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Improvement in fermentation characteristics of degermed ground corn by lipid supplementation

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Abstract With rapid growth of fuel ethanol industry, and concomitant increase in distillers dried grains with solubles (DDGS), new corn fractionation technologies that reduce DDGS volume and produce higher value coproducts in dry grind ethanol process have been developed. One of the technologies, a dry degerm, defiber (3D) process (similar to conventional corn dry milling) was used to separate germ and pericarp fiber prior to the endosperm fraction fermentation. Recovery of germ and pericarp fiber in the 3D process results in removal of lipids from the fermentation medium. Biosynthesis of lipids, which is important for cell growth and viability, cannot proceed in strictly anaerobic fermentations. The effects of ten different lipid supplements on improving fermentation rates and ethanol yields were studied and compared to the conventional dry grind process. Endosperm fraction (from the 3D process) was mixed with water and liquefied by enzymatic hydrolysis and was fermented using simultaneous saccharification and fermentation. The highest ethanol concentration (13.7% v/v) was achieved with conventional dry grind process. Control treatment (endosperm fraction from 3D process without lipid supplementation) produced the lowest ethanol concentration (11.2% v/v). Three lipid treatments (fatty acid ester, alkylphenol, and ethoxylated sorbitan ester 1836) were most effective in improving final ethanol concentrations. Fatty acid ester treatment produced the highest final ethanol concentration (12.3% v/v) among all lipid supplementation treatments. Mean final ethanol concentrations of alkylphenol and ethoxylated sorbitan ester 1836 supplemented samples were 12.3 and 12.0% v/v, respectively.

Keywords Modified dry grind process · Anaerobic fermentation · Ethanol · Lipid supplements · Distillers dried grain with solubles

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Introduction

United States imports 150 billion gal/year of petroleum [8]. Ethanol production from corn is an attractive alternative to foreign oil as it promotes local economies and reduces dependence on foreign oil. About 65% of US domestic fuel ethanol production (3.9 billion gal/year) is from the corn dry grind process industry which is one of the fastest growing industries [13]. Most of the growth in production capacity has been from small, farmer owned cooperative plants (45–60 million gal/year) due to lower capital costs and federal and state government tax incentives [4]. Small dry grind plants strengthen the rural economy by generating jobs and ensuring adequate markets for US corn [22].

In a conventional dry grind process, corn is ground and mixed with water to produce slurry. The slurry is cooked; slurry starch is liquefied, saccharified and fermented to produce ethanol. The remaining nonfermentables in corn (germ, fiber and protein) are recovered as a mixture at the end of the dry grind process as an animal food coproduct called distillers dried grains with solubles (DDGS). In a typical dry grind process, one bushel (25.4 kg or 56 lb) of corn produces 10.6 L (2.8 gal) of ethanol and 7.2 kg (1 lb) of DDGS. Due to high fiber content, DDGS is used primarily as ruminant animal food. Due to the rapid growth of the dry grind industry, the ruminant market for DDGS is reaching saturation.

Fractionation of corn prior to the fermentation is one approach to reduce DDGS volume and improve quality. With lower fiber and higher protein contents, modified DDGS from these processes can be used as food for nonruminants such as poultry and swine. Fractionation technologies that have been developed for dry grind ethanol industry can be divided broadly into wet and dry technologies based on the presence/absence of a corn soaking step. Dry technologies such as dry degerm, defiber process (3D process) [5] do not involve corn soaking and produce germ and pericarp fiber as additional coproducts (Fig. 1).

These coproducts can be used as a source of other coproducts such as corn oil (obtained from corn germ) and corn fiber oil (obtained from pericarp and endosperm fiber) [21]. Corn oil is a valuable coproduct used as cooking oil and in other food applications. Corn fiber oil contains unique serum cholesterol lowering compounds such as ferulate phytosterol esters (FPE), free phytosterols (St) and phytosterol fatty acyl esters (St:E) [14, 15].

Most corn kernel lipids are in germ and aleurone layer below the pericarp. Removal of germ and pericarp fiber in dry fractionation processes results in loss of these nutrients in fermentation medium. Removal of the lipids could result in poor fermentation characteristics of the endosperm fraction produced from dry fractionation process. Loss of starch in coproducts, due to imperfect separation of coproducts, reduces germ quality [10] and could lower ethanol yields.

