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Methods to Recover Value-Added Coproducts from Dry Grind Processing of Grains into Fuel Ethanol

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ABSTRACT: Three methods are described to fractionate condensed distillers solubles (CDS) into several new coproducts, including a protein-mineral fraction and a glycerol fraction by a chemical method; a protein fraction, an oil fraction and a glycerol-mineral fraction by a physical method; or a protein fraction, an oil fraction, a mineral fraction, and a glycerol fraction by a physicochemical method. Processing factors (ethanol concentration and centrifuge force) were also investigated. Results show that the three methods separated CDS into different fractions, with each fraction enriched with one or more of the five components (protein, oil, ash, glycerol and other carbohydrates) and thus having different targeted end uses. Furthermore, because glycerol, a hygroscopic substance, was mostly shifted to the glycerol or glycerol-mineral fraction, the other fractions had much faster moisture reduction rates than CDS upon drying in a forced air oven at 60 °C. Thus, these methods could effectively solve the dewatering problem of CDS, allowing elimination of the current industrial practice of blending distiller wet grains with CDS for drying together and production of distiller dried grains as a standalone coproduct in addition to a few new fractions.

KEYWORDS: *condensed distiller solubles, fuel ethanol, dry grind processing, DDGS, coproducts, animal feed, dewater, CDS*

INTRODUCTION

Fuel ethanol production in the United States and elsewhere is an important growing industry. In 2012, despite a severe drought, the U.S. ethanol plants converted 4.5 billion bushels (114.3 million metric tons) of corn (about 40% of total U.S. supply) into an estimated 13.3 billion gallons (50.3 billion liters) of ethanol and 34.4 million metric tons of coproducts as livestock feed, including distillers dried grains with solubles (DDGS), corn gluten feed, and corn gluten meal.[1] Of that production, 91.8% of ethanol facilities employed some variation of the dry-grind processing. In the dry-grind processing, the whole grain kernels are processed through several sequential steps, including grinding, cooking, liquefaction, scarification, fermentation, distillation, and coproduct recovery.[2,3]

Coproduct recovery, which consists of several additional steps to convert nonfermentable residues into value-added coproducts, is an important aspect of ethanol manufacturing since the sale of all types of coproducts as livestock feed substantially increases the economic viability of ethanol plants. In a typical dry-grind processing plant, coproduct recovery begins with whole stillage,[2−4] which results from distillation of fermented mash for ethanol removal. Upon leaving distillation, whole stillage contains 6−16% solids in both dissolved and suspended forms. It is a hot, acidic, and viscous fluid with limited shelf life and must be dried for easy handling, storage, and end use. The common practice to dry whole stillage consists of separating it into a liquid fraction (thin stillage) and a solid fraction (distillers wet grains, DWG), evaporating thin stillage (90−95% moisture) into condensed distillers solubles (CDS) (50−75% moisture), combining CDS with DWG, and drying them together to produce DDGS. There are multiple reasons to have these steps instead of drying the whole stillage or its two fractions directly. First, a significant portion (10−50%) of thin stillage is returned to the cooking step as a source of water.[3−5] This is known as back setting, which saves water and reduces evaporative load (saves energy). Second, removing water in a dryer uses 4 to 5 times more energy than removing water in an evaporator, since evaporators allow reuse of some thermal energy.[4] Third, CDS contains 25−50% solids and is very viscous and difficult to dry but the mixture of CDS and DWG is easier and less expensive to dry than CDS alone.[4]

This common industrial practice has drawbacks. First, because the same material is recycled and dried many times, it is prone to excessive heat exposure, which results in a loss of nutritional quality in the final product.[6,7] Second, the ratio of DWG to CDS is hard to control and usually varies among plants and even batches within a plant. This causes great variation in chemical composition[2,6−8] and other properties[6,7,9] of DDGS. Third, by mixing the two fractions into one, DDGS has been the only dried coproduct available in the current market, thus limiting market opportunity.

In recent years, efforts have been made to monitor chemical changes of biomass during the entire dry grind process[10,11] or quantify the physical and chemical properties of selected streams, such as CDS[12−14] and thin stillage[14−16] in order to identify potential valuable components. One important development in recovering valuable components from processing streams has been the effort to separate the oil from whole stillage,[17] thin stillage,[18] or CDS.[17,19] Among the reported methods, the one by Cantrell and Winsness[19] has been successfully commercialized by GreenShift Corp (Alpharetta, GA, greenshift.com) and is being used by some ethanol plants in the U.S. The functional lipid composition of the oil extracted

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by the GreenShift method was subsequently documented.[20] To enhance oil recovery from CDS, Majoni et al.[21] treated CDS with proteases and other enzymes before centrifugation. In a separate study,[22] the same group attempted to extract oil from the CDS residual (following the initial centrifugation for oil removal) with isopropanol, butanol, or a mixture of hexane and ethanol in order to increase total oil recovery. The main objective of all these reported studies[17−22] has been to recover oil from stillage and use it as a biodiesel feedstock. The remaining material is combined with DWG and dried together to produce deoiled DDGS.

