3-25-2009

Effect of Low-Shear Extrusion on Corn Fermentation and Oil Partition

Hui Wang
Iowa State University

Tong Wang
Iowa State University, tongwang@iastate.edu

Lawrence A. Johnson
Iowa State University

Follow this and additional works at: http://lib.dr.iastate.edu/fshn_ag_pubs

Part of the Food Chemistry Commons, and the Human and Clinical Nutrition Commons

The complete bibliographic information for this item can be found at http://lib.dr.iastate.edu/fshn_ag_pubs/2. For information on how to cite this item, please visit http://lib.dr.iastate.edu/howtocite.html.

This Article is brought to you for free and open access by the Food Science and Human Nutrition at Iowa State University Digital Repository. It has been accepted for inclusion in Food Science and Human Nutrition Publications by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
Effect of Low-Shear Extrusion on Corn Fermentation and Oil Partition

HUI WANG, TONG WANG,* AND LAWRENCE A. JOHNSON

Department of Food Science and Human Nutrition, 2312 Food Sciences Building, Iowa State University, Ames, Iowa 50011

To study oil distribution in fermentation liquid and solids for the purpose of recovering oil from corn stillage by centrifugation, a low-shear single-screw extruder was used to treat corn for dry-grind ethanol fermentation. Five different treatments for corn were used, and their effects on ethanol fermentation, oil distribution, and oil extractability were studied. Extruded corn with different particle sizes had similar ethanol yields (33% based on corn) because the starch was equally gelatinized by extrusion. Pretreatment with larger particle size before extrusion tended to have higher free oil than pretreatment with smaller particle sizes, but the effect was not dramatic, which indicates that manipulating particle size has limited effect on oil distribution in the liquid. Autoclaved flaked corn had lower ethanol yield because autoclaving at 28% moisture did not fully gelatinize the starch. Addition of protease and cellulase significantly increased the ethanol yield by at least 4%. A significant amount of bound oil became more extractable after enzyme treatment. Such oil can be effectively extracted into liquid phase by using a surfactant. In general, oil tended to be strongly associated with the solids in the thin stillage. By enzymatic treatment, 70% oil distribution was achieved in the thin stillage, compared to the conventional fermentation, where only 50% oil goes into the liquid. It was also demonstrated that mass loss after fermentation can be used to accurately quantify ethanol yield.

KEYWORDS: Dry-grind; extrusion; flaking; fuel ethanol; oil partition; thin stillage; surfactant

INTRODUCTION

Two processes are used to produce fuel ethanol from corn: one is a dry-grind process, in which the corn is ground and all components are fermented together; the other is a wet-milling process, whereby only the starch fraction is fermented after the corn is fractionated into different components. After years of expansion, the dry-grind process accounts for about 82% of the total corn fuel ethanol (Renewable Fuels Association, 2008) because it has relatively simple processing steps and requires lower capital investment compared to the wet-milling process. In a typical dry-grind process, the corn kernels are ground into meal, slurried with water and enzymes, cooked, and then inoculated with enzymes and Saccharomyces cerevisiae yeast. The hydrolyzed starch is then converted to ethanol during anaerobic fermentation. After the ethanol is removed by distillation, the ethanol-free mash is separated into dewatered solids of wet cake and liquid phase of thin stillage, which are dried and concentrated, respectively, and combined into the distiller's dried grains with solubles (DDGS). The major components in DDGS are protein, fiber, and oil. DDGS is used mainly for livestock feed. Due to the disappearance of starch during hydrolysis and yeast fermentation, the oil content is increased from 4% in original corn to about 14% in DDGS.

Removal of the oil will not only improve the feed quality but also increase the oil feedstock for the biodiesel and biolubricant production (1).

One way to remove the oil from DDGS is by solvent extraction. A patent application claimed that DDGS with 2–4% of crude fat can be produced after the oil was removed by hexane extraction and the extracted oil contained 10–15% of free fatty acids (2). However, due to the relatively low concentration of oil in the DDGS and the high capital investment for large-scale solvent extraction facility, solvent extraction has not seen widespread application near dry-grind ethanol plants. Another strategy is removing the oil from the liquid system by centrifugal force after fermentation, especially during the decanting step. This method uses only physical means without organic solvent, which should be relatively more possible for industry to adopt than the solvent extraction system. In the current industrial process, about 50% of the total oil distributes in the liquid phase (thin stillage) and the remainder goes with the solids (wet cake) (data from our own laboratory). It is believed that more oil could be partitioned into the thin stillage if proper physical/enzymatic treatments are used.