In this study, fermentation characteristics of a dry fractionation process (3D process, Fig. 1) were compared to the conventional dry grind process. Additionally, the effect of lipid supplement addition on improving endosperm fraction fermentation characteristics obtained from 3D process was investigated.

Materials and methods

Materials

Yellow dent corn grown during the 2004 crop season at the Agricultural and Biological Engineering Research Farm, University of Illinois at Urbana-Champaign was used. Samples were hand cleaned and moisture content was determined using a standard two stage convection oven method [2].

The α-amylase (α-amylase solution *Bacillus licheniformis*, type XII-A saline solution 500–1,000 units/mg protein, 1,4-α-D-glucan-glucanohydrolase, 9000-85-5, Sigma-Aldrich, St. Louis, MO, USA) and glucoamylase (amyloglucosidase from *Aspergillus niger*, glucoamylase, 1,4-α-D-glucan glucohydrolase, exo-1,4-α-glucosidase, 9032-08-0, Sigma-Aldrich, St. Louis, MO, USA) with activities of 21,390 and 300 units/ml, respectively, were used for liquefaction and saccharification, respectively. Ten different lipid supplements (Table 1) obtained from Cognis Corporation (Cincinnati, OH, USA) were used to supplement the 3D process (Fig. 1) endosperm fraction. Some of the supplements were experimental samples. The supplements consisted of fatty acid esters, alcohols and phenols as primary active compounds.

The effect of lipid supplementation on fermentation characteristics of endosperm fraction obtained from 3D process was studied using a simultaneous saccharification and fermentation (SSF) processes. Fermenter solids level was constant at 25% (wb) for all treatments.

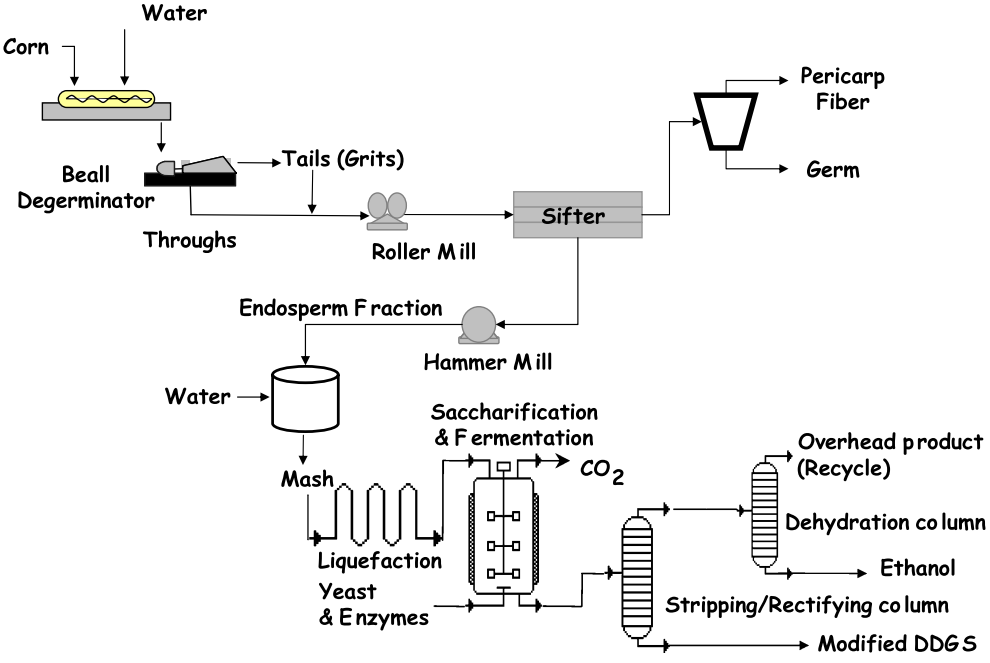

Fig. 1 Dry degerm defiber (3D) process

Table 1 Lipid supplements and sample nomenclature

Treatment name	Product name
FE1	Fatty acid methyl ester
FE2	Ethoxylated fatty alcohol 400
FE3	Sorbitan ester 428
FE4	Ethoxylated sorbitan ester 1308
FE5	Alkylphenol
FE6	Ethoxylated sorbitan ester 1836
FE7	Fatty acid ester
FE8	Ethoxylated fatty alcohol 488
FE9	Ethoxylated fatty acid
FE10	Lipophilic sterol supplement
3D	Untreated 3D process endosperm fraction
CDG	Conventional dry grind

