Quantification of Physical and Chemical Properties, and Identification of Potentially Valuable Components from Fuel Ethanol Process Streams

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ABSTRACT

Wider exploration of ethanol coproduct uses is necessary as the ethanol industry continues to face challenges. Currently, process streams such as thin stillage and condensed distillers solubles (CDS) are processed into distillers dried grains with solubles and used as animal feeds, but other higher value opportunities may exist. The objective of this study was to identify chemical components and quantify physical properties of CDS and thin stillage. Protein, organic acid, and sugar profiles were determined. Zein protein was identified, and glycerol was determined to have a concentration of 18.8 g/L in thin stillage and 63.2 g/L in CDS. Physical properties including density, thermal conductivity, thermal diffusivity, and rheological behaviors were also examined. Thermal conductivity of thin stillage and CDS was approximately 0.54 and 0.45 W/m°C, respectively. Quantification of the physical properties and identification of the chemical constituents pave the way for exploration of new value-added uses for thin stillage and CDS.

U.S. ethanol demand from 2005 to 2008 grew by 5.6 billion gallons (21.2 billion liters); meanwhile, from 2005 to 2010 the production grew by 9.3 billion gallons (35.2 billion liters) and the number of operating ethanol plants increased from 81 to 189 (RFA 2011b). To produce a record 13.2 billion gallons (50.0 billion liters) in 2010, 4.8 billion bushels of corn was utilized (RFA 2011b). If, for every 56 lb (25.4 kg) of corn consumed, 17 lb (7.71 kg) of distillers dried grains with solubles (DDGS) was produced (Jacques et al 2003), then nearly 37.0 million metric tons of DDGS was produced in 2010, and that figure grew even more in 2011. Because DDGS is such a large-volume product from the ethanol industry, its marketability and that of other coproducts serve as important assets to the economic viability of ethanol plants.

DDGS is often composed of approximately 86.2–93.0% dry matter, 25–35% protein, 3–13% fat, and some unfermented starches, sugars, and other materials (Weigel et al 1997; Rosentrater and Muthukumarappan 2006; Ganesan et al 2008; Shurson and Alhamdi 2008; Bhadra et al 2009b). Because of the moderate-to-high nutrient values found in the DDGS and its high digestibility, feeding it to livestock animals is a proven method for DDGS utilization (Rosentrater and Muthukumarappan 2006). Currently, the beef, dairy, swine, and poultry feed industries are the largest consumers of DDGS (Shurson and Noll 2005; RFA 2011a). Studies have also explored the use of DDGS in aquaculture feeds (Rosentrater et al 2009a, 2009b; Schaeffer et al 2009; Kannadhason et al 2010). A very small percentage of the DDGS market includes deicers, cat litter, "lick barrels," and worm food (Bothast and Schlicher 2005). In addition to being used as a livestock feed source, new value-added industrial products derived from DDGS are being explored (Rosentrater 2007). DDGS may even find a place in the human food market eventually, as research is improving its viability as a human food ingredient (Rosentrater and Krishnan 2006; Rosentrater 2007). Studies are also being conducted to determine the efficacy of producing plastics from DDGS (Bothast and Schlicher 2005; Tatara et al 2006, 2007).

Although there are numerous possibilities for the utilization of DDGS, its physical and flowability characteristics can prove to be problematic during storage and transport (Rosentrater and Giglio 2005; Schlicher 2005; Rosentrater 2006). Poor flowability of the DDGS can cause it to harden during transport (known as caking and bridging). When this happens within the shipping container, funnel flow can occur, which makes unloading troublesome because the flow can be unpredictable and uncontrollable. The flowability behavior of DDGS can be influenced by any number of interrelated factors, including moisture content, fat content, particle size distribution, storage temperature, relative humidity, storage time, compaction pressure distribution, and vibrations during transport (Craik and Miller 1958; Johansson 1978; Moreyra and Peleg 1981; Teunou et al 1991; Fitzpatrick et al 2004). Various studies have been performed to determine the cause and find solutions to the flowability problem (Bhadra et al 2009a, 2009b, 2010; Keierleber and Rosentrater 2010). A key factor appears to be the level of condensed distillers solubles (CDS) that is used during DDGS production. One potential solution is to extract lipids from the DDGS, the thin stillage, or the CDS (Ganesan et al 2009).

