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Use of surfactant and enzymes in dry-grind corn ethanol fermentation improves yield of ethanol and distillers corn oil

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Abstract
Distillers corn oil (DCO) is a valuable co-product of dry-grind corn ethanol process. It can be used for bio-fuel production and in animal feed. DCO can be present in different forms in the fermentation matrix, including oil adhering to solid surfaces such as cell wall and protein matrix, and oil contained in unbroken cells, which is difficult to partition to thin stillage by decanting. Effects of using surfactant (Tween® 80) and enzymes during fermentation on DCO partition to thin stillage and DCO recovery from the condensed corn distillers with solubles (CCDS) were investigated. There was more than 8 ~ 10% DCO adhered to wet cake solids in whole stillage produced by conventional procedure, and this part of DCO was moved to thin stillage when 500 ppm of Tween® 80 was used during fermentation. Enzymes reduced the particle size of wet cake solids and released more DCO from wet cake to thin stillage. However, the use of protease reduced oil recovery (4.0% versus 7.9% and 17.9%, protease versus control and non-starch polysaccharide hydrolyzing enzymes) by producing partially hydrolyzed protein, which may have acted as emulsifier. Moreover, a synergistic effect between the use of enzymes and Tween® 80 was found on DCO partition in thin stillage and its recovery from CCDS

Keywords
Dry-grind ethanol process, Distillers corn oil (DCO), Oil partition, Oil recovery Tween® 80, Hydrolyzing enzymes

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USE OF SURFACTANT AND HYDROLYZING ENZYMES IN DRY-GRIND CORN PROCESSING IMPROVES ETHANOL YIELD AND DISTILLERS CORN OIL RECOVERY

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Abstract:

Distillers corn oil (DCO) is a valuable co-product of dry-grind corn ethanol process and can be used in animal feed and for bio-fuel production. DCO can be in different forms in the fermentation matrix, including oil adhering to solid surfaces such as cell wall and protein matrix and oil contained in unbroken cells, which was difficult to partition to thin stillage by decanting. Effects of using surfactant (Tween® 80) and hydrolyzing enzymes during fermentation on DCO partition after decanting and DCO recovery from condensed corn distillers with solubles (CCDS) were investigated. There was about 8~10% DCO adhered to wet cake solids in whole stillage produced by conventional procedure, and this part of DCO partitioned to thin stillage when 500 ppm of Tween® 80 was added in corn slurry. Enzymes reduced the particle size of wet cake and released more DCO from wet cake to thin stillage. However, the use of protease reduced oil recovery (4.0% versus 7.9% and 17.9%, protease versus control and non-starch polysaccharides hydrolyzing enzymes) by producing partially hydrolyzed protein, which may have worked as emulsifier. Moreover, a synergistic effect between the use of enzymes and Tween® 80 was found on DCO partition in thin stillage and recovery from CCDS.

Keywords: dry-grind ethanol process, distillers corn oil (DCO), oil partition, oil recovery, Tween® 80, hydrolyzing enzyme.

Highlights:

- The use of Tween® 80 at the concentration of 500 ppm in corn slurry led to highest oil partition in thin stillage;
- The use of hydrolyzing enzymes during fermentation tended to move more oil from wet cake to thin stillage;
- The synergistic effect between surfactant and hydrolyzing enzyme on oil recovery from CCDS was demonstrated;
- The use of Tween® 80 had no negative effect on ethanol production.

1 Introduction

Dry-grind ethanol industry has become the second largest corn user in the United States, producing 90% of the ethanol in U.S. in 2015. This industry was very profitable in the past. However, due to the sharp decline in crude oil price, the price of dry-grind corn ethanol fell sharply from $2.18 per gallon in December 2014 to $1.25 per gallon in February 2015 (Irwin and Good, 2015; Wisner, 2015). The additional revenue streams from co-products of the dry-grind ethanol process are becoming more important. One such co-product is the distillers corn oil (DCO), which is the oil recovered from post-fermentation streams. The revenue from DCO has become more and more important for U.S. ethanol plants, particularly in the low margin times (Jayasinghe, 2015).

The most widely used method for DCO recovery in dry-grind ethanol process is separating the oil from the condensed corn distillers solubles (CCDS) by centrifuge (Moreau et al., 2012). The oil recovery procedure was described in a U.S. patent 7601858. In brief, after collecting ethanol by distillation (Figure 1), the ethanol-removed whole stillage is separated by decanting into thin stillage and wet cake. In general, 40~60% of total oil in corn whole kernel is left in wet cake and the rest goes to thin stillage after decanting. The thin stillage is further evaporated to produce CCDS with 60~85% moisture content, and the DCO is extracted from the CCDS by using a disk stack centrifuge (Cantrell and Winsness, 2009). Many efforts have been made to improve the DCO recovery from CCDS. Centrifugation coupled with oil

recovery aid is easy to use and relatively effective in improving oil recovery from the CCDS. A number of commercial oil recovery aids have been designed for large-scale process, including FoodPro SA9843 corn oil yield improver (General Electric, Trevose, PA, USA), PTV M-5309 corn oil extraction aid (Ashland Chemical, Covington, KY, USA), Ashland DPI-428 (Ashland Hercules Water Technologies, Wilmington, DE, USA), and Hydri-Maize Demulsifier 300 (Hydrite Chemical Co., Waterloo, IA, USA). However, these products are designed for oil recovery from CCDS only, and do not affect the partitioning of oil during decanting and oil from wet cake.

DCO is present in several different forms during the dry-grind ethanol process, including the oil adhering to surface of wet cake solids, like cell wall and protein matrix (Majoni et al., 2011). Based on our preliminary experiments, about 8~10% w/w of total corn oil was adhered to solid wet cake particles, which did not partition to thin stillage fraction by decanting. The oil adhering to the wet cake surface is very similar to the oil stain on a fabric surface of clothes. Surfactants as cleaning agents work by reducing the surface tension and removing the oil as micelles. In our previous study on the distribution of different types of oil in CCDS, the use of surfactant mix resulted in a higher recovery of oil partially coming from surface adhering oil (Fang et al., 2015). This gives the basis for our hypothesis that the cleaning function of surfactants could be applied in dry-grind ethanol process to partition the oil from wet cake to the thin stillage.