This communication describes three methods, which are based on chemical, physical, or physicochemical principles, respectively, to fractionate CDS during coproduct recovery of the dry grind process. The objectives were (1) to make fractions from CDS easier to dewater than CDS and thus eliminate the step of blending of CDS with DWG for drying together into DDGS and (2) at the same time to generate several new coproducts with unique composition and added values. Unlike the previous methods known in the literature for processing thin stillage or CDS,[17−22] the methods reported in this communication directly process CDS into value-added coproducts without returning any of the fractionated products to the existing processing stream. The present study was also the first to examine general chemical composition and glycerol content in all resulting fractions, monitor their drying behaviors, and compare them to those of CDS.

MATERIALS AND METHODS

Materials. The CDS sample was kindly provided by Golden Grain Energy, Inc. (Mason City, IA). The commercial sample was frozen after collection for transportation and storage, and thawed in the laboratory just before further processing and/or analysis. Duplicate analysis showed that the CDS sample had a moisture content of 71.17%. The 95% ethanol was purchased from Pharmco-AAPER (Shelbyville, KY).

Fractionation of CDS by a Chemical Method. The method consisted of mixing CDS directly with an aqueous ethanol for extraction, followed by a solid−liquid separation (Figure 1). Three ethanol concentrations in the final extraction system, 55, 65 and 75% v/v, were used. For 55%, 80 g of CDS was mixed with 156.9 mL of 95% ethanol and 57.1 mL of water; for 65%, with 185.5 mL of 95% ethanol and 28.5 mL of water; and for 75%, with 214 mL of 95% ethanol and 0 mL of water. After mechanical mixing for 10 min at room temperature, each mixture was poured into a centrifuge bottle (750 mL total capacity). The bottles were balanced and placed into a swing bucket rotor fitted into a centrifuge (Sorvall RC-12BP, Thermo Fisher Scientific, Waltham, MA) and centrifuged at 1000× *g* for 10 min at room temperature. The precipitate was collected as a protein-mineral fraction. The ethanol in the supernatant was recovered with a laboratory rotary vacuum evaporator at 80 °C. The supernatant was further condensed using the same evaporator at 90 °C and then collected as a glycerol fraction. The experiment was duplicated.

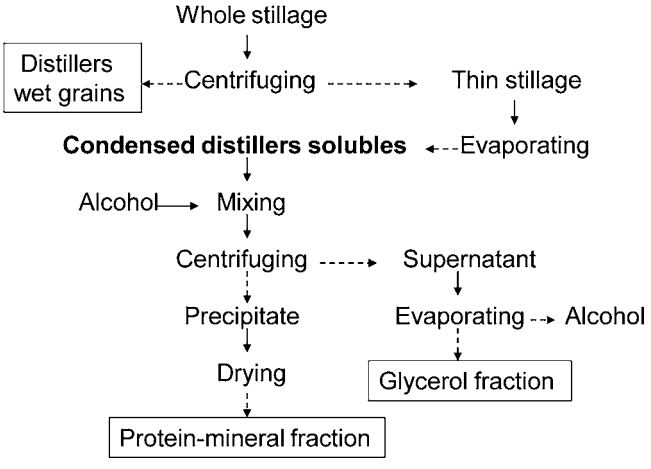

Figure 1. Flow diagram illustrating the chemical method to fractionate condensed distillers solubles for coproduct recovery during dry grind processing of grains into ethanol.

Fractionation of CDS by a Physical Method. The method consisted of diluting CDS with water followed by centrifugation (Figure 2). For making one batch of diluted CDS (moisture increased to 85%), 160 g CDS was mixed with 147.5 mL of tap water. Three centrifuge forces, 1000, 3000, and 6000× *g*, were used, with duplication for each force. Thus, a total of 6 batches of diluted CDS were made in the same way. Diluted CDS was poured into the aforementioned centrifuge bottle. For each centrifuge force, two bottles were balanced and centrifuged for 10 min at room temperature. The top layer was skimmed off to become an oil fraction. The precipitate was collected as a protein fraction. The middle layer (supernatant) was condensed with the evaporator at 90 °C until the density of the mixture reached to about 1.2 g/mL and collected as a glycerol-mineral fraction.