Conventional dry grind process

Corn (1,000 g) was milled in a cross beater mill (model MHM4, Glen Mills, Clifton, NJ, USA) at 500 rpm equipped with a 0.5 mm hole sieve. Moisture content of milled corn was analyzed using a two stage conventional oven method [2]. Milled corn (200 g db) was mixed with water at 60°C to form 25% solids mash. The mash was liquefied using 2.8 ml α-amylase for 90 min in a water bath maintained at 90°C. After adjusting mash pH to 4.2 using 1.0*N* sulfuric acid, the mash was simultaneously saccharified and fermented as described in the section below.

Dry degerm defiber (3D) process

Corn (15,000 g) was tempered to a moisture content of 22.5% (wb) for 18 min at 25°C. Corn was passed through a horizontal drum degermination mill (model SPL56CC17 F20 51EP, Marathon Electric, Wausau, WI, USA) and dried at 49°C for 2 h to 15% (db) moisture. After four passes through a roller mill (fluted rollers 150 mm diameter, 240 mm width, 155 rpm) driven by an electric motor (2 hp; model EVGD, US Electrical Motors, Los Angeles, CA, USA), corn was sieved over a 10 mesh sieve for 5 min. Germ and fiber fractions retained on the sieve were separated by aspiration (6DT4, Kice Metal Products, Wichita, KS, USA). The remaining endosperm fraction was milled in a cross beater mill (model MHM4, Glen Mills, Clifton, NJ, USA) at 500 rpm using a 0.5 mm sieve with round holes. Milled endosperm fraction was analyzed for moisture [2]. Milled endosperm fraction was used for all lipid supplementation treatments.

Treatments to endosperm fraction from the 3D process

Each 200 g sample of endosperm fraction was mixed with water at 60°C to form 25% solids (wb) slurry which was liquefied using 2.8 ml α-amylase for 90 min in a water bath maintained at 90°C. Lipid supplements (Table 1) were added at 5,000 ppm to the liquefied mash. Each sample was fermented using SSF process as described below.

Simultaneous saccharification and fermentation

Liquefied mash was cooled to 30°C and pH adjusted to 4.2 using 1.0*N* sulfuric acid. The mash was saccharified by adding 2.8 ml glucoamylase. Simultaneously, mash was inoculated with active dry yeast (0.022 g/g dry solids, Fleischmann's Yeast, Fenton, MO, USA). To provide 500 ppm free amino nitrogen, $(NH_4)_2SO_4$ was added. The SSF process was performed at 30°C for 72 h with continuous agitation at 50 rpm and monitored by withdrawing 5 ml samples of fermentation broth at 12 h intervals. Sugar and ethanol concentrations were measured using HPLC method described below.

HPLC analyses

Samples (5 ml) drawn from fermentation vessels were centrifuged (model Durafuge 100, Precision, Winchester, VA, USA) at 1,476*g* for 5 min to obtain supernatant which was filtered through a 0.2 μm filter. Filtered supernatant liquid (5 μl) was injected into an ion exclusion column (Aminex HPX-87H, Bio-Rad, Hercules, CA, USA) maintained at 50°C. Sugars (glucose, fructose, maltose and maltotriose), organic acids (lactic, succinic, and acetic acid) and alcohols (ethanol, methanol and glycerol) were eluted from the column with HPLC grade water containing 5 mM sulfuric acid. Separated components were detected with a refractive index detector (Model 2414, Waters, Milford, MA, USA). The elution rate was 0.6 ml/min; a calibration standard was used prior to each set of samples. Data were processed using HPLC software (Version 3.01, Waters, Milford, MA, USA).

Statistical analyses

A complete randomized block design was used. Two replicate fermentations were conducted for each treatment. Fermentation samples from all experiments were analyzed using a mean of two values from HPLC analyses.