Surfactants have been applied in aqueous extraction processing to improve vegetable oil recovery. Sodium dodecyl sulfate was used to improve recovery of soybean oil (Campbell and Glatz, 2009) and canola oil (Tuntiwiwattanapun et al., 2013) by extracting the oil trapped in disrupted cellular matrix. The extended-surfactants, which is a recently developed new class of surfactants that works by significantly reducing the interfacial tension, extracted 93-95% of total oil from the insufficiently ground peanut and canola seeds (Do and Sabatini, 2010). However, due to the safety issue of sodium dodecyl sulfate and extended-surfactants, they are not allowed for human or animal consumption. To date, only a few reports on destabilization of oil-in-water emulsion by using food-grade-surfactants are available. Fang et al. (2015) attempted to improve oil recovery from CCDS by using Tween® 80-Span® 80-silica nanoparticle mixture. Zhang and Wang (2016) used Tween® 20 to improve peanut oil recovery. Both works explained the improved oil recovery as the result of unstable emulsion formation by surfactant-protein competition on the emulsion interface. Thus, we believed that using surfactant at the beginning of dry-grind ethanol process could not only improve oil partition in thin stillage but also increase the oil recovery from CCDS by demulsification.

There is a large portion of oil (40~60% of total oil in corn) remaining in intact cells and protein/polysaccharide matrices of wet cake solids; enzyme hydrolysis of the solids might be an efficient way to release this part of the oil. Luangthongkam et al. (2015) reported that using a combination of cellulolytic enzymes, protease, and

64 phytase during fermentation led to a higher oil partition in thin stillage. However, no
65 research has been reported to confirm if hydrolyzing the non-fermentable components
66 during fermentation step can improve oil recovery from CCDS. Therefore, the
67 objectives of this research were 1) to determine the optimum level and best processing
68 stage to add the surfactant (Tween® 80) and 2) to investigate the synergistic effects of
69 surfactant and hydrolyzing enzymes (protease, cellulase, and pectinase) on ethanol
70 production and oil recovery.

71 **2 Materials and Methods**

72 **2.1 Materials**

73 Ground whole corn meal (average particle size of 0.44 mm), α-amylase
74 (Novozymes, Franklinton, NC), glucoamylase (liquid, Spirizyme Excel XHS,
75 Novozymes, Franklinton, NC), dry yeast (Saccharomyces cerevisiae; commercial
76 grade currently being used in the ethanol plant) and antibacterial chlorine dioxide
77 (commercial grade) were donated by Lincolnway Energy LLC, Ames, IA. Cellulase
78 (75,000 CU/g) and pectinase (3500 ENDO-PG/g) were provided by Bio-Cat (Troy,
79 VA). In this study, pectinase and cellulase were used as a mix (PC) with ratio 1:1 (w:
80 w). Fermgen^{TM} (Acid protease, liquid, activity 1,000 SAP units/g) was provided by
81 DuPont Industrial Biosciences (Palo Alto, CA). The other chemicals, including
82 Tween® 80 (polysorbate 80), hydrochloride acid, petroleum ether, and ethyl ether
83 were purchased from Fisher Scientific (Fairlawn, NJ, USA).

84

2.2 Corn fermentation

The procedure of lab-scale fermentation is shown in Figure 2. The liquefaction and simultaneous saccharification and fermentation of the corn slurry were performed in 250-mL round bottom flasks with Tornado IS6 Overhead Stirring System (Radleys Discovery Technologies, Shire Hill, Saffron Walden, UK) equipped with an anchored stirring shaft. Ground corn was mixed with cold DI water (or Tween® 80 water solution) at a 1:2 ratio, w: w.. The total amount of slurry was maintained at 225-230 g. α-Amylase (0.15 mL) was added to the slurry and mixed at 81°C for 3h. After that, the flasks were cooled to 30°C in an ice bath, and the pH of the cooled slurry was adjusted to 4.0 with 3 M sulfuric acid. Chlorine dioxide (0.021 mL), ammonium sulfate (0.065 mL of 0.2 g/g water), gluco-amylase (0.15 mL) and dry yeast (0.15 g) were added. Fermentation was carried out at 30°C for 64 h with continuous stirring (190 rpm). During fermentation, ethanol production was estimated by mass loss according to the following equation (Wang et al., 2009).

$$\text{Ethanol yield } (\text{g}/100\,\text{g dry corn}) = 100 \times \frac{46 \times (g\ of\ mass\ loss)}{44 \times (g\ of\ dry\ corn)}$$

Where 46 and 44 are molecular weights of ethanol and CO_2, respectively.

For the experiments of adding hydrolyzing enzymes during fermentation, 0.375 mL of Fermgen or 0.3 g of PC was added before starting the fermentation. For experiments of adding Tween® 80 in fermentation, Tween® 80 water solutions of 200, 300, 400, 500, 600, 700, 800, 1000 ppm were prepared and ground corn meal was mixed with Tween® 80 solutions instead of DI water.

106 **2.3 Post-fermentation processing**

107 The rotary evaporation (Rotavapor R-210 and Vacuum Pump V-700, Buchi,
108 Switzerland) at 82 °C for 10 min was used to simulate the industrial distillation step.
109 After the distillation, water was added to make up for the weight loss during rotary
110 evaporation, giving the stillage a final solids content of 13% w/w. The whole stillage
111 was subjected to decanting following a procedure that simulates the industrial
112 decanting process (Wang et al., 2009) to obtain the thin stillage and wet cake
113 fractions. The wet yields, solid content and oil content of thin stillage and wet cake
114 were measured. CCDS was made by condensing thin stillage with rotary evaporation
115 at 75°C for 30 min. The solid content of CCDS was adjusted to 28% with water. All
116 CCDS samples were stored at 4°C until use.

117 **2.4 Oil recovery from CCDS**

118 Oil recovery from CCDS was simulated by using the method of Fang et al.
119 (2015). To compare the effect of surfactant addition, 2300 ppm Tween® 80 was
120 added in CCDS before heating and shaking. In brief, 40g of CCDS in a 250-mL
121 centrifuge bottle was heated at 80-85°C for 10 min at 100 rpm shaking in a shaker
122 water bath (Model R-76, New Brunswick Scientific Co. Inc., NJ, USA). Immediately
123 following heating and shaking, oil was separated using a Centra MP4 centrifuge
124 (International Equipment Company, Needham Heights, MA, USA) at 3000 xg for 10
125 min. The oil layer was collected by washing the oil on the top layer with hexanes (5
126 washes with 20, 20, 10, 10, and 5 mL respectively). The solvent was removed by

127 evaporation then by vacuum drying. The weight of the oil was determined
128 gravimetrically.