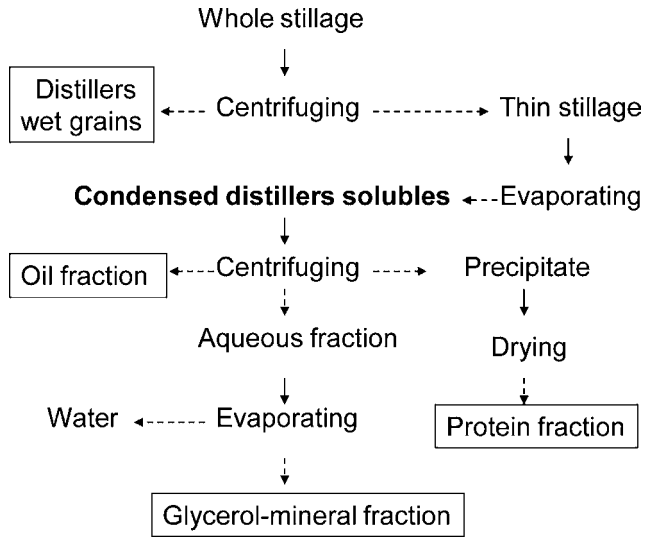

Figure 2. Flow diagram illustrating the physical method to fractionate condensed distillers solubles for coproduct recovery during dry grind processing of grains into ethanol.

Fractionation of CDS by a Physicochemical Method. The physicochemical method (Figure 3) basically combined the physical method (Figure 2) with the chemical method (Figure 1). In the physical method just described above, after the condensed supernatant obtained under each centrifuge force was collected as the glycerol-mineral fraction, it was divided equally into two portions. One portion was used for a drying experiment and chemical analysis, and the other portion was used as the starting material for this experiment. Basically, the portion of glycerol-mineral fraction was further fractionated by pouring into the plastic centrifuge bottle and mixing with a proper amount of 95% ethanol by mechanical mixing for 10 min at room temperature. The mixture had a final ethanol concentration of 65% v/v. It was centrifuged at 1000× *g* for 10 min at room temperature. The precipitate was collected as a mineral fraction. The ethanol from the new supernatant was recovered with the rotary evaporator under a vacuum at 80 °C. The remaining liquid was further condensed and collected as another glycerol fraction. Because a total of six glycerol-mineral fractions were obtained by the three different centrifuge forces

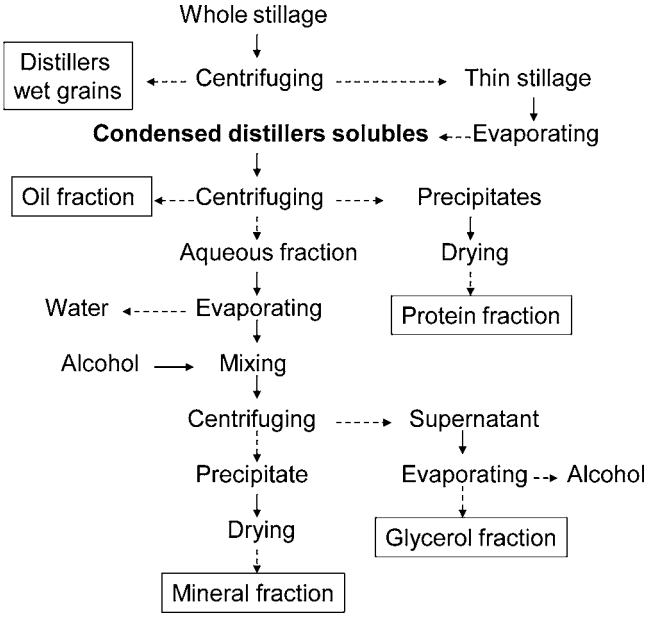

Figure 3. Flow diagram illustrating the physicochemical method to fractionate condensed distillers solubles for coproduct recovery during dry grind processing of grains into ethanol.

with duplication at each force in the physical method, they were all subjected to the same treatment in this experiment.

Drying of CDS and Its Fractions. The original CDS (Control); the protein-mineral and glycerol fractions obtained by the chemical method under 65% ethanol concentration; the oil, protein, and glycerol-mineral fractions by the physical method under 3000× *g* centrifuge force; and the two new fractions, mineral and glycerol fractions, by the physicochemical method under the 3000× *g* centrifuge force and 65% ethanol concentration, were all dried under a forced air oven at 60 °C. In order to compare their relative difficulty to dry, each sample in a preweighed pan was weighed at the intervals of 0:00, 0:20, 0:40, 1:00, 1:30, 2:00, 3:00, 4:00, 6:00, 8:00, 24:00 (hr:min) throughout the drying period. The wet basis moisture content at each interval was calculated. Other fraction samples, which were not associated with the drying experiment, were dried in the same forced air oven at 60 °C overnight before chemical analysis.

Chemical Analysis. The original CDS and all of its fractions were analyzed for mass and contents of moisture, protein, oil, ash, glycerol, and carbohydrates. All measurements were conducted in duplicate. Moisture and ash contents were determined according to official methods.[23] The moisture content was used to convert concentrations of other components into a dry matter (dm) basis. The total nitrogen/protein content in samples was measured by a combustion method,[23] using a protein analyzer (Model FT528, Leco Corp. St. Joseph, MI). The protein content was calculated with a conversion factor of 6.25. The oil content was determined by an AOCS Official Procedure,[24] using a fat analyzer (Model XT 10, Ankom Technology, Macedon, NY). However, instead of using petroleum ether, hexane was used as the extracting solvent. Glycerol was measured based on a kit (K-GCROL, Megazyme Intl., Wicklow, Ireland). It was based on use of ADP-glucokinase and increase in absorbance on conversion of NAD^+ to NADH. Carbohydrate content was calculated based on difference between 100% and sum of contents of protein, oil, ash and glycerol, % dry matter basis.