In our previous studies, we tested different physical breaking methods, such as grinding to different particle sizes, flaking, extrusion, and the combination of them, on oil distribution (1). The data showed that extrusion of the flaked corn with twin-screw extruder released most free oil. However, the extruded
material was too fine to be practically applicable in the corn dry-grind industry. The attempt to adjust extrusion conditions using the same high-shear twin-screw extruder was fruitless because only dry corn flakes (as-is moisture of about 14%) can be extruded. Corn meal with higher moisture (>20%) caused the extruder to jam instantly. To further investigate the effects of extrusion treatment on oil distribution, a single-screw extruder was used in this study. Such an extruder generates lower shear force and less kneading action than the twin-screw extruder. Interestingly, the single-screw extruder we used can extrude only corn materials with relatively high moisture (above 24%). The extrudate was a strand-shaped product, which is very different from that produced by the twin-screw extruder, which was powderly. If the corn particle in the fermentation is too fine, the majority of the solids will go to thin stillage, which will need more energy to concentrate than what is currently seen in the industry. The objective of this study was to investigate how this new extrusion treatment would influence ethanol fermentation and oil distribution into the thin stillage. The hypothesis for this study was that lower shear extrusion can free more oil without producing excessive meal fines that would go to the fermentation liquid; pretreatment with larger particle sizes before extrusion would distribute more oil in the liquid. Using extrusion has another major advantage in corn fermentation, which is the effective mixing and gelatinization of the starch. Therefore, this could be an improved processing strategy with more desirable end products than those from the conventional method.

Our previous studies also showed that a considerable percentage of oil in the corn mash was trapped oil, which was defined as the oil that cannot be centrifuged out but can be extracted after mixing with hexane (J). This oil is believed to exist on the surface of the solid particles and in fine droplets. An experiment was designed to test the hypothesis that a surfactant can displace the less extractable oil and make it free and recoverable by centrifugation.

**MATERIALS AND METHODS**

**Corn 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 α-amylase SPEZYME Xtra (13642 α-amylase units/g, optimal pH of 5.0–6.7) and a saccharifying enzyme G-ZYME 480 Ethanol (401 glucoamylase units/g, optimal pH of 4.0–4.5), both from Genencor Inc. (Cedar Rapids, IA), were used in the liquefaction and saccharification of the corn slurry, respectively. Additional cellulase and protein hydrolyzing enzymes were used in one treatment. These are one pectinase (Multifect Pectinase FE, activity of 145–180 pectinase units/g, optimal pH of 4.2–4.7), one β-glycanase (Multifect CX B, activity of 2250 GLU/mL, optimal pH of 5.0), one cellulase (Multifect CX GC, activity of 3200 EU/g, optimal pH of 4.0), and two proteases (Protex 15 L with activity of 1000 SAPU/g and optimal pH of 3.75, and Protex 89 L with activity of 3000 GSU/mL, optimal pH of 8.0), which were all from Genencor Inc. Lactrol (462 g of virginiamycin bioactivity/lb), an antibiotic extract, was from PhibroChem (Ridgefield Park, NJ). Dry yeast (S. 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 of these materials were of industrial grade, and most of them are being used today in dry-grind ethanol plants in the Midwest.

**Sample Treatments and Fermentation.** Five treatments, as illustrated in Table 1, were designed using four corn meals produced by different pretreatments. The extruded samples were made from corn with different pretreatments, that is, grinding with different particle sizes before extrusion. The first pretreatment was the finely ground corn meal using a hammer mill Fitz Mill model DAS 06 (Fitzpatrick Co., Elmhurst, IL) at 7,000 rpm with a 1 mm screen opening. The second pretreatment was the flaked corn produced by using a Roskamp Roller Mill model K (Roskamp Manufacturing, Inc., Waterloo, IA) set at a 0.25 mm (0.010 in.) gap between the rollers. The material had intermediate size (only a relative term in this study) before extrusion. The third pretreatment was the cracked dry corn using the same roller mill but with the roller gap fully open (the gap between the rollers was 3.45 mm or 0.136 in.). This corn was broken in such a way that one kernel was cracked into a few large pieces. It had the largest particle size. Before extrusion, the three particulated corn materials were tempered to increase the moisture from as-is of 14–28% to 30–40%. Tempering was done by placing the materials in a 2 gal bucket. A specific amount of water was added gradually by spraying with a spray bottle while the bucket was tilted and rotated manually. Intermittent stirring was done to prevent materials from caking. The tempered materials were mixed thoroughly, sealed in plastic bags, and set in a 5 °C cooler for about 12 h to equilibrate the moisture.