129 **2.5 Surfactant recyclability with backset**

130 Thin stillage sample was collected from corn fermentation with 500 ppm
131 Tween® 80 as described in Section 2.2 and was used as backset to replace part of the
132 incoming water and made the 150 g total volume liquid in the new batch of
133 fermentation. Batches of fermentation were performed with corn slurries prepared
134 with 100% DI water (Treatment 1), 100% fresh made 500 ppm Tween® 80
135 (Treatment 2), 50% fresh made 500 ppm Tween® 80 solution + 50% backset
136 (Treatment 3), and 50% fresh made 1000 ppm Tween® 80 solution + 50% backset
137 (Treatment 4). The thin stillage decanted from fermentation as backset was collected
138 as described in Section 2.3.

139 **2.6 Analytical methods**

140 The adhering oil droplets on wet cake surface were observed using a light
141 microscope (BX40, Olympus Corporation, Tokyo, Japan) after staining in Sudan IV
142 ethanol solution.

143 The water holding capacity of wet cake was measured to figure out the reason for
144 the low solid content in thin stillage resulted by using surfactant. Wet cake samples
145 were dried overnight at 105 °C. The water holding capacity (WHC) was analyzed by
146 soaking 250 mg of dried wet cake in 10 mL of water for 24 h at room temperature.
147 Samples were centrifuged at room temperature at 5000 xg for 20 min, and inverted

and subsequently drained for 15 min. WHC was calculated as the amount of water retained per gram of dry material.

The solid content was determined by weight difference after oven-drying at 105°C for 5 h. Total oil content was determined by acid hydrolysis method (AOAC Official Method 922.06).

2.7 Calculations

The calculations of wet yield of thin stillage, solid distribution in thin stillage, oil partition in thin stillage, and oil recovery from CCDS are described as below:

$$\text{Wet yield of thin stillage, \%} = 100\% \times \frac{g\ of\ thin\ stillage}{g\ of\ whole\ stillage, before\ decanting}$$

$$\text{Solid partition in thin stillage, \%} = 100\% \times \frac{g\ of\ dry\ solids\ in\ thin\ stillage}{g\ of\ dry\ solids\ in\ whole\ stillage, before\ dacanting}$$

$$\text{Oil partition in thin stillage, \%} = 100\% \times \frac{g\ of\ oil\ in\ thin\ stillage}{g\ of\ oil\ in\ whole\ stillage, before\ decanting}$$

$$\text{Oil recovery from CCDS, \%} = 100\% \times \frac{g\ of\ free\ oil}{g\ of\ total\ oil\ in\ thick\ stillage}$$

2.8 Statistical analysis

All the treatments and analysis were triplicated. The data were analyzed by using SAS (Version 9.4, SAS Institute Inc. Cary, NC) to test treatment difference at 95% significant level.

3 Results and Discussion

3.1 Oil partition in thin stillage as affected by adding surfactant in corn slurry

The optimal Tween® 80 concentration for increasing oil partition in thin stillage was investigated. The concentration of Tween® 80 in corn slurry had a significant effect on oil partition in thin stillage after decanting. As shown in Figure 2, the oil partition in thin stillage was at 40% of total corn oil for surfactant concentrations below 300 ppm, but the partition significantly improved to 50% when the Tween® 80 concentration was increased to 500 ppm. However, no more improvement was seen over 500 ppm. Since surfactant cannot release the oil from the unbroken cells, the extra oil partitioned in the thin stillage should be coming from the adhered oil on the wet cake surface. This hypothesis is supported by microscopic observations in Figure 3, in which Sudan IV stained oil droplets can be seen in wet cake surface of the control, but hardly seen in 500 ppm Tween® 80 treated samples.

The adhering oil on the surface of wet cake is very similar to oily dirt on surface of clothes. Surfactant works as detergent to move adhering oil into water during washing. The lipophilic ends of the surfactant molecules attach themselves to the oily dirt, and the hydrophilic heads attach to the water. With continuous whirling, the oily

dirt is pulled away from the surface. In the fermentation tank, Tween® 80 worked as a detergent, the adhering oil droplets were moved into the aqueous phase from the surface of the wet cake with the help of continuous mixing in the process.

3.2 Ethanol production rate and yield as affected by using enzyme and surfactant

There was no adverse effect of adding Tween® 80 in corn slurry on ethanol yield and production rate. When only Tween® 80 was added to fermentation (C500), a significant increase in ethanol yield (from 28.13 in the control to 30.71 g/100 g dry corn in C500) was observed (Table 1). The maximum ethanol production rate of C500 was similar with the control, but a higher average ethanol production rate was observed (Figure 4). The mechanism behind this finding was not clear. However, similar results were reported in studies of cellulosic ethanol production when non-ionic surfactants were added, for the purpose of reducing enzyme-substrate interaction (Alkasrawi et al., 2003). They reported that surfactant adsorption onto lignin prevented unproductive binding of enzymes to lignin. Park et al. (1992) also concluded that surfactants help the enzyme to desorb from the binding site on the substrate surface after the completion of saccharification at that site. Though, the dry-grind ethanol process has a different circumstance from cellulosic ethanol process, the high fiber content in dry-grind ethanol process might have similar side-effects on enzyme activity as by cellulosic matters. In this study, the non-ionic surfactant may have the same mechanism to improve saccharification efficiency in dry-grind ethanol process.

When only Fermgen (mainly a protease enzyme) was used during fermentation (Table 1 and Figure 5), both ethanol production rate and yield increased significantly compared to the control. The increased ethanol production rate suggested that fermentation time could be reduced to approximately 40 h from 65 h. These findings agreed with the results reported in literature in which protease was shown to increase ethanol production rate and decrease supplemental N requirements (Johnston and McAloon, 2014). Protease enzyme hydrolyzed corn protein and increased the concentration of free amino acid and peptides which can be used as N source for yeast. Moreover, protein matrix embeds corn starch in corn endosperm (Watson, 1987) and can be broken by a protease to make starch more available for producing ethanol (Lamsal and Johnson, 2012).

