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

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 **1 Introduction**

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

12 The most widely used method for DCO recovery in dry-grind ethanol process is
13 separating the oil from the condensed corn distillers solubles (CCDS) by centrifuge
14 (Moreau et al., 2012). The oil recovery procedure was described in a U.S. patent
15 7601858. In brief, after collecting ethanol by distillation (Figure 1), the ethanol-
16 removed whole stillage is separated by decanting into thin stillage and wet cake. In
17 general, 40~60% of total oil in corn whole kernel is left in wet cake and the rest goes
18 to thin stillage after decanting. The thin stillage is further evaporated to produce
19 CCDS with 60~85% moisture content, and the DCO is extracted from the CCDS by
20 using a disk stack centrifuge (Cantrell and Winsness, 2009). Many efforts have been
21 made to improve the DCO recovery from CCDS. Centrifugation coupled with oil

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

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

43 Surfactants have been applied in aqueous extraction processing to improve
44 vegetable oil recovery. Sodium dodecyl sulfate was used to improve recovery of
45 soybean oil (Campbell and Glatz, 2009) and canola oil (Tuntiwiwattanapun et al.,
46 2013) by extracting the oil trapped in disrupted cellular matrix. The extended-
47 surfactants, which is a recently developed new class of surfactants that works by
48 significantly reducing the interfacial tension, extracted 93-95% of total oil from the
49 insufficiently ground peanut and canola seeds (Do and Sabatini, 2010). However, due
50 to the safety issue of sodium dodecyl sulfate and extended-surfactants, they are not
51 allowed for human or animal consumption. To date, only a few reports on
52 destabilization of oil-in-water emulsion by using food-grade-surfactants are available.
53 Fang et al. (2015) attempted to improve oil recovery from CCDS by using Tween®
54 80-Span® 80-silica nanoparticle mixture. Zhang and Wang (2016) used Tween® 20
55 to improve peanut oil recovery. Both works explained the improved oil recovery as
56 the result of unstable emulsion formation by surfactant-protein competition on the
57 emulsion interface. Thus, we believed that using surfactant at the beginning of dry-
58 grind ethanol process could not only improve oil partition in thin stillage but also
59 increase the oil recovery from CCDS by demulsification.

60 There is a large portion of oil (40~60% of total oil in corn) remaining in intact
61 cells and protein/polysaccharide matrices of wet cake solids; enzyme hydrolysis of the
62 solids might be an efficient way to release this part of the oil. Luangthongkam et al.
63 (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™ (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

85 2.2 Corn fermentation

86 The procedure of lab-scale fermentation is shown in Figure 2. The liquefaction
87 and simultaneous saccharification and fermentation of the corn slurry were performed
88 in 250-mL round bottom flasks with Tornado IS6 Overhead Stirring System (Radleys
89 Discovery Technologies, Shire Hill, Saffron Walden, UK) equipped with an anchored
90 stirring shaft. Ground corn was mixed with cold DI water (or Tween® 80 water
91 solution) at a 1:2 ratio, w: w.. The total amount of slurry was maintained at 225-230 g.
92 α -Amylase (0.15 mL) was added to the slurry and mixed at 81°C for 3h. After that,
93 the flasks were cooled to 30°C in an ice bath, and the pH of the cooled slurry was
94 adjusted to 4.0 with 3 M sulfuric acid. Chlorine dioxide (0.021 mL), ammonium
95 sulfate (0.065 mL of 0.2 g/g water), gluco-amylase (0.15 mL) and dry yeast (0.15 g)
96 were added. Fermentation was carried out at 30°C for 64 h with continuous stirring
97 (190 rpm). During fermentation, ethanol production was estimated by mass loss
98 according to the following equation (Wang et al., 2009).

$$99 \quad \text{Ethanol yield (g/100 g dry corn)} = 100 \times \frac{46 \times (\text{g of mass loss})}{44 \times (\text{g of dry corn})}$$

100 Where 46 and 44 are molecular weights of ethanol and CO₂, respectively.

101 For the experiments of adding hydrolyzing enzymes during fermentation, 0.375
102 mL of Fermgen or 0.3 g of PC was added before starting the fermentation. For
103 experiments of adding Tween® 80 in fermentation, Tween® 80 water solutions of
104 200, 300, 400, 500, 600, 700, 800, 1000 ppm were prepared and ground corn meal
105 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

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

150 The solid content was determined by weight difference after oven-drying at
151 105°C for 5 h. Total oil content was determined by acid hydrolysis method (AOAC
152 Official Method 922.06).

