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Effects of Kernel Breakage and Fermentation on Corn Germ Integrity and Oil Quality

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To investigate the ability of corn germ to withstand the fuel ethanol fermentation process without major damage to germ integrity and germ oil quality, five treatments were designed to explore degerming before fermentation (front-end) and after fermentation (tail-end), and the feasibility of breaking the kernel with minimum shear forces (wet-split). Germ from low-shear (wet-split) tail-end degerming maintained its integrity during the process. The wet-grind pretreatment caused 22% germ damage, and the subsequent fermentation caused 18% additional germ damage. The germ recovered after fermentation showed physical strength similar to that of those isolated by wet means before fermentation. The oils extracted from the tail-end germ fractions had the same low free fatty acid (FFA) content (2%) and similar low peroxide value (2 meq/kg) as those extracted at the front end. The good oil quality of the tail-end germ fraction was attributed to excellent germ integrity. The oil recovered after traditional dry-grind ethanol production was highly deteriorated, with 22% FFAs and 9 meq/kg peroxide value because the germ was broken into small pieces during dry grinding. So long as kernel-breakage or size-reduction pretreatments are conducted to retain intact germs or keep them in large pieces before fermentation, the germ can survive the cooking, starch hydrolysis, and yeast metabolism during the ethanol fermentation process. These findings lay a foundation for developing new degerming strategies where the germ can be isolated during or after fermentation, which could be easily integrated into the conventional dry-grind corn ethanol process.

KEYWORDS: Corn germ; corn oil; degerm; dry-grind fermentation; free fatty acid; fuel ethanol; kernel breakage; oxidation of oil

INTRODUCTION

The Renewable Fuels Association (RFA) indicates that 21% of the total US corn crop (equivalent to about 3.8 billion bushels) was used to make fuel ethanol in 2009 (*1*). About 82% of the corn used in fuel ethanol production was processed by dry-grind ethanol plants in 2007, with the remaining 18% by wet-mill ethanol plants (*2*). The major difference between the dry-grind ethanol process and the wet-mill ethanol process is that dry-grind does not fractionate the botanical components (including germ, bran, starch, and endosperm protein) while wet-milling does. The dry-grinding process produces only one coproduct, distiller's dried grains with solubles (DDGS), a mixture of nonfermentable residues, used as low-value dairy and beef cattle feed due to its high fiber content. Wet-milling, however, produces more coproducts including high-value gluten protein products, germ oil, and germ meal. However, wet milling requires sophisticated equipment, high capital cost, and high inputs of energy and water, and thus is usually operated at a larger scale in order to achieve commercial efficiency. The wet-milling industry is dominated by about 13 companies and has changed little through the corn fuel ethanol boom (*3*).

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Compared to the wet-mill ethanol process, the dry-grind ethanol process is much simpler and requires less capital, energy, and water inputs. For this reason, the majority of new fuel ethanol production capacity in the past decade is from dry-grind plants. Since germ is ground into flour-like small particles along with the other parts of the whole corn kernel in the dry-grind process, it is difficult to recover and is lost into low-value DDGS.

One possible strategy to recover oil is from the downstream liquid phase in the conventional dry-grind ethanol process since some of the oil may be released from the finely broken germ pieces during fermentation. Our previous studies, however, showed that once the oil is mixed with and diluted by the oil-lean components including fiber, endosperm proteins, and residual starch, it is difficult to extract with nonsolvent methods (*4*, *5*). In addition to low extraction efficiency, the oil from the conventional dry-grind process contains high levels of free fatty acid (FFA) (9−12%) (*6*, *7*) (unpublished data).