When only the mixture of pectinase and cellulase was added (PC in Table 1 and Figure 6), ethanol production rate and final yield were significantly increased compared to the control. However, its effect was less significant than using Fermgen. The mechanism of using PC to improve ethanol production rate and final yield is different from using protease enzyme. PC hydrolyzed non-starch polysaccharides and produced fermentable monosaccharides for yeast. However, products of PC hydrolysis cannot stimulate the activity of yeast as the products of protease hydrolysis.

When both Tween® 80 and PC were used, the ethanol production yield of using PC500 (34.98 g ethanol per 100 g dry corn) was not significantly different with PC

alone (34.52 g ethanol per 100 g dry corn). Similar pattern was observed when Fermgen and Tween® 80 were used, in which using Fermgen (35.19 g ethanol per 100 g dry corn) had no significant different compared to F500 (35.25 g ethanol per 100 g dry corn). Improvement in ethanol yield by using Tween® 80 was not observed when enzymes were added during fermentation. As we speculated in the previous section, non-ionic surfactant may improve ethanol yield by affecting enzyme-substrate interaction. The enzyme hydrolysis reduces the particle size and makes the substrate more available to saccharification enzyme, and this effect might have made Tween® 80 unnecessary in this step to further reduce enzyme-substrate interaction.

3.3 Thin stillage yield, and solid and oil partitions in thin stillage as affected by enzyme and surfactant treatments

The composition and properties of thin stillage are very important for the performance of piping, centrifuge efficiency, oil recovery and energy cost in ethanol plant. The wet yield of thin stillage was significantly improved in Fermgen treatment (85.9%) and PC treatment (87.1%) compared to the control (79.3%). Whereas, PC treatment had similar solid content (7.2%) and Fermgen treatment had lower solid content (6.8%) compared to the control (7.2%). Similar results were observed by Yao et al. (2014) and Luangthongkam et al. (2015) when fiber hydrolyzing enzymes were used in fermentation. Likely, the hydrolysis action of the enzymes reduced the water holding capacity of insolubles in the wet cake by interrupting the large protein matrix and cell wall network. In addition, the enzyme actions might have reduced the particle

size of the solid substrates. Both functions of the enzymes resulted in more liquid and finer solids partition in thin stillage, which contributed to the high thin stillage yield and solid partition in thin stillage from the PC and Fermgen treated process.

A significant reduction of solid content in thin stillage was observed when Tween® 80 was added in corn slurry, which are 7.2% versus 6.8% (Control versus C500), 6.8% versus 6.6% (Fermgen versus F500), and 7.2% versus 6.9% (PC versus PC500) in Table 2. Lower solid content in thin stillage is preferable in ethanol plants, due to low energy cost in piping and less fouling of evaporators. To further understand the reason for this observation, we tested the WHC of the dried wet cake for Tween® 80 solution and water. The dried wet cake can hold more DI water (4.39 ± 0.17 g water/g dried wet cake) than Tween® 80 solution (3.87 ± 0.02 g solution/ g dried wet cake). This may be explained by the reduced capillarity of solution due to the action of surfactant, which make the liquid separation easier from the dried wet cake by centrifugation.

Enzymes and Tween® 80 treatments significantly increased oil partition in thin stillage. Since more oil is in thin stillage, more oil can potentially be extracted. Table 2 shows that using hydrolyzing enzymes significantly increased oil partition in thin stillage from 40.8% (Control) to 52.6% (Fermgen) and 52.3% (PC). Comparing with using enzyme alone, Tween® 80 further improved the oil partition in thin stillage, which were 40.8% versus 49.3% (Control versus C500), 52.6% versus 58.5% (Fermgen versus F500), and 52.3% versus 54.9% (PC versus PC500). Before

decanting, the oil maybe present in different forms and a large proportion of oil remains in unbroken cells and large matrix, like protein and cell wall components. This part of oil can be released by enzyme hydrolyzing the solids or large particles. These findings agreed with the reported observations in literatures (Luangthongkam et al., 2015; Yao et al., 2014), which shown an improved oil partition in thin stillage by adding NSPs hydrolyzing enzymes during fermentation. Different from the action of enzymes, the use of surfactant improved the oil partition in a different way, i.e. washing the adhering oil from wet cake surface into the aqueous phase. However, when hydrolyzing enzymes were added, the improvement of oil partitioning in thin stillage by adding surfactant in corn slurry was reduced comparing with non-enzyme treatments, which partitioned 8.5% more of total corn oil from the control to C500, 5.9% from Fermgen to F500, and 2.6% from PC to PC500. The use of hydrolyzing enzymes weakened the function of surfactant, probably due to the enzymatic hydrolysis of protein and cell wall that freed the adhering oil into thin stillage. This hypothesis is supported by the parallel improvements in solid partition and oil partition when enzymes were used (Table 2).

3.4 Oil recovery from CCDS as affected by surfactant and enzyme

The use of hydrolyzing enzymes significantly improved oil content in CCDS, as shown in Table 3. When Tween® 80 was used, the significant oil content increase was found in the control (6.8% versus 9.3%, Control versus C500), whereas, only

294 numerical but not significant improvements were observed on Fermgen and PC
295 treatment.
296 In the lab-scale experiment of oil recovery from CCDS, the use of PC in
297 fermentation significantly improved oil recovery from 7.9% (control) to 17.4% (PC)
298 when Tween® 80 was not added (Table 4). A similar finding has been reported by
299 Yao et al. (2014) who found an increased free oil recovery from thin stillage after
300 polysaccharide hydrolyzing enzyme treatment in fermentation. As expected, because
301 of the polysaccharides being partially broken by the PC enzymes, the trapped oil
302 would be released and present in the form of free oil. However, a significantly lower
303 oil recovery was found when Fermgen was added in fermentation. The partial
304 hydrolysis of protein maybe the reason for this observation. It has been reported that
305 the emulsification ability of rice protein (Paraman et al., 2007), soy protein isolate
306 (Kim et al., 1990) and pea protein isolate (Barac et al., 2011) can be enhanced by
307 partial enzyme hydrolysis. The duration of proteolytic treatment and enzyme type
308 played very important roles in the properties of enzyme modified proteins. In this
309 study, the protease hydrolyzed corn protein may have worked as a good emulsifier to
310 stabilize oil-in-water emulsion.
311 When Tween® 80 was added in corn slurry, the significant improvement in oil
312 recovery from CCDS was found in all treatments comparing with the non-surfactant
313 treatments (Table 4). Especially for the Fermgen treatment, adding surfactant in the

process significantly improved oil recovery from 4.0% (Fermgen) to 24.9% (F500) without significant change in oil content of CCDS (Table 3).