153 2.7 Calculations

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

156 Wet yield of thin stillage, %

$$157 = 100\% \times \frac{\textit{g of thin stillage}}{\textit{g of whole stillage, before decanting}}$$

158

159 Solid partition in thin stillage, %

$$160 = 100\%$$

$$161 \times \frac{\textit{g of dry solids in thin stillage}}{\textit{g of dry solids in whole stillage, before dacanting}}$$

162

163 Oil partition in thin stillage, %

$$164 = 100\% \times \frac{\textit{g of oil in thin stillage}}{\textit{g of oil in whole stillage, before decanting}}$$

165

$$166 \text{ Oil recovery from CCDS, \%} = 100\% \times \frac{\textit{g of free oil}}{\textit{g of total oil in thick stillage}}$$

167

168

169 **2.8 Statistical analysis**

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

173 **3 Results and Discussion**

174 **3.1 Oil partition in thin stillage as affected by adding surfactant in corn slurry**

175 The optimal Tween® 80 concentration for increasing oil partition in thin stillage
176 was investigated. The concentration of Tween® 80 in corn slurry had a significant
177 effect on oil partition in thin stillage after decanting. As shown in Figure 2, the oil
178 partition in thin stillage was at 40% of total corn oil for surfactant concentrations
179 below 300 ppm, but the partition significantly improved to 50% when the Tween® 80
180 concentration was increased to 500 ppm. However, no more improvement was seen
181 over 500 ppm. Since surfactant cannot release the oil from the unbroken cells, the
182 extra oil partitioned in the thin stillage should be coming from the adhered oil on the
183 wet cake surface. This hypothesis is supported by microscopic observations in Figure
184 3, in which Sudan IV stained oil droplets can be seen in wet cake surface of the
185 control, but hardly seen in 500 ppm Tween® 80 treated samples.

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

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

193 **3.2 Ethanol production rate and yield as affected by using enzyme and surfactant**

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

211 When only Fermgen (mainly a protease enzyme) was used during fermentation
212 (Table 1 and Figure 5), both ethanol production rate and yield increased significantly
213 compared to the control. The increased ethanol production rate suggested that
214 fermentation time could be reduced to approximately 40 h from 65 h. These findings
215 agreed with the results reported in literature in which protease was shown to increase
216 ethanol production rate and decrease supplemental N requirements (Johnston and
217 McAloon, 2014). Protease enzyme hydrolyzed corn protein and increased the
218 concentration of free amino acid and peptides which can be used as N source for
219 yeast. Moreover, protein matrix embeds corn starch in corn endosperm (Watson,
220 1987) and can be broken by a protease to make starch more available for producing
221 ethanol (Lamsal and Johnson, 2012).

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

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

232 alone (34.52 g ethanol per 100 g dry corn). Similar pattern was observed when
233 Fermgen and Tween® 80 were used, in which using Fermgen (35.19 g ethanol per
234 100 g dry corn) had no significant different compared to F500 (35.25 g ethanol per
235 100 g dry corn). Improvement in ethanol yield by using Tween® 80 was not observed
236 when enzymes were added during fermentation. As we speculated in the previous
237 section, non-ionic surfactant may improve ethanol yield by affecting enzyme-
238 substrate interaction. The enzyme hydrolysis reduces the particle size and makes the
239 substrate more available to saccharification enzyme, and this effect might have made
240 Tween® 80 unnecessary in this step to further reduce enzyme-substrate interaction.