The extrusion was carried out using a C. W. Brabender single-screw extruder model PL2000 (C. W. Brabender Instruments, Inc., South Hackensack, NJ) with a 125-25 HC extrusion barrel, which has five heating blocks. The temperature profile of the heating blocks, from feeding to die sections, was 70–85–100–110–120 °C. A single-stage mixing screw was used. The diameter and the length of the screw were 3.175 and 76.2 cm (1.25 and 30 in.), and the maximum torque, temperature, and pressure were 240 mN, 400 °C, and 10,000 psi, respectively. The diameter of die opening was 3 mm. The speed of extrusion was maintained at 60 rpm. After they had cooled and dried at room temperature (25 °C), the extrudates were cut into 2.5 cm (1 in.) long pieces. The three extrusion treatments were designated “fine-grinding—extrusion”, “flaking—extrusion”, and “cracking—extrusion”, respectively.

The fourth treatment was autoclaving of the tempered corn flakes (with moisture content of 28%) at 120 °C for 20 min in a plastic bag. The autoclaved flakes were cooled at room temperature before fermentation similar to the extruded materials. It was called “flaking—autoclaving”. This treatment was designed to examine the effect of extrusion shear force on oil release because both were treated under similar temperature and moisture content, but one was with shear and the other without.

To study the effectiveness of protein and cellulose hydrolyzing enzymes on oil release, treatment 5 was designed. It had the same pretreatment as “flaking—extrusion” except that a cocktail of proteases and cellulase enzymes as listed in the previous section was added during fermentation. The addition levels were 0.5 mL/500 g of original corn materials for each enzyme after the pH of the slurry was adjusted to 4.7 and before the inoculation of the yeast. This treatment was named “flaking—extrusion enzymes”, which was designed to evaluate the effect
of enzymatic hydrolysis on the oil release during fermentation and the solid–liquid separation (J).

A similar 1.5 L laboratory-scale fermentation procedure as designed and used in our previous studies was used in this research except that we did not use an additional cooking step because the starch was expected to be gelatinized during extrusion (for treatments 1–3 and 5) or autoclaving (for treatment 4) (J). After the materials were slurried with water, all of the liquefaction and saccharification enzymes, yeasts, and other ingredients were added together. The fermentation was carried out in an incubator, LAB-LINE Incubator-Shaker, model 3525 (Lab-line Instruments Inc., Melrose Park, IL), for 60 h at 34 °C with shaking at 100 rpm. After fermentation, the finished beer was heated to 70 °C in a water bath for 20 min to destroy 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-pressure liquid chromatography (HPLC) after the samples (J) had been cleaned. After ethanol sampling, 100 ppm of sodium azide (Sigma Chemical Co., St. Louis, MO) was added to prevent microbial spoilage before the beer was sent to storage 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. Mass loss was used to calculate the ethanol yield, which was compared to the ethanol yield derived from HPLC ethanol concentration.

Thin Stillage Preparation, Oil Extractability, and Oil Partition Quantification. The preparation of thin stillage was carried out using the multiple-wash–centrifugal–filtration device and procedure designed in our laboratory. Detailed information about the device and the decanting procedure can be found in our earlier paper (4). Oil extractability after different treatments was examined using our previous methods (J). Three types of oil (in the unit of percentage) were defined: the free oil (FO) is the portion of the oil that can be recovered by centrifugation directly; the trapped oil (TO) is the fraction of oil that cannot be recovered by centrifugation alone but can be recovered by centrifugation after the slurry is mixed with hexane; the bound oil (BO) is the fraction of oil that cannot be recovered by centrifugation after the slurry is mixed with hexane; the bound oil (BO) cannot be recovered by centrifugation alone but can be recovered by centrifugation after the slurry is mixed with hexane; the bound oil (BO)