Similarly, when Tween® 80 was added in CCDS directly, the significant improvement of oil recovery was found in all treatments comparing with non-surfactant treatments (Table 4). Protein is a major stabilizer for oil-in-water emulsion in CCDS. The oil recovery improvement of adding Tween® 80 in CCDS has been explained as a result of surfactant and protein competition which formed an unstable emulsion (Fang et al., 2015), when proteins were replaced by surfactants. However, these improvements were significantly lower than when surfactant added in corn slurry.

The enhanced oil recovery from CCDS by adding surfactant in corn slurry is explained with two proposed mechanisms 1) more available oil is present in CCDS, and 2) formation of more unstable emulsion. In the first explanation, surface adhering oil could have been moved from wet cake surfaces during 64 h of fermentation into the aqueous phase (Figure 1) and partitioned in thin stillage after decanting. This part of oil was present in thin stillage as suspended oil droplet (free or in emulsion) and can be recovered by centrifugation. In explanation for the second mechanism, when surfactant was introduced to corn slurry or CCDS, a protein-surfactant co-stabilized emulsion was formed (Figure 5). A higher concentration of protein on interfacial surfaces contributes to a stronger interaction among protein molecules and this interaction stabilizes the protein enabled emulsion (Mackie et al., 1999). When

Tween® 80 was added in CCDS, Tween® 80 competed and replaced protein from the interface of emulsion. Although surfactants like Tween® 80 can stabilize oil-in-water emulsions, they are not as strong as protein-protein interactions (Wilde et al., 2004). In this case, this emulsion has lower stability than protein stabilized emulsion (Wustneck et al., 1996). Zhang and Wang (2016) reported that the replacement between surfactant and protein was not be an instant process. Thus, after dispersing the Tween® 80 into CCDS by heating and shaking for 10 min in lab-scale experiment, only a relatively small proportion of protein-surfactant co-stabilized emulsion was formed, and this contributed to the improved oil recovery from CCDS. However, the oil-in-water emulsion was not fully formed yet in corn slurry. Thus, more Tween® 80 are involved in co-stabilizing oil-in-water emulsion with protein during fermentation. After 64 h of fermentation with desirable temperature and mechanical mixing, a relatively large proportion of protein-surfactant co-stabilized emulsion was formed. This emulsion was not stable, thus it contributed to a significantly higher oil recovery from CCDS after centrifugation (Table 4). These findings agreed with the observations from Zhang and Wang (2016), who also suggested adding Tween® 20 at the initial stage of aqueous extraction of peanut oil. Based on the current observations and previous reports, the secondary mechanism might have the primary contribution to improved oil recovery.

3.5 Tween® 80 recyclability in backset

The 50% of water (w/w) was replaced by Tween® 80 containing thin stillage (backset) for making new batch of corn slurry. Based on the experiment design, Treatment 3 had 250 to 500 ppm Tween® 80 and Treatment 4 had 500~750 ppm Tween® 80, depending on the concentration of active Tween® 80 in thin stillage backset. As shown in Table 5, Treatment 4 had significantly higher oil partition than Treatment 3, and the improvement (45.9 to 53.8%) was very similar to that between non-surfactant fermentation (Treatment 1, no backset) and 500 ppm surfactant fermentation (Treatment 2, no backset) (40.8 to 49.6%). This observation indicated that the Tween® 80 in thin stillage backset cannot make the concentration of active Tween® 80 to 500 ppm in Treatment 3, and the final concentration of Tween® 80 in Treatment 3 might be even lower than 300 ppm based on the oil partition trend in Figure 1. The recycled Tween® 80 in thin stillage backset may have lost its function as detergent. Since the effects of Tween® 80 on oil partition and oil recovery were observed, we believe that Tween® 80 was still in the thin stillage with entire molecular structure, and most of Tween® 80 molecules were located on the interface of oil-in-water emulsion in the backset and no free Tween® 80 worked as detergent to wash adhering oil in next batch of fermentation.

4 Conclusion

The large portion of oil stayed in the wet cake should be moved to thin stillage and to be recovered by centrifugation. Tween® 80 and hydrolyzing enzymes have

shown to have the potential to increase DCO yield. Oil partition in thin stillage and the oil recovery from CCDS were also significantly improved without any negative effects on ethanol production. An additional benefit of using hydrolyzing enzymes and surfactant during fermentation is that the application of these technologies would not require any change in the design of a current ethanol plant. However, scale-up experiments are needed to further confirm the effectiveness on commercial scale. Moreover, research on the recyclability of the surfactant is needed to reduce process cost and study of treatment effects on quality of DDGS is needed.

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Abbreviations used

CCDS, condensed corn distillers solubles; DDGS, dried distillers grain with solubles; DCO, distillers corn oil; C500, treatment of 500 ppm surfactant added in corn slurry; PC, treatment of pectinase and cellulase added in fermentation; PC500, treatment of pectinase and cellulase added in fermentation, and 500 ppm surfactant added in corn slurry; F500, treatment of Fermgen added in fermentation and 500 ppm surfactant added in corn slurry; WHC, water holding capacity.