Statistical Treatment of Data. The three methods to fractionate CDS were duplicated at the processing stage. Data were analyzed with JMP software, version 6 (JMP, a Business unit of SAS, Cary, NC). Analysis of variance (ANOVA) was conducted within each fractionation in order to determine the effect of fractionation and ethanol concentration (or centrifuge force for the physical or physicochemical method) on the content of each constituent. Tukey's honestly significant difference test was conducted for pairwise comparisons of all means of fractions and the original CDS for each constituent. A significance level was set at $p < 0.05$.

RESULTS AND DISCUSSION

The dry grind process of grains converts starch to glucose, which is then fermented by yeast into ethanol and carbon dioxide, leaving many other components in feedstock relatively unchanged.[2,3,9,10] Besides the components from the original feedstock (protein, oil, carbohydrate, and minerals), CDS contains metabolites of both yeast and bacteria. These metabolites include glycerol, lactic acid, ethanol, acetic acid, and isopropanol, succinic acid, etc.[12,14,15]

Among the components found in CDS, glycerol, a byproduct of yeast fermentation, is considered the major one responsible for the dewatering difficulty of CDS. There are two reasons for this difficulty. First, glycerol is very hygroscopic, so a large amount of energy is needed to drive off moisture. Second, the concentration of glycerol in CDS, when expressed on dry matter basis, was found to be as high as 21.89% in this study (Table 1). The value was consistent with previous reports on CDS[12,14] or thin stillage.[4,15,16] Therefore, a key strategy in the present study was centered on removing or reducing this substance by chemical, physical, or physicochemical processes. In the chemical process, an alcohol solvent was a natural choice since it has been demonstrated to extract glycerol from DDGS.[25] Among alcohols, ethanol is considered most convenient since it is readily available in an ethanol production plant.

The Chemical Method. In the first experiment, CDS was mixed with 95% ethanol and water in proportions so that the final solution could reach three different ethanol concentrations: 55, 65 and 75% v/v. Centrifugation of the mixture at each ethanol level generated two fractions, a precipitate and a

Table 1. Mass and Composition of Condensed Distiller Solubles and Its Fractions Made by the Chemical Method[a]

coproducts	ethanol concentration (%)	wet mass (g)	moisture (%)	dry mass (g)	oil (%)	protein (%) 6.25 × N	glycerol (%)	ash (%)	carbohydrate (%)
condensed distillers solubles		80.00 a	71.17 b	23.06 a	18.23 b	22.87 c	21.89 d	11.83 d	25.17 b
protein-mineral fraction	55	60.50 b	80.19 a	11.99 d	16.53 c	29.65 a	8.05 e	13.54 c	34.24 a
	65	47.66 d	68.02 b	15.25 c	17.00 c	25.99 b	7.19 e	15.20 b	36.62 a
	75	54.32 c	69.83 b	16.39 b	16.13 c	25.94 b	7.31 e	16.25 a	36.37 a
glycerol fraction	55	15.64 e	29.59 e	11.01 e	20.18 a	11.81 e	39.37 c	9.59 e	19.05 c
	65	13.26 f	41.01 d	7.82 f	20.06 a	14.60 d	51.56 b	7.54 f	6.24 d
	75	11.89 f	46.25 c	6.39 g	20.57 a	13.93 d	56.69 a	5.37 g	3.44 e

[a]Compositional data were expressed as % dry matter basis except for the moisture content. In dried form, the glycerol fraction still had a thick paste consistency. Carbohydrate content excluded glycerol content. Column means bearing different letters differed significantly at $p < 0.05$.

Table 2. Mass and Composition of Condensed Distiller Solubles and Its Fractions Made by the Physical Method[a]

coproducts	centrifuge force (× g)	wet mass (g)	moisture (%)	dry mass (g)	oil (%)	protein (%) 6.25 × N	glycerol (%)	ash (%)	carbohydrate (%)
condensed distillers solubles		160.00 a	71.17 c	46.12 a	18.23 d	22.87 c	21.89 b	11.83 c	25.17 d
oil fraction	1000	23.66 g	61.00 d	9.23 e	65.82 c	7.52 e	7.96 e	4.26 f	14.44 f
	3000	17.25 h	53.91 e	7.95 e	69.48 b	3.10 f	2.78 f	2.90 g	21.74 e
	6000	16.85 h	51.93 e	7.73 e	73.17 a	1.14 g	1.14 g	0.87 h	23.68 a
protein fraction	1000	103.98 b	80.52 a	20.26 b	4.08 f	33.49 b	19.73 c	10.82 d	31.89 c
	3000	84.83 c	76.01 b	20.36 b	5.16 f	34.67 ab	16.56 cd	8.73 e	34.87 b
	6000	50.40 d	62.36 d	18.97 b	5.58 f	35.72 a	14.98 de	7.90 e	35.82 b
glycerol-mineral fraction	1000	26.25 f	35.88 g	16.83 d	7.63 e	15.10 d	31.52 a	15.65 b	30.10 c
	3000	30.47 e	41.63 f	17.79 c	7.58 e	14.49 d	34.42 a	17.08 a	26.43 d
	6000	33.80 e	42.97 f	19.28 b	7.84 e	14.02 d	34.75 a	17.99 a	25.39 d