Table 2. Fermentation Results after Various Treatments*

<table>
<thead>
<tr>
<th>sample/treatment</th>
<th>treatment</th>
<th>mass loss (g)</th>
<th>HPLC ethanol (g/L)</th>
<th>acetic acid (g/L)</th>
<th>lactic acid (g/L)</th>
<th>solid (% in finished beer) based on mass loss</th>
<th>based on HPLC</th>
</tr>
</thead>
<tbody>
<tr>
<td>fine-grinding</td>
<td>1</td>
<td>162 b</td>
<td>120 b</td>
<td>0.90 a</td>
<td>0.57 ab</td>
<td>15.3 b</td>
<td>33.8 b</td>
</tr>
<tr>
<td>flaking</td>
<td>2</td>
<td>160 b</td>
<td>119 b</td>
<td>0.73 a</td>
<td>0.23 b</td>
<td>15.8 b</td>
<td>33.4 b</td>
</tr>
<tr>
<td>cracking</td>
<td>3</td>
<td>159 b</td>
<td>121 b</td>
<td>1.42 a</td>
<td>0.88 a</td>
<td>13.1 c</td>
<td>33.2 b</td>
</tr>
<tr>
<td>flaking</td>
<td>4</td>
<td>144 c</td>
<td>110 c</td>
<td>1.23 a</td>
<td>0.78 a</td>
<td>17.2 a</td>
<td>30.1 c</td>
</tr>
<tr>
<td>flaking</td>
<td>5</td>
<td>182 a</td>
<td>131 a</td>
<td>1.29 a</td>
<td>0.79 a</td>
<td>12.6 c</td>
<td>38.0 a</td>
</tr>
</tbody>
</table>

*Means with same letter within the same column are not significantly different (P = 0.05). N = 2. **Ethanol yield (%) is the ethanol mass based on the starting corn mass.

![Figure 1.](image-url)
for each sample. Statistical analysis was performed using General Linear Model procedures of SAS 9.1 (6).

RESULTS AND DISCUSSION

Fermentation Results. When mixed with water before fermentation, the extruded corn materials swelled greatly, absorbing most of the water, apparently due to starch gelatinization by extrusion. The semisolid mixture was gradually liquefied during the 60 h fermentation process. For all fermentations, microbial contamination was under control as shown by the low concentrations of acetic and lactic acids in the finished beer (Table 2).

Because the solids content of the starting slurries are not the same across the ethanol plants or laboratories, the ethanol concentration in the finished beer may not always be a good parameter for starch conversion comparison. In this study we used the ethanol yield, defined as the percentage of pure ethanol mass based on the mass of the original corn material (on dry weight basis). Two types of ethanol yields were calculated: one was based on the ethanol concentration in the finished beer as measured by HPLC, and the other was based the mass loss during fermentation.

Ethanol Yield Based on HPLC Concentration. Because the unit of the ethanol concentration by HPLC was w/v basis (g of ethanol/L of filtered finished mash), it needs to be converted to w/w basis. The ethanol concentrations in this study ranged from 130 to 160 g/L. The densities of such aqueous ethanol solutions at 20 °C are within the range of 0.9700–0.9800 g/cm³ (7). Therefore, the density of 0.9750 g/cm³ (975 g/L) was chosen for the calculation. Another assumption we made was about ethanol distribution. Ethanol forms strong hydrogen bonds with water molecules and is uniformly distributed in the beer system including inside and between the hydrated solid particles.

Therefore, ethanol yield by HPLC (%) = 100 × total beer mass × ethanol concentration in the beer (%) / original corn material mass

Thus, ethanol yield by mass loss (%)

= 100 × {total beer mass ×

HPLC ethanol concentration in the beer (g/L) / 975 (g/L)}/original corn material mass (g)

Ethanol Yield Based on Mass Loss. The following justifications and assumptions were used for calculation of ethanol yield based on mass loss after fermentation:

1. The mass loss was due to the production of CO₂ during fermentation. Multiple control tests were carried out to evaluate possible mass loss due to moisture and ethanol evaporation under the same fermentation condition. About 1500 g of 16% aqueous ethanol was put in a similar flask and shaken in the same incubator along with the treatment samples at 34 °C for 60 h. The average mass loss due to the evaporation of water, ethanol, and other volatiles was about 0.2 g/1,500 g of ethanol solution, or 0.013%; thus, this loss was ignored in this study.

2. Because the microbial contamination was under control during the fermentation, no non-CO₂ gas was produced and the CO₂ produced was solely from the respiration of yeast.