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Tables and Figures

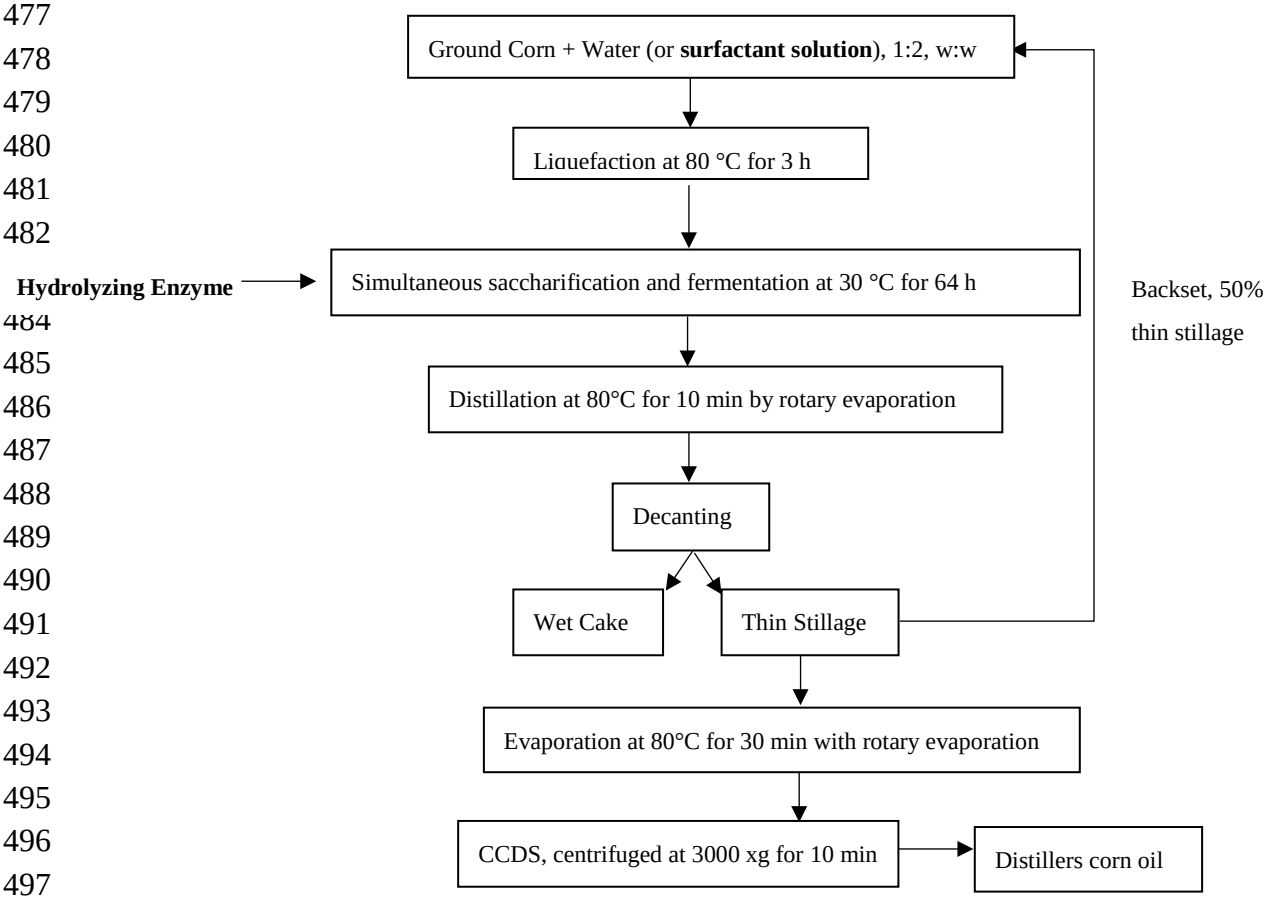

Figure 1. Lab scale dry-grind ethanol process

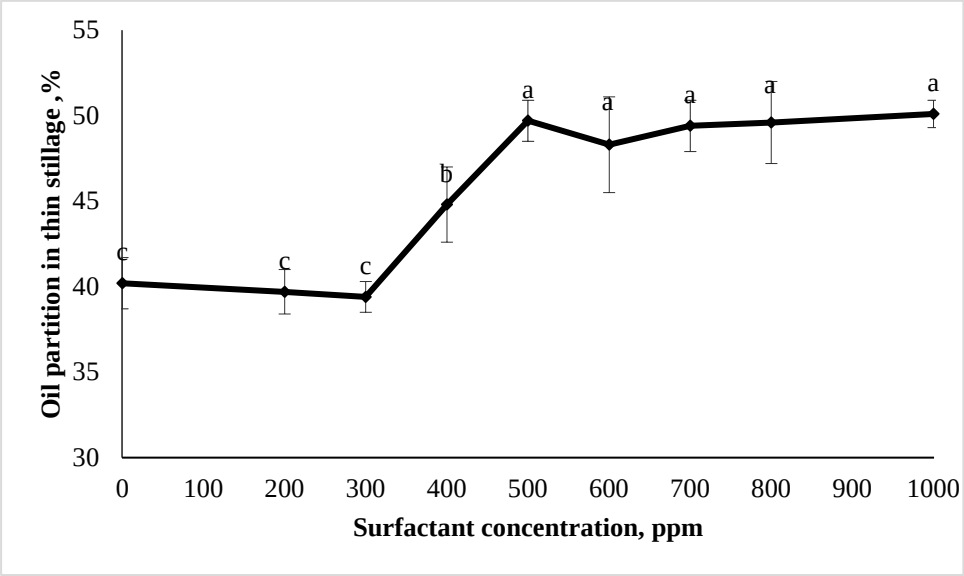

Figure 2. Effect of surfactant concentrations on oil partition in thin stillage by decanting. Data sharing the same letter has no significant difference ($p>0.05$).

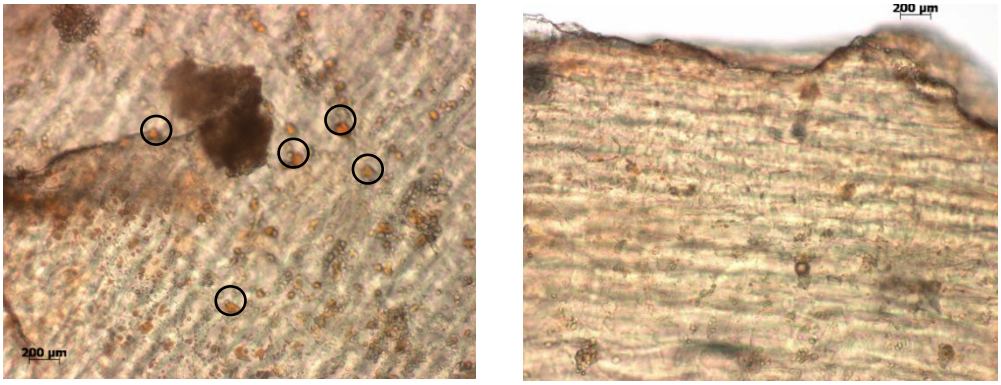

Figure 3. Microscopic observations for surface of wet cake. Left: Control; Right: 500 ppm Tween® 80 added treatment