[a]Compositional data were expressed as % dry matter basis except for the moisture content. In dried form, the glycerol fraction still had a thick paste consistency. Carbohydrate content excluded glycerol content. Column means bearing different letters differed significantly at $p < 0.05$.

supernatant (Figure 1). The precipitate was collected and dried to become a protein-mineral fraction. The supernatant was evaporated to recover ethanol first, and further condensed until the density of the mixture reached to about 1.2 g/mL. The condensed mixture was collected as a glycerol fraction. Results show that the two new fractions produced by the chemical method had compositions different from each other and from the original CDS (Table 1). Compared to CDS, the protein-mineral fraction was higher in protein, ash and carbohydrate contents but slightly lower in oil and much lower in glycerol. In contrast, the glycerol fraction was slightly higher in oil and much higher in glycerol than CDS. This fraction also contained noticeable amounts of protein, ash, and carbohydrate.

The mass and composition of these new fractions were also affected by the final concentration of aqueous ethanol used in the extraction process. More specifically, the ethanol concentration had a significant effect on dry mass and contents of protein and ash but no effect on oil content in both fractions. It also had a significant effect on glycerol and carbohydrate contents of the glycerol fraction but no effect on these attributes in the protein-mineral fraction. When the wet masses of the two fractions were summed, the value varied with ethanol concentration, but all were less than the initial wet mass of CDS. This is because during processing, a small amount of water was added to CDS as 95% ethanol while a substantial amount of water in the glycerol fraction was evaporated out later on, and the net effect was loss of some water. However, the sum of two fractions' dry mass was conserved regardless of ethanol concentration (i.e., the values were close to the initial dry mass of CDS).

A water miscible organic solvent, such as ethanol, is known to precipitate a protein and ionic salts (soluble minerals) from an aqueous solution since it affect the dielectric constant of the medium, the intermolecular attraction, and the solute−solvent interaction.[26] In the case of protein precipitation, the solvation layer around the protein will decrease as the organic solvent progressively displaces water from the protein surface and binds it in hydration layers around the organic solvent molecules. With smaller hydration layers, the proteins can aggregate by attractive electrostatic and dipole forces. In the case of mineral precipitation, ionic salts are dissolved in water by forming dipole interactions with ions. Since the dipole interaction between ions and water is weaker than the hydrogen bonds between alcohol and water molecules, when ethanol is added to the solution, the weaker dipole moments are broken, and the stronger hydrogen bonds form, causing precipitation of minerals

Singh and Cheryan[25] subjected DDGS to anhydrous ethanol extraction at 50 °C for 30 min at various ethanol (ml) to DDGS (g) ratios of 2, 4, 6, 8, and 10, and found that protein, fat, and glycerol accounted for more than 90% of total solids extracted. With increasing ethanol/DDGS ratio (ethanol concentration remained anhydrous since DDGS was a dry particulate material), the amount of protein, fat and glycerol extracted from DDGS increased, but their concentrations in the extract decreased. Noureddini et al.[17] extracted oil from whole stillage and CDS with hexane at room temperature, respectively, and converted the extracted oils into biodiesels. In this study, a different extraction system was used, which featured aqueous alcohol extraction of CDS at room temperature with a fixed CDS to solvent ratio but varying ethanol concentration in the extractant. So the outcomes of these studies cannot be easily compared.

The Physical Method. The protein-mineral fraction from the chemical method was higher in protein and lower in glycerol than CDS, but contained a substantial amount of ash (13.54−16.25% dry matter, depending on the ethanol concentration). Its oil content (16.13−17.00%, dm) was also higher than DDGS (typically around 11%).[6,8,10] So, in the second experiment, CDS was simply centrifuged with an objective to produce a fraction with reduced ash and oil contents (Figure 2). For improving flowability and separation, the moisture content of CDS was first adjusted from the original 71.17 to 85% by mixing with a calculated amount of water just before the centrifugation. If the starting material were thin stillage, evaporation would have been stopped when the moisture level of the product reached this level. Unlike centrifugation of the CDS−ethanol mixture, centrifugation of CDS alone produced three layers. The bottom layer (precipitate), which comprised mostly suspended solids, was collected as a protein fraction. The top layer was an oil fraction. The middle layer, the major fraction by volume, was an aqueous layer. It was condensed by evaporation to reduce its moisture levels and collected as a glycerol-mineral fraction.