Another possible strategy for recovering oil is to recover the oil in the form of whole germ as in wet milling, and it has to be adopted to a dry-grind plant. A number of new processes have been proposed over the past decade. These processes can be summarized into two categories, i.e., dry-degerming processes and wet-degerming processes. The dry-degerming process typically involves tempering the corn for a short period, reducing the

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Table 1. Summary of Different Corn Degerming and Fermentation Treatments

		treatment component (in time sequence)				
trt no.	treatment	steeping	kernel breaking	degerming before fermentation	fermentation	degerming after fermentation
1	front-end hand-dissect degerm fermentation[a]	Y	hand dissecting + wet grinding	Y	Y	N
2	front-end wet-grind degerm fermentation	Y	wet grinding	Y	Y	N
3	tail-end wet-grind degerm fermentation	Y	wet grinding	N	Y	Y
4	tail-end wet-split degerm fermentation	Y	wet splitting	N	Y	Y
5	no degerm fermentation (dry-grind)	N	dry grinding	N	Y	N

[a] For treatment 1, the kernel was first broken by hand-dissection, then the germ-free fraction was wet-ground as in treatments 2 and 3.

particle size by dry milling, then separating different components by size or density (*8*−*11*). The corn moisture before size reduction typically ranges from 15 to 35% (*12*). The oil content in the germ fraction from dry milling was only about 20% compared to about 40% from wet milling (*13*, *14*) because the separation of germs and other components was not complete. Less than 50% of germ was recovered by dry-degerming (*10*), which compromised the economy of these processes.

Wet-degerming processes involve soaking or steeping the corn in water for 4−12 h followed by size-reduction and fractionation in the liquid phase. The germs can be isolated by floatation, hydrocloning, or centrifugation. Only the germ-free fraction is used in ethanol fermentation. The steeping or soaking times were dramatically reduced from 24 to 36 h at 52 °C in conventional wet milling (*13*) to less than 12 h at 59 °C (*15*). The oil content in the germ was about 30%. This technique is known as Quick Germ Process (*15*, *16*). A similar process, HydroMilling (*17*), has been tested in at least one commercial plant. If the coarse fiber was also recovered before fermentation, the process was then termed Quick Germ Quick Fiber (*16*, *18*, *19*). There are other minor modifications to wet-degerming processes, such as those in Enzymatic Milling or E-Milling, where enzymes were used to replace part or all of the chemicals used in corn steep (*20*).

Nevertheless, these novel wet-degerming techniques have yet to achieve widespread adoption probably because they still need major pieces of wet-milling equipment and/or probably because of the lower ethanol yield resulting from starch loss to the germ fraction. The oil content in the germ fraction from the Quick Germ process was about 30%, lower than that in germ from the conventional wet-milling process.

All of these known degerming processes involve germ separation before fermentation. We proposed a series of new degerming strategies in which the germ fraction is recovered during or after ethanol fermentation, attempting to overcome some of the shortfalls of the front-end degerming processes. A few potential benefits are anticipated: (1) higher ethanol yield; (2) higher germ yield; (3) easier recovery of the germ because the fermentation process eats away the starch between the germ and other components; and thus (4) the need for less expensive machinery.

These proposed new degerming strategies will be possible and desirable only if the germs remain intact, and the germ oil is unhydrolyzed after fermentation. Our hypothesis was that the intact germs can survive starch gelatinization, hydrolysis, and ethanol fermentation and that the oil will not undergo major deterioration if the germs remain intact or in large pieces. Our objective was to investigate the fate of corn germ and oil in post-fermentation (tail-end) degerming for fuel ethanol production.

MATERIALS AND METHODS

Corn Samples and Fermentation Materials. No. 2 yellow dent corn from the 2007 crop year was acquired from the Heart of Iowa Cooperative (Nevada, IA). The corn was cleaned by using a KICE laboratory aspirator Model 6DT4 (KICE Metal Products Co. Inc., Wichita, KS). Liquid α-amylase SPEZYME Xtra (13,642 α-amylase units/g, optimum pH of 5.0−6.7) and a saccharifying enzyme G-ZYME 480 Ethanol (401 glucoamylase units/g, optimum pH of 4.0−4.5), both from Genencor Inc. (Cedar Rapids, IA), were used to liquefy and saccharify the corn slurry, respectively. 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 fermentation materials were of industrial grade.