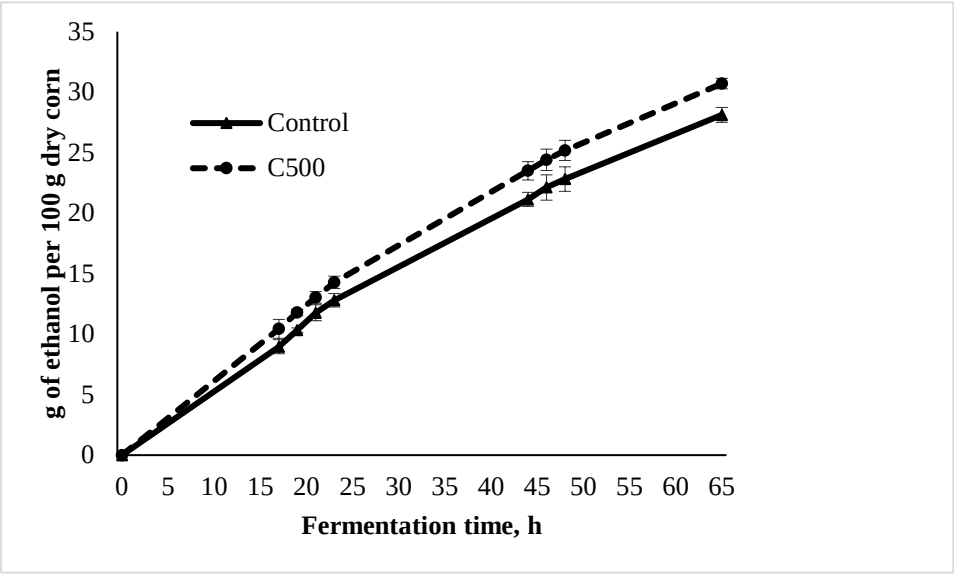

Figure 4. Effect of Tween® 80 addition on fermentation rate and ethanol yield. C500: 500 ppm Tween® 80 added in fermentation step of control group

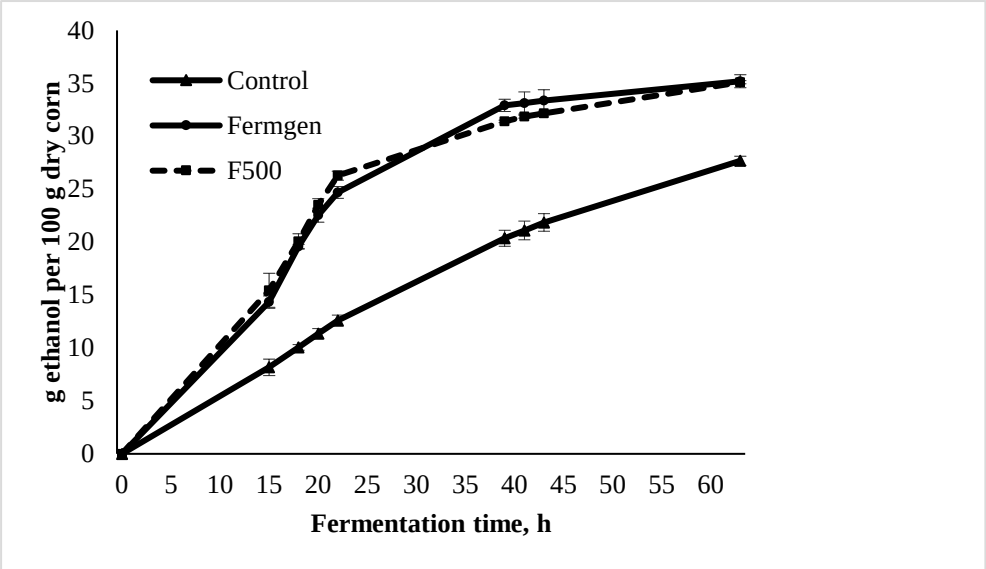

Figure 5. Effect of protease and Tween® 80 addition on ethanol production rate and ethanol yield. Fermgen: 0.5% of dry corn weight protease was added; F500: 0.5% of dry corn weight protease and 500 ppm Tween® 80 were added

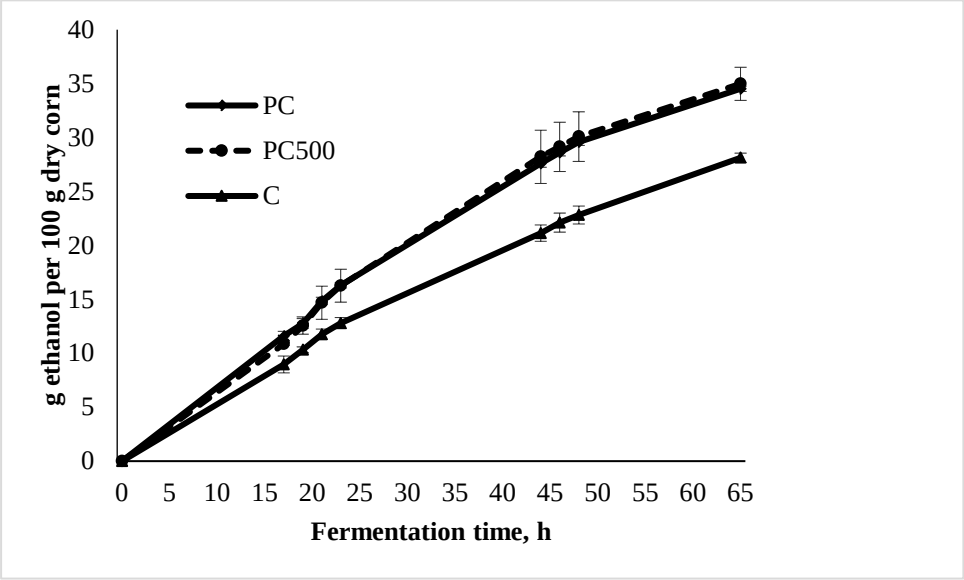

Figure 6. Effect of pectinase-cellulase (PC) and Tween® 80 addition on ethanol production rate and ethanol yield. C: control; PC: 0.2% of dry corn weight pectinase and 0.2% of dry corn weight cellulase were added; PC500: 0.2% of dry corn weight pectinase, 0.2% of dry corn weight cellulase and 500 ppm Tween® 80were added