Thus, by the physical method, CDS was fractionated into three coproducts: the protein fraction, the oil fraction and the glycerol-mineral fraction. Results show that the three new fractions produced by the physical method had compositions different from each other and from the original CDS and that each of these new coproducts had a unique chemical composition on dry matter basis (Table 2). Compared to the

Table 3. Mass and Composition of Condensed Distiller Solubles and Its Fractions Made by the Physicochemical Method[a]

coproducts	centrifuge force (× g)	wet mass (g)	moisture (%)	dry mass (g)	oil (%)	protein (%) 6.25 × N	glycerol (%)	ash (%)	carbohydrate (%)
condensed distillers solubles		160.00 a	71.17 c	46.12 a	18.23 d	22.87 c	21.89 c	11.83 d	25.17 d
oil fraction	1000	23.66 e	61.00 d	9.23 cd	65.82 c	7.52 g	7.96 f	4.26 f	14.44 f
	3000	17.25 f	53.91 e	7.95 d	69.48 b	3.10 h	2.78 g	2.90 g	21.74 e
	6000	16.85 f	51.93 e	7.73 d	73.17 a	1.14 i	1.14 h	0.87 h	23.68 de
protein fraction	1000	103.98 b	80.52 a	20.26 b	4.08 f	33.49 b	19.73 d	10.82 d	31.89 c
	3000	84.83 c	76.01 b	20.36 b	5.16 f	34.67 ab	16.56 de	8.73 e	34.87 b
	6000	50.40 d	62.36 d	18.97 b	5.58 f	35.72 a	14.98 e	7.90 e	35.82 b
mineral fraction	1000	16.48 f	40.42 g	9.82 c	7.14 e	18.01 d	7.02 f	25.22 c	42.61 a
	3000	17.82 f	41.12 g	10.48 c	7.27 e	16.98 de	7.29 f	27.06 b	41.39 a
	6000	18.14 f	38.24 h	11.20 c	7.69 e	15.63 e	7.44 f	30.05 a	39.19 a
glycerol fraction	1000	13.46 g	48.07 f	6.98 d	8.33 e	11.17 f	65.62 b	2.06 g	12.82 f
	3000	15.36 fg	52.03 e	7.36 d	7.99 e	10.80 f	73.22 a	2.72 g	5.27 g
	6000	16.52 f	51.06 e	8.08 d	7.91 e	11.59 f	72.54 a	1.41 h	6.54 g

[a]Compositional data were expressed as % dry matter basis except for the moisture content. In dried form, the glycerol fraction still had a thick paste consistency. Carbohydrate content excluded glycerol content. Column means bearing different letters differed significantly at $p < 0.05$. The mass (wet or dry) of the mineral fraction and the glycerol fraction was doubled to match that of the starting material (CDS) since the supernatant obtained by centrifugation of CDS was condensed and halved before being further processed into the two fractions (see Materials and Methods).

protein-mineral fraction obtained by the chemical method (Table 1), the protein fraction was lower in ash and oil contents but higher in protein content. Compared to CDS, the protein fraction was lower in ash, oil, and glycerol but also higher in protein content. The oil fraction contained much higher levels of oil than CDS. It also contained a substantial amount of carbohydrate and measurable amounts of protein, glycerol, and ash. The glycerol-mineral fraction had higher glycerol and higher ash contents when compared to CDS.

Three centrifuge forces were used: 1000, 3000, and 6000× *g*. Results show that centrifuge force had a significant effect on the oil content of the oil fraction but no effect on that of other two fractions (Table 2). Increasing centrifuge force improved oil content of the oil fraction. For the rest of the constituents, centrifuge force had little effect on the glycerol-mineral fraction but significant effect on other two fractions.

When the wet masses of the three fractions obtained for each centrifuge force were added together, the value decreased with increasing centrifuge force, but all were less than the initial wet mass of CDS. Again, this is because during processing, some water was added for diluting CDS before centrifugation while some water in the glycerol fraction was evaporated out after centrifugation, and the net effect was loss of some water. However, similar to the chemical method, the sums of the three fractions' dry masses were conserved regardless of centrifugation force (i.e., all values were close to the initial dry mass of CDS).