Results and discussion

Fermentation rates and final ethanol concentrations increased when lipid supplements were added to the 3D process endosperm fraction for SSF process (Fig. 2, Table 2). Conventional dry grind fermentation had the highest ethanol concentration (13.73% v/v) and lowest residual glucose (0.28% w/v) among all samples. Of the ten lipid supplements evaluated, three gave highest

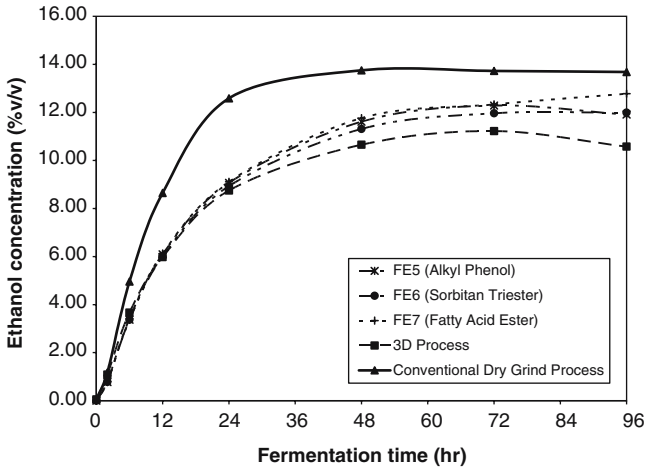

Fig. 2 Ethanol profiles (ethanol concentration vs. fermentation time) for conventional dry grind (CDG), 3D process and three treatments lipid supplements

increase in the final ethanol concentration compared to control treatment (3D endosperm fraction with no added lipid supplements). Lipophilic sterol supplement was most effective in increasing ethanol concentration during the first 24 h of fermentation. Fatty acid ester supplement and ethoxylated sorbitan ester 1836 were effective in latter stages of fermentation (>24 h). The final ethanol concentration was highest for fatty acid ester treatment (12.33% v/v) compared to control (11.22% v/v). Mean final ethanol concentrations of alkylphenol and ethoxylated sorbitan ester 1836 treatments were 12.29 and 11.96% v/v, respectively, and were 1.07 and 0.74% v/v more than control treatment using SSF process.

Fatty acid ester and alkylphenol treatments improved glucose utilization by 8.47 and 6.15% and produced 10.38 and 8.99% more ethanol than control treatment, respectively. Ethoxylated sorbitan ester 1836 treatments produced 6.15% more ethanol however, glucose was under utilized (+0.84%) as compared to control treatment. Ethanol production improved for all lipid treatments indicating a beneficial effect of lipid supplements (Table 2). Glucose was under utilized in all treatments except for fatty acid ester and alkylphenol treatments, indicating under utilized potential for improving ethanol concentrations (Table 3).

Most corn kernel lipids are in germ and aleurone layers. Recovery of pericarp fiber and corn germ prior to fermentation step, as in the 3D process, reduces lipid content of the fermentation substrate (3D process endosperm fraction). Lipids are essential components of yeast cell membranes and adequate lipid levels are required to maintain cell membrane integrity. Yeast cannot manufacture necessary lipids under strictly anaerobic conditions and require external lipid supplements for proper growth [12, 20]. Supplementation of fermentation medium with ergosterol has been demonstrated to improve yeast cell viability in wine fermentations [6]. Different fatty acids have varied efficacy in improving cell viability [23]. Some authors have investigated periodic oxygen purging as a means to stimulate intracellular lipid synthesis by yeast [19]. Inactivated yeast cells have been shown to be effective in improving fermentation characteristics of nitrogen rich, lipid deficient synthetic medium [3]. High ethanol concentrations as encountered in fuel ethanol fermentations cause irreversible inactivation of cell membrane constituents and cause damage to the yeast cell membrane [16]. Hence, adequate supply of lipids is essential to maintain cell membrane integrity, yeast cell viability and rapid growth phase of yeast. Actively growing yeast produce ethanol more than 30 times faster than yeast in stationary phase [11]. Therefore abundant supply of essential lipids can improve final ethanol concentrations and fermentation rates of yeast in a modified dry grind ethanol process using the 3D process to fractionate corn prior to fermentation.