241 **3.3 Thin stillage yield, and solid and oil partitions in thin stillage as affected by** 242 **enzyme and surfactant treatments**

243 The composition and properties of thin stillage are very important for the
244 performance of piping, centrifuge efficiency, oil recovery and energy cost in ethanol
245 plant. The wet yield of thin stillage was significantly improved in Fermgen treatment
246 (85.9%) and PC treatment (87.1%) compared to the control (79.3%). Whereas, PC
247 treatment had similar solid content (7.2%) and Fermgen treatment had lower solid
248 content (6.8%) compared to the control (7.2%). Similar results were observed by Yao
249 et al. (2014) and Luangthongkam et al. (2015) when fiber hydrolyzing enzymes were
250 used in fermentation. Likely, the hydrolysis action of the enzymes reduced the water
251 holding capacity of insolubles in the wet cake by interrupting the large protein matrix
252 and cell wall network. In addition, the enzyme actions might have reduced the particle

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

256 A significant reduction of solid content in thin stillage was observed when
257 Tween® 80 was added in corn slurry, which are 7.2% versus 6.8% (Control versus
258 C500), 6.8% versus 6.6% (Fermgen versus F500), and 7.2% versus 6.9% (PC versus
259 PC500) in Table 2. Lower solid content in thin stillage is preferable in ethanol plants,
260 due to low energy cost in piping and less fouling of evaporators. To further
261 understand the reason for this observation, we tested the WHC of the dried wet cake
262 for Tween® 80 solution and water. The dried wet cake can hold more DI water (4.39
263 ± 0.17 g water/g dried wet cake) than Tween® 80 solution (3.87 ± 0.02 g solution/
264 g dried wet cake). This may be explained by the reduced capillarity of solution due to
265 the action of surfactant, which make the liquid separation easier from the dried wet
266 cake by centrifugation.

267 Enzymes and Tween® 80 treatments significantly increased oil partition in thin
268 stillage. Since more oil is in thin stillage, more oil can potentially be extracted. Table
269 2 shows that using hydrolyzing enzymes significantly increased oil partition in thin
270 stillage from 40.8% (Control) to 52.6% (Fermgen) and 52.3% (PC). Comparing with
271 using enzyme alone, Tween® 80 further improved the oil partition in thin stillage,
272 which were 40.8% versus 49.3% (Control versus C500), 52.6% versus 58.5%
273 (Fermgen versus F500), and 52.3% versus 54.9% (PC versus PC500). Before

274 decanting, the oil maybe present in different forms and a large proportion of oil
275 remains in unbroken cells and large matrix, like protein and cell wall components.
276 This part of oil can be released by enzyme hydrolyzing the solids or large particles.
277 These findings agreed with the reported observations in literatures (Luangthongkam et
278 al., 2015; Yao et al., 2014), which shown an improved oil partition in thin stillage by
279 adding NSPs hydrolyzing enzymes during fermentation. Different from the action of
280 enzymes, the use of surfactant improved the oil partition in a different way, i.e.
281 washing the adhering oil from wet cake surface into the aqueous phase. However,
282 when hydrolyzing enzymes were added, the improvement of oil partitioning in thin
283 stillage by adding surfactant in corn slurry was reduced comparing with non-enzyme
284 treatments, which partitioned 8.5% more of total corn oil from the control to C500,
285 5.9% from Fermgen to F500, and 2.6% from PC to PC500. The use of hydrolyzing
286 enzymes weakened the function of surfactant, probably due to the enzymatic
287 hydrolysis of protein and cell wall that freed the adhering oil into thin stillage. This
288 hypothesis is supported by the parallel improvements in solid partition and oil
289 partition when enzymes were used (Table 2).

