxide during beer fermentation. *Technical Quarterly - Master Brewers Association of the Americas* **1995**, *32*, 126–131.

Received for review June 27, 2008. Revised manuscript received August 15, 2008. Accepted August 29, 2008. This research was funded by the Grow Iowa Values Fund from the State of Iowa and Feed Energy of Des Moines, IA.

JF801970N