Corn Treatments. Five degerming treatments were studied. All treatments are summarized in **Table 1**. For treatments 1 (front-end hand-dissect degerm fermentation) and 2 (front-end wet-grind degerm fermentation) germs were recovered before fermentation, while in treatments 3 (tail-end wet-grind degerm fermentation) and 4 (tail-end wet-split degerm fermentation), the germs were recovered after fermentation. Treatment 5 simulated a typical dry-grind ethanol fermentation without degerming where the whole kernel including the germ was ground, and the germ pieces were too small to be recovered. For better understanding, the treatment details are described in the sequence of processing across different treatments as follows below.

Corn steeping. All treatments started with 500 g of corn (dry weight basis). In treatments 1−4, the corn was steeped in deionized water at 52 °C for 36 h at a 2:3 (w/w) corn/water ratio. After steeping, the steeping liquid was drained, leaving the corn kernels with about 33% moisture content (determined by drying at 130 °C for 3 h). No chemical, such as sulfur dioxide or lactic acid applied in conventional wet-milling steeping, was used. The steeping liquor generated during each steeping was incorporated in subsequent fermentation of the same corn in order to recover the corn solubles. No steeping was conducted for treatment 5.

Kernel Breaking and Size Reduction. In treatment 1, 750 g of steeped corn (500 g on dry basis) was hand-dissected to remove the germ. The germ-free fraction was mixed with 750 g of water/steeping liquor, then divided, and ground in 6 equal batches by using a commercial Waring Blender Model 51BL31 (Waring Products, Inc., Torrington, CT) equipped with a customer-made blunt blade inside a glass cup with a capacity of 1,183 mL (40 oz). The blender was operated at the low setting by using a Staco variable autotransformer Type 3PN2210 (Staco Energy Products, Co., Dayton, OH) set at 35% of the 120 voltage for 5 min. The same grinding step was applied to treatments 2 and 3 (750 g of steeped corn with 750 g of water/steeping liquor and ground in 6 batches). This method was a modification of the one by Eckhoff et al. (*21*), which simulates coarse grinding in wet milling. For treatment 4, the steeped corn was split by using a Roskamp smooth-surfaced roller mill Model K (Roskamp Manufacturing, Inc., Waterloo, IA) with the roller gap fully open (the gap between the rollers was 3.45 mm or 0.136 in.). This gap setting was chosen to slightly break or crack open the corn kernels without causing major damage to the germs. In treatment 5, the corn was ground by using a Fitz Mill Model DAS 06 (Fitzpatrick Co., Elmhurst, IL) at 5,000 rpm with a screen no. of 1531−0125 which has an opening size of 3.18 mm (0.125 in.). The resulting corn meal had a particle size distribution profile of 4, 22, and 74% of particles retained on the no. 20 sieve, no. 12 sieve, and pan, respectively, which was similar to corn ground in a commercial dry-grind ethanol plant.

Degerming. For treatments 1 and 2, the germs were recovered before fermentation (front-end). In treatment 1, the kernels were hand-dissected to expose the germ, and the germ was removed by using pointed tweezers. Care was taken to ensure the integrity and clean separation of the germ

Table 2. Fermentation Results of Different Corn Degerming and Fermentation Treatment[a]

trt. no.	treatment	ethanol concn in beer by HPLC (%, w/v)	ethanol yield, based on mass loss (%)	lactic acid concn in beer by HPLC (%, w/v)	acetic acid concn in beer by HPLC (%, w/v)
1	front-end hand-dissect degerm	16.57 ab	34.76 a	0.16 a	0.08 c
2	front-end wet-grind degerm	16.99 a	34.78 a	0.16 a	0.10 b
3	tail-end wet-grind degerm	16.31 ab	35.33 a	0.18 a	0.08 c
4	tail-end wet-split degerm	14.82 c	31.36 b	0.12 ab	0.08 c
5	no degerm (dry-grind)	16.10 b	34.67 a	0.04 b	0.14 a