290 **3.4 Oil recovery from CCDS as affected by surfactant and enzyme**

291 The use of hydrolyzing enzymes significantly improved oil content in CCDS, as
292 shown in Table 3. When Tween® 80 was used, the significant oil content increase
293 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

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

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

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

335 Tween® 80 was added in CCDS, Tween® 80 competed and replaced protein from the
336 interface of emulsion. Although surfactants like Tween® 80 can stabilize oil-in-water
337 emulsions, they are not as strong as protein-protein interactions (Wilde et al., 2004).
338 In this case, this emulsion has lower stability than protein stabilized emulsion
339 (Wustneck et al., 1996). Zhang and Wang (2016) reported that the replacement
340 between surfactant and protein was not be an instant process. Thus, after dispersing
341 the Tween® 80 into CCDS by heating and shaking for 10 min in lab-scale
342 experiment, only a relatively small proportion of protein-surfactant co-stabilized
343 emulsion was formed, and this contributed to the improved oil recovery from CCDS.
344 However, the oil-in-water emulsion was not fully formed yet in corn slurry. Thus,
345 more Tween® 80 are involved in co-stabilizing oil-in-water emulsion with protein
346 during fermentation. After 64 h of fermentation with desirable temperature and
347 mechanical mixing, a relatively large proportion of protein-surfactant co-stabilized
348 emulsion was formed. This emulsion was not stable, thus it contributed to a
349 significantly higher oil recovery from CCDS after centrifugation (Table 4). These
350 findings agreed with the observations from Zhang and Wang (2016), who also
351 suggested adding Tween® 20 at the initial stage of aqueous extraction of peanut oil.
352 Based on the current observations and previous reports, the secondary mechanism
353 might have the primary contribution to improved oil recovery.
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356 **3.5 Tween® 80 recyclability in backset**

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

374 **4 Conclusion**

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

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

385 **Acknowledgment**

386 The authors would like to thank the ISU Fermentation Institute and Bioeconomy
387 Institute for funding and supporting this research, and Bio-Cat and DuPont Industrial
388 Biosciences companies for providing enzyme samples.