3. It is known that yeast can undergo two types of respirations: one is aerobic when oxygen is available, the other is anaerobic when oxygen is depleted. During aerobic respiration, glucose is fully oxidized into CO₂ and water, and no ethanol is produced. Only during anaerobic respiration is glucose partially oxidized into CO₂ and ethanol. It is understandable that when the yeast was inoculated in the corn slurry in the flask, the only oxygen the yeast can use is the oxygen solubilized in the slurry and that remained in the headspace of the fermentation flask. Once the oxygen is depleted, the diffusion of oxygen from outside the flask can be assumed to be limited because the flask was loosely capped and the generated CO₂ filled the headspace of the flask, preventing the atmospheric oxygen from coming in. Therefore, the fermentation continues to be ethanologenic until the starch is all consumed. According to aerobic respiration reaction

\[ 6O_2 C_6H_{12}O_6 (\text{glucose}) + 38ADP \rightarrow 2CO_2 + 6CO_2 + 38ATP \]

Then the nonethanologenic CO₂ production (g)

\[
= 44 \times (\text{oxygen in 1.5 L of corn slurry and 0.5 L of headspace at 34 °C}) / 32
= 44 \times (0.007 \times 1.5 + 1.2 \times 0.5 \times 21%) / 32
= 0.14 \ (g)
\]

(Note: 44 and 32 are the molecular weights of carbon dioxide and oxygen, respectively, 0.007 g/L is the oxygen solubility in water at 34 °C (8).) 1.2 g/L is the density of air at 34 °C atmospheric pressure, and 21% is the oxygen content in the air).

Compared to 140–160 g of CO₂ produced during one batch of fermentation, 0.14 g of nonethanologenic CO₂ was insignificant. Therefore, the total mass loss after fermentation was considered to be solely due to ethanologenic metabolism, that is, the anaerobic respiration of the yeast.

On the basis of the above justification, mass loss during fermentation can be considered to be from the ethanologenic production of CO₂. According to the anaerobic respiration reaction

\[ C_6H_{12}O_6 (\text{glucose}) + 2ADP \rightarrow 2C_2H_6O (\text{ethanol}) + 2CO_2 + 2ATP \]

so ethanol yield by mass loss (%)

= 100 × {46 × total CO₂ production (g) / 44}

/ original corn material mass

= 100 × {46 × total CO₂ production (g) / 44}

/ original corn material mass (g)

The ethanol yields based on HPLC concentration and mass loss are shown in Figure 1. Results obtained from the two methods are very similar and show a strong correlation. Ethanol yields at the highest (far left data) showed a 2-percentage-point difference between HPLC yield and mass loss yield. One possible reason may be that HPLC slightly underestimated the ethanol concentration when the ethanol level was high. Considering the variation in HPLC analysis (2 ± 5%, based on our data), the time-and-effort-consuming preparation of samples for analysis, and the need for a high-cost-high-maintenance HPLC system, mass loss is a much quicker and more economical ethanol yield quantification method with similar accuracy as HPLC analysis. It is especially useful for real-time monitoring of ethanol production during the fermentation process. It eliminates the periodic sampling of the fermentation slurry, which not only saves the fermentation materials but also avoids the danger of contamination or disturbance of the fermentation process. Using mass loss or CO₂ production was also reported by other researchers (9, 10).

Ethanol yields from fermentation treatments 1–3 were similar to the typical fermentation in our previous study, and they were also similar to industry data, about 120 g/L ethanol in the...
different letters represent significant difference at $P = 0.05$.

finished beer. As expected, beers with higher ethanol yield had lower solids content because more starch was used for fermentation (Table 2). Fermentation of flaking—extrusion with hydrolyzing enzyme cocktail produced the highest ethanol yield, about 131 g/L or 38% yield based on the starting corn material. The hydrolyzing enzymes may have led to more fermentable sugars that were not available for yeast if only amylase was used. Ethanol from flaking—autoclaving fermentation had the lowest ethanol level, implying that moisture content of 28% in the corn material was not enough to gelatinize the starch when it was autoclaved at 120 °C for 20 min. On the contrary, starch in the corn materials with the same moisture content was fully gelatinized by the extrusion cooking because of the shear and mixing actions.