[a] Means within a column followed by different lower case letters are significantly different at $P < 0.05$.

from the endosperm and pericarp. For treatment 2, the majority of the germ fraction was recovered by collecting germs that were floating, while the ground slurry was constantly stirred by using a scoop made of copper mesh with 2.80 mm (0.11 in.) openings, which is equivalent to a standard 7-mesh sieve. Any nonfloating large germ pieces were hand-picked from the retained solids after the slurry was filtered through a 7-mesh sieve (2.80 mm openings). The degermed solids (mainly consisting of large pericarp and endosperm pieces) and liquid (starchy slurry containing fine solids) were then combined to form the degermed slurry for fermentation. For treatments 3 and 4, the germ fractions were isolated after fermentation (tail-end) by hand-picking after the beer was filtered through a 7-mesh sieve. The recovered germ fractions included the intact germs and large broken germ pieces that were retained on the 7-mesh sieve. A few intact germs of similar size and shape from each treatment were sampled and stored at 5 °C in a sealed plastic bag for physical strength analysis. The germ fraction from each treatment was dried at 80 °C for 3 h and placed into a sealed plastic bag until oil extraction and analysis. The drying condition was chosen to minimize possible heat damage to the germ oil.

Ethanol Fermentation. A modified laboratory dry-grind corn-ethanol fermentation procedure based on our previous research (*4*) was used for all the corn samples. No additional autoclaving or jet-cooking was used in this study. The cooking and liquefaction were carried out simultaneously at 82 °C for 4 h with constant stirring using a setup consisting of a stirrer (Fisher Scientific, Dubuque, IA), a button-type glass shaft, and a polytetrafluoroethylene (PTFE) blade. Two milliliters of α-amylase was used. The fermentation was carried out in an incubator-shaker at 34 °C with 100 rpm shaking for 60 h. After fermentation, the finished beer was heated at 70 °C for 20 min to inactivate the yeast. An elastic film was used to seal the mouth of the flask to prevent ethanol loss during heating.

Ethanol Yield Quantification. The ethanol yield was calculated on the basis of mass loss during fermentation (*5*). The ethanol and lactic and acetic acid concentrations in the finished beer were measured by high pressure liquid chromatography (HPLC) (*4*).

Germ Characterization. *Germ Yield, Oil Content, and Germ Breakage*. Germ yield was calculated as the percentage of germ fraction based on original corn. After grinding the germs by using a mortar and pestle to about 20-mesh, germ oil was extracted with hexane at a 1:5 (w/v) germ/solvent ratio under constant stirring for 30 min. Solids were separated from liquid by using vacuum filtration with filter paper. Each sample was extracted four times. The four extracts were combined, and the solvent was removed by using a rotary evaporator. In order to achieve the maximum recovery of the FFAs, chloroform−methanol (2:1, v/v) was also tested at the same ratio four times for comparison. Hexane recovered 94% of the total lipid and 90% of the FFAs based on the extraction with chloroform−methanol. Because the mixture of chloroform−methanol and ground sample was difficult to filter and such a solvent extracted significant amounts of nonlipid components, which need multiple purification steps, hexane was used as the extraction solvent. Oil content in the germ was the percentage of oil in dry germ.

Germ breakage was derived from oil extraction data by the following equation:

$$\text{Germ breakage } (\%) = \left(1 - \frac{\text{total oil in the recovered germ}}{\text{total theoretical germ oil}}\right) \times 100$$

where the total oil in the hand dissected germ fraction was considered the total theoretical germ oil, and the recovered germ was the amount of germ larger than the opening of the 7-mesh sieve. This parameter was used to quantify the amount of broken fine germ pieces that ended up in the degermed fractions.

An acid hydrolysis method was used to quantify the oil content in the degermed mass or DDGS equivalent after the degermed beer was evaporated at 80 °C. It is used to verify the germ oil partitioning between recovered germs and degermed DDGS.