389 **Abbreviations used**

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

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475 **Tables and Figures**

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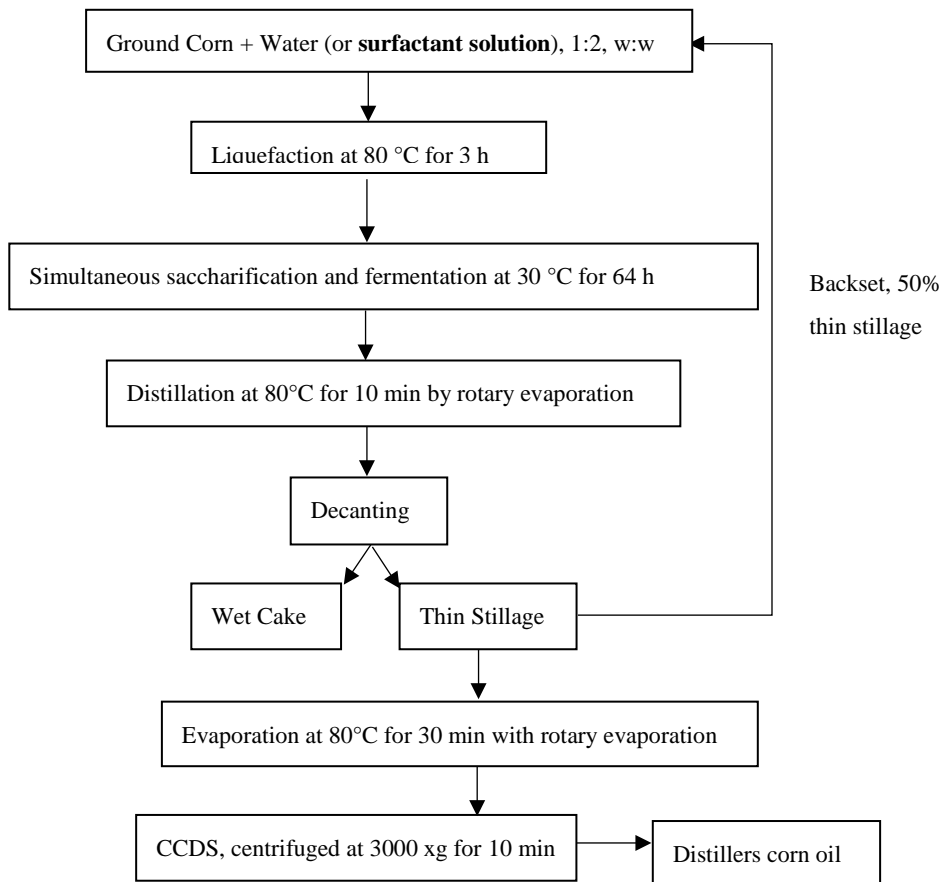
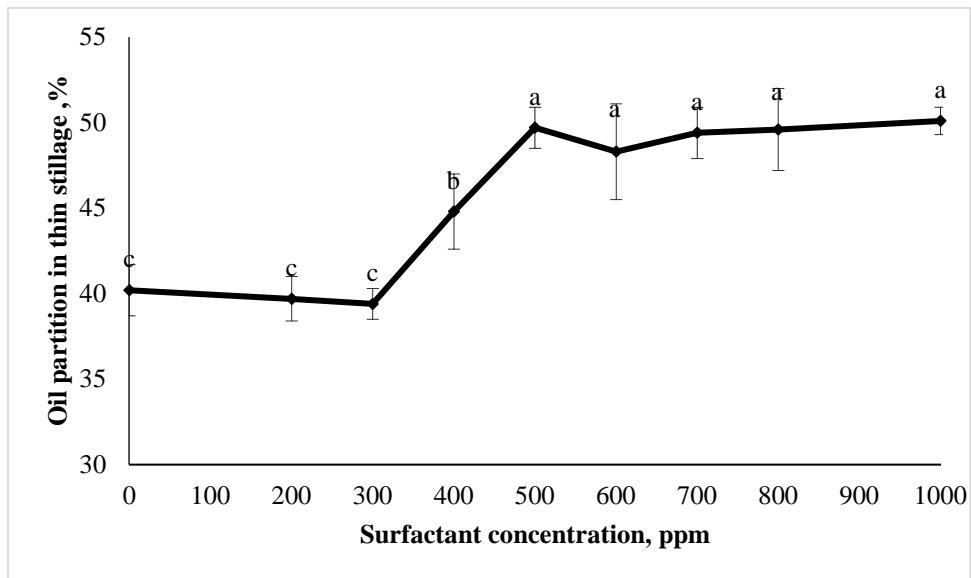


Figure 1. Lab scale dry-grind ethanol process

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

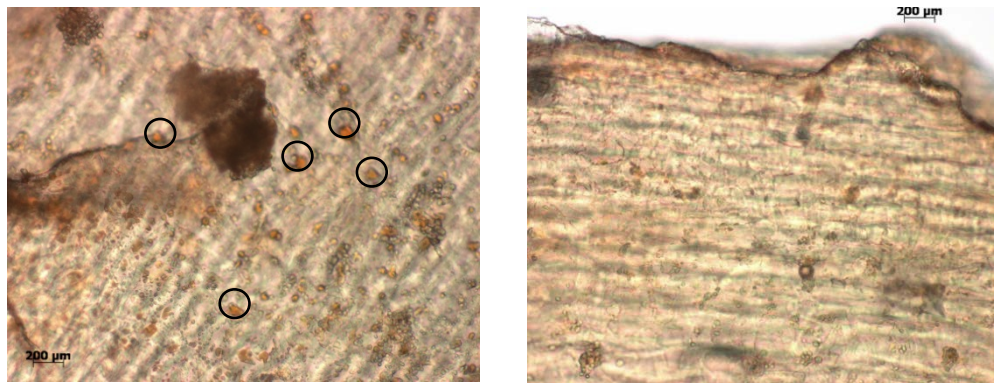
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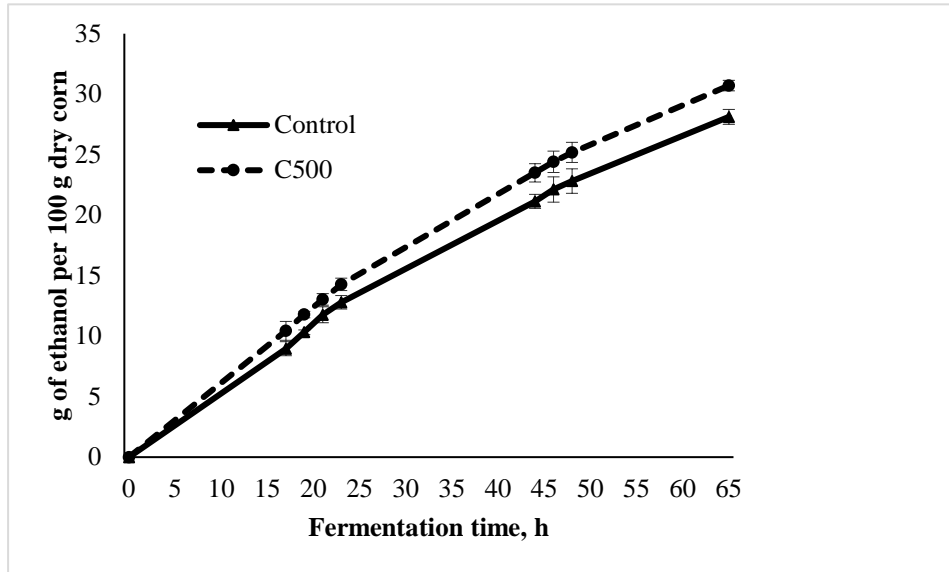
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512 **Figure 3.** Microscopic observations for surface of wet cake. Left: Control; Right: 500
513 ppm Tween® 80 added treatment