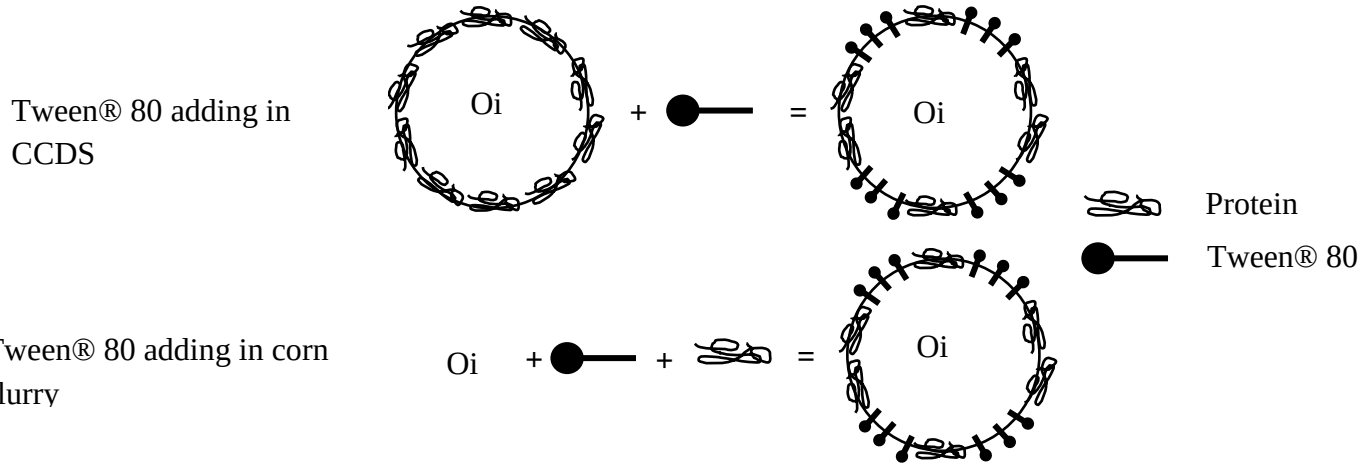

Figure 7. The formation of unstable emulsion in different condition

549 **Table 1.** Effect of Tween® 80 and hydrolyzing enzymes in dry-grind
550 fermentation on ethanol production.

Treatment	Maximum ethanol yield, g/100 g dry corn at 64 h	Maximum ethanol production rate, g/100 g dry corn/ h
Control	28.13 ± 0.43 c	0.67 ± 0.11 d
500 ppm Tween® 80 (C500)	30.71 ± 0.61b	0.67 ± 0.04 d
Pectinase and Cellulase (PC)	34.52 ± 1.54 a	1.04 ± 0.05 c
500 ppm +Pectinase and Cellulase (PC500)	34.98 ± 0.25 a	1.05 ± 0.10 c
Fermgen	35.19 ± 0.61 a	2.63 ± 0.20 a
500 ppm + Fermgen (500F)	35.25 ± 0.15 a	2.31 ± 0.13 b

551 Data sharing the same letter in the same column have no significant different
552 ($p>0.05$).
553
554
555 **Table 2.** Thin stillage production, solid and oil partition after decanting

	Thin stillage wet yield, %	Solid content in thin stillage, %	Solid partition in thin stillage, %	Oil partition in thin stillage, %
Control	80.9±0.5 c	7.2±0.3 a	41.0±1.6 c	40.8±0.6 e
500 ppm Tween® 80 (C500)	79.3±1.2 c	6.8±0.2 b	36.02±1.8 d	49.3±0.7 d
Fermgen	85.9±1.3 b	6.8±0.1 b	47.1±2.1 b	52.6±0.1 c
500 ppm + Fermgen (500F)	86.2±0.5 b	6.6±0.09 c	47.9±1.5 b	58.5±1.8 a
Pectinase and Cellulase (PC)	87.1±0.3 a	7.2±0.1 a	52.2±1.0 a	52.3±1.6 c
500 ppm +Pectinase and Cellulase (PC500)	87.8±0.5 a	6.9±0.3 b	53.1±0.06 a	54.9±1.3 b

556 Data sharing the same letter in the same column has no significant different
557 ($p>0.05$).
558

Table 3. Total oil content in CCDS, % dry basis

	Oil content in CCDS, db%
Control	6.8±0.3 d
500 ppm Tween® 80 (C500)	9.3±0.9 c
Fermgen	13.3±0.1 a
500 ppm + Fermgen (500F)	13.8±0.1 a
Pectinase and Cellulase (PC)	10.7±0.3 b
500 ppm +Pectinase and Cellulase (PC500)	11.5±0.3 b

Data sharing the same letter in the same column have no significant different (p>0.05).

Table 4. Oil recovery from CCDS

	Non-surfactant, %	Surfactant added in corn slurry, %	Surfactant added in CCDS, %
Control	7.9±0.7 Cb	31.8±0.9 Aa	11.0±0.9 Bb
Fermgen	4.0±3.8 Cc	24.9±5.9 Ab	9.3±0.4 Bc
PC	17.4±1.7 Ca	24.5±1.8 Ab	19.3±0.9 Ba

C: Control; PC: Pectinase/Cellulase added. Data sharing the same lower-case letter on the same column have no significant different (p>0.05). Data sharing the same upper-case letter on the same row have no significant different (p>0.05).

Table 5. Tween® 80 recycling stability in thin stillage backset

	Oil distribution in thin stillage, %
Treatment 1: Corn + 100% water	40.8 ± 0.6 b
Treatment 2: Corn + 100% 500ppm	49.6 ± 0.7 a
Treament 3: Corn + 50% 500ppm + 50% B	45.9 ± 1.1 b
Treament 4: Corn + 50% 1000ppm + 50% B	53.8 ± 0.9 a

500ppm: 500 ppm Tween® 80 water solution; 1000 ppm: 1000 ppm Tween® 80water solution; B: Backset from 500 ppm Tween® 80 treated fermentation. Data sharing the same letter in the same column have no significant different (p>0.05).