Physical Strength of the Germ. The physical strength of the wet germ was analyzed using a texture analyzer Model TA-XT2i (Texture Technologies Corp., Scarsdale, NY) equipped with a TA-10 probe and a TA-90A plate at ambient temperature. The strength is expressed as the resistance force profile during the process in which the probe pressed the germ to 80% of the original thickness at a constant speed of 0.20 mm/s.

Germ Oil Quality Analyses. *FFA Content*. FFAs were isolated and quantified by using thin layer chromatography (TLC) plates, Silica Gel G 500 (Analtech Inc., Newark, DE) with hexane/ethyl ether/acetic acid (80/20/1, v/v/v) as the mobile phase. The FFA band was detected under UV light after spraying with 2′,7′-dichlorofluorescein, and the silica was collected by scraping the band. The FFAs were then converted to methyl esters by reacting with 3% sulfuric acid in methanol (v/v) at 65 °C for 3 h. Methyl heptadecanoate was used as an internal standard. The composition of FFAs was analyzed using a gas chromatograph Model 589 Series II (Hewlett-Packard Co., Avondale, PA). The column used was a 15 m × 0.25 mm i.d., 0.2 μm film, Model SP-2423 fused-silica capillary column (Supelco, Inc., Bellefontaine, PA). The carrier gas (He) was set at 5.4 mL/min, H_2 at 13.9 mL/min, and air at 426 mL/min. The injector split ratio was 24:1. The temperatures were as follows: injector temperature, 230 °C; detector temperature, 230 °C; oven temperature program, 150 to 180 °C at 5 °C/min with no holding time.

Peroxide Value. The peroxide value of the germ oil was measured according to a standard AOCS redox titration method (*22*).

Experimental Design and Statistical Analysis. All treatments were randomized with two replicates for each treatment. Statistical analysis was performed by using General Linear Model procedures of SAS 9.1 (*23*).

RESULTS AND DISCUSSION

Treatment 1 represents ideal (complete) degerming and the best oil quality since the germs were isolated by hand-dissection before cooking and fermentation, while treatment 5 represents the most extreme degerming possibility and potentially the worst oil quality since the germ was broken into fine pieces and went through the entire fermentation process. Treatments 2 and 3 were used to compare the effect of yeast fermentation on the germ breakage and oil quality since the corn was wet-ground in the same manner, but the germs were recovered at different stages of the process (before and after fermentation, front-end, and tail-end, respectively). Treatment 4 was designed to test both the effect of fermentation on germ breakage and germ oil quality and the effect of low-shear kernel breaking method (wet-split), which was expected to have much less germ damage compared to grinding in the wet-degerming process (treatments 2 and 3).

Fermentation Performance. The low lactic and acetic levels indicate that microbial contamination during fermentation was under control. The ethanol yields for most of the treatments were about 35%, similar to the commercial dry-grind ethanol yield (on the basis of communications with industry personnel) except for treatment 4 (tail-end wet-split degerm fermentation), which was about 10% lower (**Table 2**). The low ethanol yield of treatment 4

was attributed to incomplete hydrolysis of starch upon visual examination. The finished beer from treatment 4 contained large pericarp and endosperm pieces and a few whole kernels. Starch granules in whole kernels and in the middle of the large endosperm pieces eluded hydrolysis by amylases. This conservative wet-split condition was chosen to avoid major damage to the germ since the objective of this study was to investigate the fate of the germs during fermentation, not to maximize ethanol yield. We believe that further optimization will increase ethanol yield while maintaining the integrity of the germs.

Figure 1 shows the solids retained on the 7-mesh sieve after fermentation of treatment 4 (tail-end wet-split degerm fermentation). Intact germs, a few identified by arrows, were visible as white pieces on the darker (yellow) background, which was the color of large pericarp pieces and endosperm proteins. Some germ pieces were loosely attached to the pericarp at the tip cap. The pale color of the germ was due to the much lower carotenoid level in the germ compared to that in the endosperm (*24*). When the white starch granules disappeared during ethanol fermentation, the carotenoid pigments were concentrated and became more pronounced in the residual solids, which act as a yellow background for the pale germs in the picture.