During SSF process, both production of glucose by saccharification and consumption of glucose by fermentation proceed simultaneously. Hence it is difficult to separately analyze the glucose consumption rates from the fermenter glucose concentrations. Final residual glucose levels were lower than control indicating a more complete fermentation (Table 3). However, high glucose levels (>4.86% w/v) in all samples using the 3D process endosperm fraction represent stuck (incomplete) fermentations. While lipid supplementation improves fermentation characteristics, lipids may not be the only limiting nutrients in fuel ethanol fermentations. High residual sugars in fermentation of 3D process endosperm fraction could represent potential processing and quality issues for DDGS. Earlier research aimed at improving carbohydrate metabolism with B vitamin and germ soak water supplements resulted in more complete fermentation of 3D process endosperm fraction. Most B vitamins are essential coenzymes for carbohydrate metabolism of eukaryotes. Improving carbohydrate metabolism stimulates growth by providing an abundant supply of metabolic precursors for growth. One B vitamin, riboflavin (vitamin B2), is essential for lipid synthesis [9].

Lactic acid concentrations greater than 0.2–0.8% (w/v) stress yeast, resulting in lower growth rates and ethanol production rates [17]. Lactic acid, an indicator of infection by *Lactobacillus sp.*, had lower concentrations (<0.03% w/v) among all samples. Consistently low levels of lactic acid (Table 4) were indicative of no major bacterial infection problems.

Glycerol, which is an indicator of yeast stress, is produced in small amounts (1.2–1.5%) in dry grind ethanol fermentations [20]. Glycerol concentrations were low (<0.91% v/v) among all samples. Higher levels of residual glucose in the fermenter could result in lower glycerol levels for all treated samples. When exposed to osmotic stress, yeast produces glycerol as a compatible solute [1]. With HPLC, we can measure compounds in extracellular fluid; therefore artificially lower levels of

Table 2 Effect of lipid supplement addition on ethanol concentration (% v/v) during fermentation and simultaneous saccharification and fermentation (SSF) for the dry degerm, defiber (3D) process

Treatment												
Time (h)	FE1	FE2	FE3	FE4	FE5	FE6	FE7	FE8	FE9	FE10	3D	CDG
0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.15	0.05	0.05	0.05	0.06
2	0.93	0.94	0.92	0.95	0.78	0.78	0.80	0.84	1.10	1.12	1.09	1.18
6	3.66 bcd	3.65 bcde	3.60 cdef	3.71 bc	3.37 ef	3.37 ef	3.41 def	3.37 f	3.71 bc	3.92 b	3.67 bcd	4.96 a
12	6.15	6.12	6.07	6.21	6.09	5.99	6.13	5.97	6.14	6.29	5.98	8.65
24	9.01 bc	8.91 bc	8.87 bc	8.98 bc	9.07 bc	8.93 bc	9.11 b	8.70 c	8.77 bc	9.10 b	8.74 bc	12.59 a
48	11.30	10.83	11.10	10.91	11.61	11.31	11.76	10.96	10.79	11.28	10.66	13.75
72[a]	11.83 def	11.00 h	11.59 defg	11.40 efgh	12.29 bc	11.96 bd	12.33 b	11.45 efg	11.38 fgh	11.85 dec	11.22 gh	13.73 a
Increase (%)	6.05	1.67	4.18	2.36	8.99	6.15	10.38	2.87	1.27	5.90	–	29.08

Values are means of two replicate fermentations
[a]Values in the same row followed by same letter are not different at $\alpha = 0.05$

Table 3 Effect of lipid supplement addition on glucose concentration (% w/v) during fermentation and simultaneous saccharification and fermentation (SSF) for the dry degerm, defiber (3D) process