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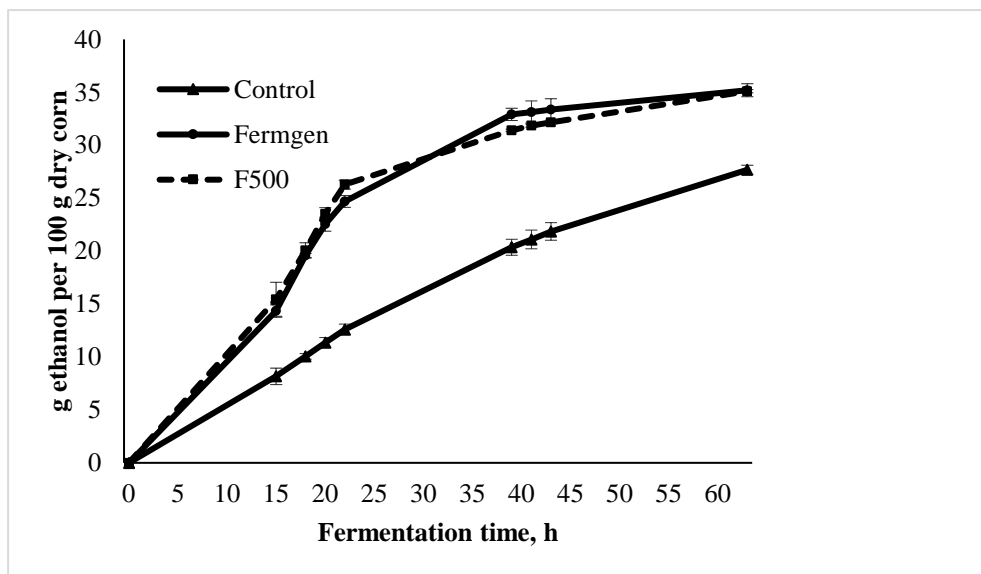
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Figure 4. Effect of Tween® 80 addition on fermentation rate and ethanol yield. C500: 500 ppm Tween® 80 added in fermentation step of control group

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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