Degerm Results and Germ Characterization. *Germ Yield, Oil Content, and Germ Breakage*. Since front-end hand-dissection theoretically removed all the germs, treatment 1 had the highest germ and oil yields. Treatment 4 (tail-end wet-split) achieved the same oil yield, indicating that all germs were recovered. For treatment 4, the germ yield seemed to be slightly lower, but the germ oil content was slightly higher (although not statistically significant) than those of treatment 1 (**Table 3**). This was attributed to more nonlipid components being leached into the liquid during fermentation, similar to that in the steeping step of conventional wet milling (*3*). These results confirmed that the germs remained as whole pieces during fermentation as shown in **Figure 1**.

Figure 1. Solids recovered in treatment 4 (tail-end, wet-split degerm fermentation) before degerming. A few germ pieces are shown by arrows.

Germ and oil yields for treatment 2 (front-end wet-grind degerm) were significantly lower than those of treatments 1 and 4, indicating that wet grinding significantly damaged the germ. When the corn was wet-ground in the same manner as that in treatment 2, but the germs were recovered at the tail-end (after fermentation), in treatment 3, the germ and oil yields were further reduced, which implied additional damage to the germs during subsequent processing steps.

Figure 2 summarizes the germ breakage or integrity data. Greater kernel breakage causes more damage to the germ. Hand-dissection (treatment 1) and dry-grinding (treatment 5) represent two opposing extremes of germ breakage (0% vs 100% breakage). The germ from tail-end wet-split fermentation (treatment 4) had near zero breakage, indicating that kernel breaking, cooking, hydrolysis, and yeast fermentation did not significantly damage the germ. Oil also did not leach out when the germ structure remained intact. Wet grinding caused 22% germ breakage (treatment 2) before fermentation. The breakage was mainly as fractured germ pieces, which were too small to be recovered by a 7-mesh sieve. When the corn was ground as in treatment 2 but with the germ removed at the tail-end after fermentation (treatment 3), germ breakage increased to about 40%. We speculate that the additional breakage was from the small germ pieces that were previously attached to the large germ pieces in the corn slurry after wet grinding but had broken loose by prolonged agitation during fermentation. It is also possible that small germ pieces were further degraded during fermentation. These data show the importance of maintaining the physical integrity of the germs during the kernel breaking step.

The germ oil yield has a strong negative linear relationship with residual oil content in the degermed DDGS: germ oil yield (%) = $-0.18 \times$ oil content in degermed DDGS (%) + 3.69, $R^2 = 0.97$. This observation confirmed that the decreased oil yield was due to the loss of fine germ pieces to DDGS, not because of metabolic consumption by the yeast. In treatment 1 (front-end hand-dissect), all germ was removed but the germ-free DDGS still

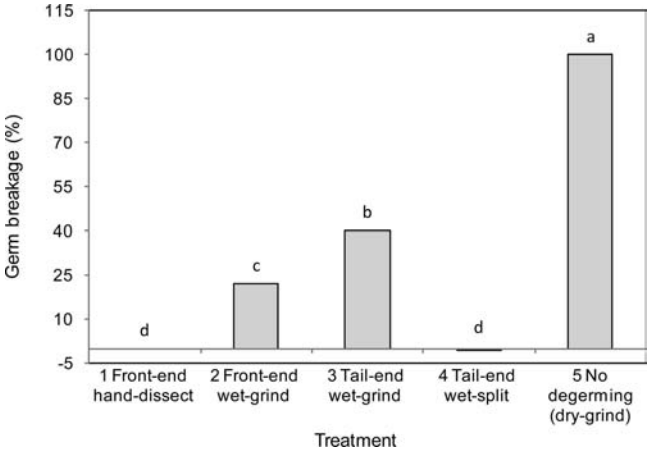

Figure 2. Germ breakage after different treatments. Different letters designate significant difference at $P < 0.05$.