There are reports using similar physical methods, such as centrifugation, to fractionate thin stillage[18] and CDS.[19−21] In particular, the method by Cantrell and Winsness [19] is patented and commercialized (GreenShift.com). It comprises heating CDS and separating the oil from it using a disk stack centrifuge. The oil fraction obtained was subsequently examined for its functional lipid composition.[20] To enhance oil recovery from CDS by the centrifugation method, Majoni et al.[21] treated CDS with proteases and other enzymes before centrifugation. Yet, the main objective of these reports[18−21] was to recover some oil from stillage and use it as a biodiesel feedstock. The rest of the fractions, that is, any nonoil fractions, were combined, mixed with DWG and dried together to produce deoiled DDGS. In contrast, the physical method reported in this study focused on fractionating CDS into different factions. None of the fractions were combined and/or returned to the existing processing system (i.e., to be mixed with DWG for drying together). Instead, each fraction was collected and dried separately except for the aqueous layer which was evaporated to become the glycerol-mineral fraction. Wood et al.[14] also fractionated thin stillage and CDS with centrifugation, but their focus was rather different. After centrifugation, they examined the distribution of protein types (zein or other proteins with different molecular weights) in the precipitates and the starting materials (thin stillage and CDC) by SDS-PAGE gel electrophoresis. They also determined the profiles of sugars and organic acids in the supernatant fraction by an HPLC method.

Furthermore, the present study was the first to examine general chemical composition and glycerol content in each of three fractions (the oil fraction, protein fraction and glycerol-mineral fraction) obtained by the physical method, monitor their drying behaviors, and compare them to that of CDS (Table 2). The same was true for fractions obtained by the chemical method (Table 1) and physicochemical method (Table 3).

The Physicochemical Method. Compared to the glycerol fraction obtained by the chemical method (Figure 1), the glycerol-mineral fraction obtained by the physical method (Figure 2) was lower in glycerol but higher in ash content (Table 2 vs Table 1). In order to reduce its ash content, in the third experiment, the physical and chemical methods were combined into a single one (Figure 3). Basically, the condensed aqueous layer (i.e., the glycerol-mineral fraction) obtained under the physical method (Figure 2) was mixed with a proper amount of 95% ethanol so that the final ethanol solution was 65%. Evaporation of the aqueous fraction obtained in the physical method was necessary in order to reduce the amount of ethanol needed to cause good precipitation. The sample was mixed well and then centrifuged. Similar to the chemical method using CDS as starting material (Figure 1), the ethanol treatment of the glycerol-mineral fraction followed by centrifugation (Figure 3) produced two subfractions: a precipitate and a supernatant. The precipitate was collected and termed as a mineral subfraction while the supernatant had

to be evaporated to remove ethanol first and then some water, and collected as a glycerol subfraction.

Therefore, by the physicochemical method, CDS was fractionated into four coproducts: the protein fraction, the oil fraction, the mineral fraction, and the glycerol fraction. Compared to CDS, the mineral fraction was much higher in ash (2−3 times), lower in protein and carbohydrate, and much lower in oil and glycerol (Table 3). The glycerol fraction by the physicochemical method had higher glycerol but lower oil and ash contents than the glycerol fraction by the chemical method (Table 1). Yet, both contained a substantial amount of glycerol.

Furthermore, compared to the glycerol-mineral fraction obtained by the physical method (Table 2), the mineral fraction obtained by the physicochemical method was higher in ash and carbohydrate but much lower in glycerol, while the glycerol fraction was much higher in glycerol and much lower in ash and carbohydrate (Table 3). The oil content did not change significantly upon the chemical treatment, while the protein content shifted, with increase in the mineral fraction and decrease in the glycerol fraction. These changes upon chemical treatment of the glycerol-mineral fraction were similar to those observed when CDS was used as the starting material (Table 1).

The effect of centrifuge force on the composition of the glycerol-mineral fraction obtained by the physical method was minimal (Table 2). So was the effect of centrifuge force on the composition of its two subfractions (mineral and glycerol fractions) obtained by the physicochemical method (Table 3). Since in carrying out the physical or physicochemical method CDS had to be diluted to about 85% moisture by adding water, thin stillage would have been a better starting material when it is evaporated to this moisture level.

Majoni et al.[22] slightly increased oil recovery from CDS by first centrifuging it to collect the oil fraction and then extracting the oil from the precipitate (which would be similar to the protein fraction in this study) with isopropanol, butanol, or a mixture of hexane and ethanol, but they did not attempt to extract oil or other components from the aqueous layer with organic solvents. In contrast, in the present study, the aqueous layer was first evaporated to become glycerol mineral fraction and then extracted with ethanol while the precipitate fraction (the protein fraction) was collected as it is (no further solvent extraction). Based on data in Table 2, by the physical method (Figure 2) the majority of the oil in CDS went into the oil fraction, while the rest of the oil from CDS was distributed more in the glycerol-mineral fraction than the protein fraction. Thus extraction of the protein fraction for oil with organic solvents could boost total oil recovery only to a limited extent. Again, the key objective of this study was to remove glycerol as much as possible so that resulting fractions could be dried directly without the need to be mixed with DWG. Therefore, the present study differed from Majoni et al.[22] not only in the second part of the procedures but also in objectives.