Treatment												
Time (h)	FE1	FE2	FE3	FE4	FE5	FE6	FE7	FE8	FE9	FE10	3D	CDG
0	1.74	1.90	1.86	1.92	1.66	1.63	1.61	1.77	1.42	1.46	1.47	1.22
2	18.29	18.81	19.31	19.61	19.90	20.08	20.14	20.03	19.12	19.03	17.82	18.33
6	14.17	14.18	14.42	14.62	14.76	14.63	14.84	14.73	13.88	13.96	12.92	11.33
12	12.61	12.42	12.48	12.55	12.42	12.54	12.50	12.19	12.09	12.00	11.22	6.75
24	9.42	9.27	9.18	9.20	9.25	9.34	9.08	8.84	9.21	9.00	8.34	1.48
48	6.41	6.92	6.20	6.59	5.83	6.15	5.51	5.90	6.54	6.21	5.90	0.23
72	5.98 b	6.85 a	5.78 bcd	6.17 b	5.10 fe	5.35 def	4.86 f	5.42 cde	5.93 bc	5.67 bcd	5.31 def	0.28 g
Residual glucose (%)	12.58	28.92	8.87	16.20	−3.92	0.84	−8.47	2.09	11.72	6.85	–	−94.70

Values are mean of two replicate fermentations. Values in the same row followed by same letter are not different at $\alpha = 0.05$

glycerol could have resulted for the treated samples. This observation is strengthened by reports by researchers that yeast can maintain intra- or extracellular ratios of glycerol up to 600-fold under osmotic stress conditions [1]. One of the many mechanisms proposed for the capacity to retain glycerol by yeast is prevention of leakage across the cell membrane through its modification [7]. The higher extracellular glycerol levels observed with the control treatment (untreated 3D process endosperm fraction) could be the result of the inability of yeast to make necessary modifications to the cell membrane due to lipid deficiency.

Under normal fermentation conditions, assuming yeast to be in the stationary phase of growth, one mole of glucose produces two moles of ethanol. The difference between the unutilized glucose levels (Table 3) in the fermenter does not match with differences in the final ethanol concentrations (Table 2) among the samples. One of the reasons for the observed difference could be due to the possible accumulation of intracellular glycerol. Glycerol production is an energy intensive process for the yeast [18]; this could be a reason for the observed discrepancy between the difference in the final ethanol levels and the residual glucose levels. There is also a lower level of secreted succinic acid and higher levels of extracellular acetic acid for all treatments were different as compared to CDG. Therefore, other pathways may have been active during fermentation of the 3D process endosperm fraction with and without lipid supplements.

Dry grind plants using the 3D process could consider using lipid supplements to improve fermentation rates and achieve lower residual sugars in DDGS. Potential

Table 4 Effect of lipid supplement addition on extracellular metabolic byproducts (% w/v) during fermentation and simultaneous saccharification and fermentation (SSF) for the dry degerm defiber (3D) process

Treatment												
	FE1	FE2	FE3	FE4	FE5	FE6	FE7	FE8	FE9	FE10	DM	DG
Lactic acid	0.03 b	0.03 b	0.02 bc	0.02 bc	0.02 c	0.02 bc	0.03 b	0.03 b	0.02 bc	0.03 bc	0.02 bc	0.03 a
Acetic acid	0.07 b	0.06 b	0.07 b	0.07 b	0.06 b	0.06 b	0.06 b	0.06 b	0.07 b	0.07 b	0.07 b	0.00 a
Succinic acid	0.05 b	0.05 b	0.04 b	0.05 b	0.07 b	0.05 b	0.05 b	0.07 b	0.05 b	0.04 b	0.05 b	0.14 a
Glycerol	0.77 b	0.75 b	0.75 b	0.76 b	0.77 b	0.75 b	0.76 b	0.76 b	0.75 b	0.75 b	0.73 b	0.91 a

Values are means of two replicate fermentations. Values in the same row followed by same letter are not different at $\alpha = 0.05$

benefits in increasing the final ethanol concentration by promoting better utilization of sugars, increased fermentation rates and lower residual sugars in DDGS may justify the use of lipid supplements.

Conclusion

Supplementation of the 3D process endosperm fraction with lipids improved fermentation rates and increased glucose utilization, resulting in higher final ethanol yields. Fatty acid esters, alkyl phenol and ethoxylated sorbitan ester 1836 provided the highest increases in final ethanol concentrations. However, high residual glucose levels could present problems in DDGS processing. Economic assessment is needed to determine whether the increase in ethanol yields justifies addition of these lipid supplements during fermentation.

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