Table 3. Germ Yields and Oil Contents for Different Corn Degerming and Fermentation Treatments

trt. no.	treatment	germ yield (%)	germ oil yield (%, on original corn)	oil content in germ (%)
1	front-end hand-dissect degerm	7.91 a	2.64 a	33.50 b
2	front-end wet-grind degerm	6.16 c	2.06 b	34.09 b
3	tail-end wet-grind degerm	4.04 d	1.58 c	39.16 a
4	tail-end wet-split degerm	7.26 b	2.64 a	36.40 ab
5	no degerm (dry-grind)	0.00 e	0.00 d	7.17 c [a]

[a] Oil was in the whole dried DDGS since no degerming was performed. Means within a column followed by different lower case letters are significantly different at $P < 0.05$.

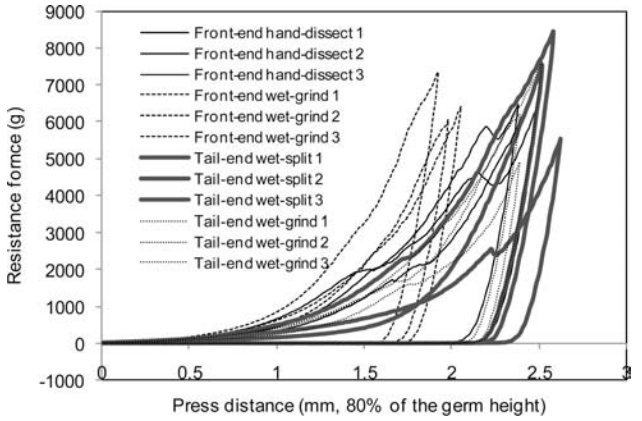

Figure 3. Germ physical strength profile during pressing by a texture analyzer.

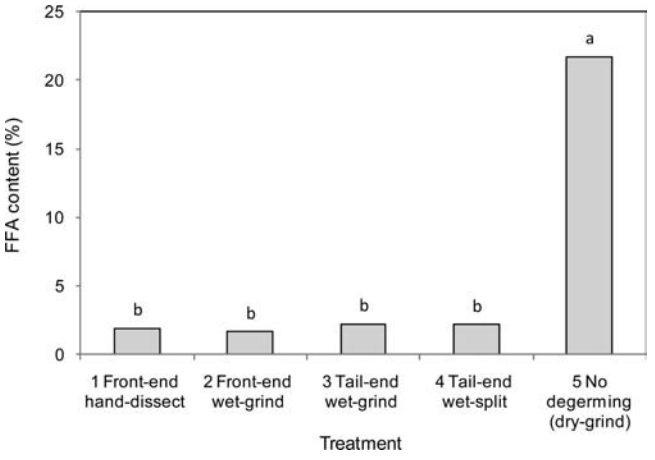

Figure 4. Free fatty acid (FFA) content in the extracted germ oils from different treatments. Different letters designate significant difference at $P = 0.05$.

contained a measurable amount of oil when using the acid hydrolysis method, which measures total lipid. This oil is mainly from corn pericarp and endosperm, and such an oil was concentrated by the disappearance of starch, which accounts for 70−74% of the corn kernel mass. Considering that the oil in the original nongerm components is very low and accounts for only a small fraction of total oil in corn, the oil outside of the germ was not the focus of present study. It is not recovered in the commercial wet-milling process either.

Physical Strength of the Germ. Comparing the physical strength of the germ before and after fermentation offers insight into why the germ can (or cannot) survive the fermentation. As the embryos of corn kernels, germs do not have homogeneous texture, nor are their shapes and structures identical from one kernel to another, which makes quantitative analysis of germ physical strength challenging, and considerable measurement variation was observed. Nonetheless, the analysis offers useful information as shown in **Figure 3**. The peaks on the Y axis represent the greatest strength when the germs were pressed to 80% of original thickness. The X axis represents the distance the probe traveled (in mm). No significant strength difference was found between germs isolated before and after fermentation. One explanation may be the composition of the germ: germ does not contain a significant amount of starch or other water-soluble or fermentable components that would cause the germ structure to collapse during cooking and fermentation.