Drying Behaviors of CDS Fractions as Compared to CDS. When the protein-mineral fraction and the glycerol fraction produced by the chemical method were dried in a forced air oven at 60 °C, along with the control sample (original CDS), moisture reduction over time varied greatly with products (Figure 4A). For CDS and the protein-mineral fraction, a sharp decrease in moisture content occurred within the first few hr but the protein-mineral fraction dried much faster than CDS. For example, after 8 h drying, the moisture content of the protein-mineral fraction was reduced to about

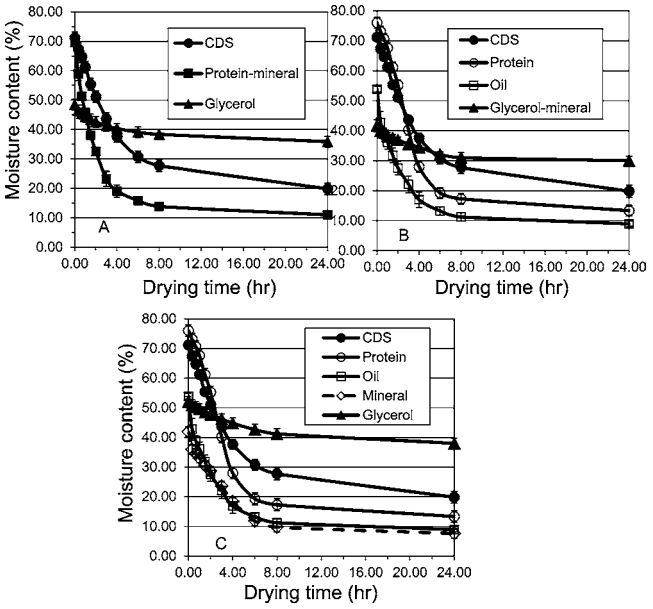

Figure 4. Comparison of moisture reduction rates of fractions obtained by the (A) chemical, (B) physical, and (C) physicochemical methods in fractionating condensed distillers solubles (control sample) during drying in a forced air oven at 60 °C. Error bars represent standard errors.

14% while that of CDS was at about 28%. After 24 h drying, the moisture of the protein-mineral fraction was further reduced to about 11% while CDS still had a moisture level of about 20%. As expected, the glycerol fraction was most difficult to dry. The differences in moisture reduction upon drying among the three coproducts can be attributed to their differences in glycerol content (Table 1). The higher the glycerol content, the more difficult it was to dry the product.

When the three fractions produced by the physical method from CDS were dried, along with CDS, the order in moisture reduction with time was oil fraction > protein fraction > CDS > glycerol-mineral fraction (Figure 4B). After 8 h drying, the oil fraction reached 11% moisture, the protein fraction had a moisture level of 17%, while CDS still had approximately 28% moisture. After 24 h drying at 60 °C the moisture of the oil fraction was further reduced to about 9% and that of the protein fraction to about 13%, but CDS had 20% moisture. The differences in moisture reduction upon drying among the four coproducts can again be attributed to their differences in glycerol content (Table 2). Higher glycerol content of a product hinders its drying process.

A total of four fractions were generated from CDS with the physicochemical method (Figure 3), including the oil, protein, mineral, and glycerol fractions. When they were dried along with CDS, the order in moisture reduction with time was: mineral fraction = oil fraction > protein fraction > CDS > glycerol fraction (Figure 4C). The mineral fraction had similar moisture reduction rate as the oil fraction. Since the oil and protein fractions in Figure 4C were same as those shown in Figure 4B, they showed the same moisture reduction rates in the two subfigures. However, the glycerol fraction in Figure 4C showed less moisture reduction upon heating as compared to the glycerol-mineral fraction in Figure 4B. This was again determined by their difference in glycerol content (Table 3).

In conclusion, three processes for coproduct recovery during the dry grind process of grains into fuel ethanol are described in

this report. These processes are based on chemical, physical, or physicochemical principles. Using CDS as a starting material, the chemical method produced a protein-mineral fraction and a glycerol fraction. The physical method produced a protein fraction, an oil fraction and a glycerol-mineral fraction. The physicochemical method generated a protein fraction, an oil fraction, a mineral fraction, and a glycerol fraction. Results of chemical analysis showed that these methods basically shifted protein, oil, ash, glycerol, and other carbohydrates—the five major components in CDS—into different fractions. Each fraction was enriched with one or more of these five components; thus each coproduct would have higher value with different targeted application. Furthermore, the three methods were able to remove substantial amounts of glycerol from CDS and collect it in the glycerol or glycerol-mineral fraction. As a result, all the other fractions from CDS, obtained by any of the three methods, could be readily dried. There was no need to mix any of them with DWG to facilitate drying. The blending step in the conventional dry grind processing could therefore be eliminated, while DWG could be dried alone to become distiller dried grains, another standalone coproduct.

All of the above developmental features could potentially boost profitability of the fuel ethanol industry. Since some of the new fractions, such as the protein fraction and the mineral fraction, obtained by the described methods had nutritional compositions favorable for aquaculture feed, either as a protein ingredient or a mineral supplement, fish feeding trials are underway to investigate their effects on fish growth and performance.

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Notes

The authors declare no competing financial interest.

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