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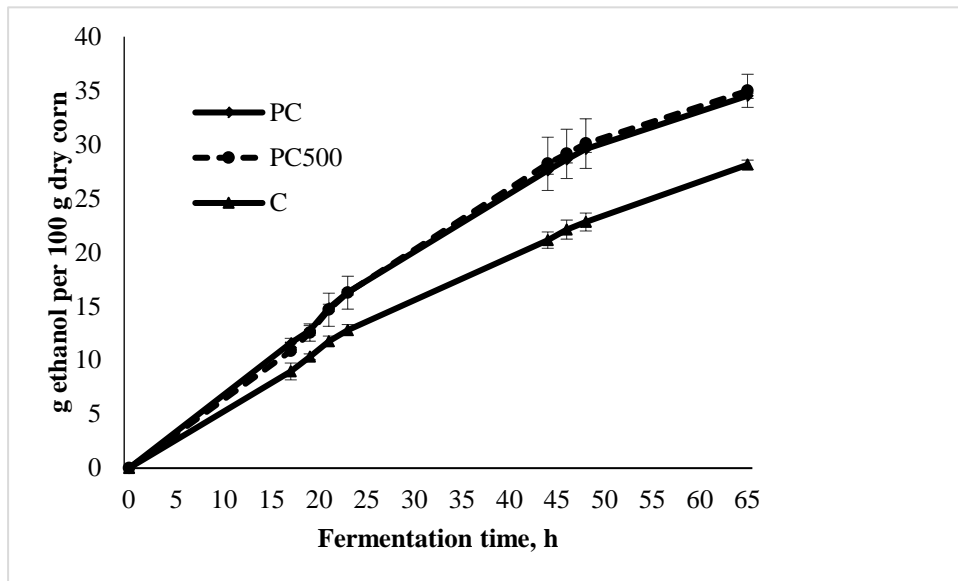


Figure 6. Effect of pectinase-cellulase (PC) and Tween® 80 addition on ethanol production rate and ethanol yield. C: control; PC: 0.2% of dry corn weight pectinase and 0.2% of dry corn weight cellulase were added; PC500: 0.2% of dry corn weight pectinase, 0.2% of dry corn weight cellulase and 500 ppm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

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Tween® 80 adding in
CCDS

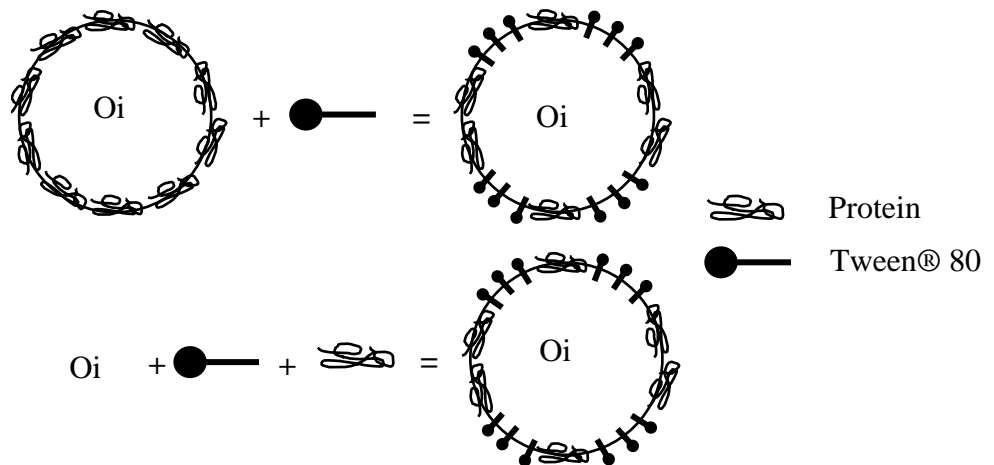


Figure 7. The formation of unstable emulsion in different condition

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Table 1. Effect of Tween® 80 and hydrolyzing enzymes in dry-grind 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

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

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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

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

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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

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

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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

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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).

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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
Treatment 3: Corn + 50% 500ppm + 50% B	45.9 ± 1.1 b
Treatment 4: Corn + 50% 1000ppm + 50% B	53.8 ± 0.9 a

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500ppm: 500 ppm Tween® 80 water solution; 1000 ppm: 1000 ppm Tween® 80 water solution; B: Backset from 500 ppm Tween® 80 treated fermentation.

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

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