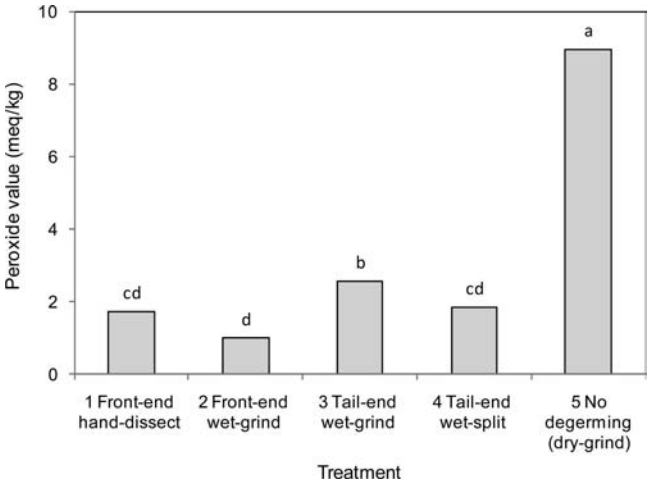

Figure 5. Peroxide value of the extracted germ oils from different treatments. Different letters designate significant difference at $P = 0.05$.

Germ Oil Quality. *FFA Content*. The FFA contents of the germ fractions from treatments 1−4 were about 2%. There was no significant difference between the oil extracted from the front-end and the tail-end germs (**Figure 4**). On the contrary, oil extracted from the dry-grind ethanol process was highly hydrolyzed, containing about 22% FFAs, even higher than literature values (*6*, *7*). It is suspected that the hydrolysis was caused by the endogenous lipase released by dry grinding or the exogenous enzymes secreted by yeast, or both. However, when the germs remain intact or in large pieces, the oil remained protected against hydrolytic enzymes.

Peroxide Value. The peroxide value showed a trend similar to that of FFAs. Germ oil from tail-end wet-split treatment, which endured 4 h of cooking and liquefication and 60 h of fermentation, had the same peroxide value as that from front-end wet-grind and front-end hand-dissection (**Figure 5**). It can be explained by two reasons: (a) when germs maintain their original structure, the oil existed in oil bodies, which were remarkably stable to oxidation and other physiochemical attacks to the oil (*25*); and (b) ethanol fermentation creates an anaerobic environment (oxygen-free in the mash). Germ oil from the tail-end wet-grind treatment had significantly higher peroxide value than that from front-end, although the difference was relatively small (<1 meq/kg). The small increase in peroxide value most likely happened during the cooking/liquefication step when the germs were partially damaged during wet grinding (**Figure 2**). However, oil from the dry-grind process had the highest peroxide value (9 meq/kg) compared to an average of 2 meq/kg for the other oils. We believe this was due to the dry-grind step breaking germs into small pieces, exposing more oil to oxygen before and after fermentation.

This study has shown that corn germs, if intact, can maintain their physical structure during starch cooking, hydrolysis, and yeast fermentation steps of the ethanol fermentation process and that the oil in the germ fraction remains largely in its native state. This suggests that the germs recovered during or after the fermentation process may be used to produce food-grade oil or a better oil feedstock for producing biodiesel than oil recovered from traditional dry-grind ethanol production. Integrating this novel degerming concept into dry-grind ethanol production may also produce value-enhanced DDGS products to meet the needs of swine and poultry feeding operations, which usually require high protein and low oil and fiber contents than are produced in typical dry-grind ethanol plants without front-end degerming or tail-end oil recovery. These findings lay a foundation for developing

a series of new degerming strategies for the dry-grind ethanol industry.

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