Effects of Extrusion Treatment on Dry Matter Yield and Oil Partition in Thin Stillage. The results show that the finer the corn was broken before single-screw extrusion, the higher the dry matter yield of the thin stillage had (Figure 2). The reason may be that even though the pretreated corn material was further agitated and heated by extrusion, the low-shear force generated by the single-screw extruder was not enough to eliminate all of the particle size difference among treatments. For the three extrusion treatments, the oil partition in thin stillage was not significantly different. Flaking—autoclaving resulted in a dramatically lower dry matter yield of the thin stillage. This probably can be attributed to two reasons: one is that the corn flake was not processed by extrusion and thus retained its fairly large particles; the other is that the particles had much undigested starch due to incomplete gelatinization (lowest ethanol yield) of starch. Therefore, during laboratory decanting, a lower amount of fine solids went to the thin stillage compared to the other treatments. The oil partition in the thin stillage made from flaking—autoclaving-treated sample was also very low because most of the oil remained in the larger particles; the other is that the particles had much undigested starch due to incomplete gelatinization (lowest ethanol yield) of starch. Therefore, during laboratory decanting, a lower amount of fine solids went to the thin stillage compared to the other treatments. The oil partition in the thin stillage made from flaking—autoclaving-treated sample was also very low because most of the oil remained in the larger particles; the other is that the particles had much undigested starch due to incomplete gelatinization (lowest ethanol yield) of starch. Therefore, during laboratory decanting, a lower amount of fine solids went to the thin stillage compared to the other treatments. The oil partition in the thin stillage made from flaking—autoclaving-treated sample was also very low because most of the oil remained in the larger particles.

or in other words, the majority of the oil in thin stillage formed certain complexes with the fine particles. The oil may be attached on the surface of the solid particles or is buried inside. Because the fiber and corn protein zein are more nonpolar than water, oil will have a high affinity to fiber and endosperm protein particles instead of existing as free oil in the water or as an oil/water emulsion, especially when the oil concentration in the thin stillage is <1% on a wet weight basis, whereas the solids content of thin stillage is typically 6–12%. Therefore, there is a need to study how to displace the oil from the solids so it can be centrifugally removed.

Effect of Extrusion Treatment on the Extractability of Oil. For the three extrusion treatments with different particle size grindings, the finer the corn was broken before extrusion, the less free oil was produced after fermentation but the more bound oil was formed. A similar observation was made in our previous study when the corn was ground into different sizes without further extrusion. We believe it can be explained by the same hypothesis, that is, the finer the grinding was, the more hydrophobic area was produced, which made the oil more nonextractable (1). Autoclaving made most of the free oil and a significant part of trapped oil into bound oil, probably because the oil formed tight complexes with fiber and protein. Interestingly, flaking—extrusion—enzymes treatment made a significant amount of bound oil into trapped oil; that is, trapped oil increased from 37 to 59% after enzyme cocktail addition using the same materials, as shown by the comparison between treatments 2 and 5 (Figure 4). This may be because the hydrolyzing enzymes destroyed the fiber—oil and protein—oil complexes, or the intact cellular structure, exposing more oil that used to be contained inside the solids. However, most of the oil still stuck on the newly produced small particles. Or more likely, although some bound oil became free oil, it may
have been absorbed by newly generated hydrophobic surfaces due to the generation of more fine fiber, protein, or their complexes by enzyme digestion. The centrifugal force used in the experiment (3,000 g) was not high enough to free the oil from the hydrophobic solid surface. However, this attached oil should be available for hexane extraction because of hexane’s high affinity for oil.

**Effect of Surfactant on Recovery of the Oil.** As indicated by [Figure 5](#), addition of surfactant in the laboratory beer from flaking—extrusion—enzymes treatment dramatically increased the distribution of oil into the supernatant after centrifugation, from 8 to about 40%. It is speculated that the surfactant disrupted the association between oil and the hydrophobic particles. The oil was literally washed off by surfactant and formed an emulsion, which remained in the supernatant after centrifugation. Addition of surfactant in the laboratory beer from flaking—autoclaving treatment only slightly increased the oil distribution in the supernatant, and the increase was not statistically significant. This is probably due to the fact that the trapped oil in the beer was much lower than in the flaking—extrusion—enzymes beer and the large variation. These observations suggest that surfactant can displace the oil which is on the surface of the solid particles. Although using Joy detergent may not be practical, it showed that detergent has potential to replace hexane to extract trapped oil in corn fermentation liquid.

In this research, we investigated the effect of low-shear extrusion in combination with other corn pretreatment on fermentation performance and on distribution of the oil in the liquid. The oil partition in the thin stillage was correlated with the dry matter yield of the thin stillage. When protein and cellulose hydrolyzing enzymes were added during fermentation, the ethanol yield was significantly increased and some of the bound oil became more extractable trapped oil, which may be extracted by the addition of surfactants. More work in enzymatic and surfactant treatment is needed to improve oil distribution and recovery from the stillage.

**LITERATURE CITED**