Much attention has been given to DDGS, but little has been given to the upstream components that make up DDGS. DDGS is the product of combining distillers wet grains (DWG) with a syrup containing approximately 55% (by weight) total solids (which is known as CDS) and then drying the mixture to produce DDGS (McAloon et al 2000). DWG is the unfermented solids remaining after the whole stillage is centrifuged and the liquid fraction (thin stillage) is removed (Kim et al 2008). Although part of this thin stillage is recycled through the ethanol production system as backset, the majority is concentrated by evaporation to produce CDS (Ganesan et al 2006). Little has been done to comprehensively determine chemical composition of DWG, CDS, or thin stillage. A few studies have determined that DWG is often composed of 30.9–44.1% dry matter, 25–39.5% protein, 8.5–14.5% fat, and 1.2–2.4% ash (Rosentrater and Muthukumarappan 2006; Kim et al 2008; Rosentrater and Lehman 2010); these studies also indicated that thin stillage can be composed of 5.0–7.7% dry matter, 1.3–16.8% crude protein, 1.3–8.1% crude fat, and 0.8–5.9% ash.

In addition to being used for producing DDGS, CDS is becoming a valuable source of corn oil. According to Majoni et al (2010), oil may be present in CDS in four different forms: 1) oil-in-water emulsion; 2) minute oil droplets attached to hydrophobic

*The *e*-Xtra logo stands for "electronic extra" and indicates that Figures 1–3, 5, and 6 appear in color online.

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proteins; 3) oil bodies in endosperm and germ particles; and 4) oil bodies from broken cellular structures. Various techniques involving centrifugation have been used to remove oil from CDS (Noureddini et al 2009; Majoni et al 2010; Moreau et al 2010). However, not all of this oil can be separated through centrifugation (Majoni and Wang 2010). The oil that can be extracted from CDS can be used to produce biodiesel (Majoni and Wang 2010) but is generally of sufficient quality for food uses. What remains of the CDS after the oil has been partially removed is then added to the DWG and dried to produce DDGS with slightly lower levels of oil, which is utilized similarly to traditional DDGS.

Not only can CDS and thin stillage be used to produce DDGS but they could also potentially be used individually, or specific components therein could be removed and used to produce higher value commodities. However, to fully understand potential value-added uses, in-depth analyses must first be conducted on these materials. Physical and chemical property data are necessary to design material handling, storage, and processing operations, including fractionation systems, bioconversion systems, and other potential value-added processes. Thus, the objectives of this study were to 1) determine various physical and chemical properties of CDS and thin stillage, and 2) explore and discuss potential opportunities for extracting value from these materials.

MATERIALS AND METHODS

Sample Collection and Experimental Design

Three samples (referred to as 1, 2, and 3) each of CDS and thin stillage were obtained from a commercial dry-grind ethanol plant in South Dakota. Each sample was collected at three different points in time, which were approximately one week apart. The samples were stored in sealed plastic containers with screw-top lids in a refrigerator at 2.5°C until needed for testing.

For each property measured, three replications (n = 3) for each test were performed on each sample for each of the coproducts, for a total of nine experimental units for each of the coproducts for each property. Physical properties included moisture content, water activity, mass density, thermal diffusivity, thermal conductivity, and apparent viscosity. Chemical properties included protein assays, sugar and organic acid profiles, and proximate analysis.

Physical Properties

With the exception of moisture content and water activity, all physical properties were determined at three different temperatures (10, 25, and 40°C). Three different temperatures were used to understand how these products behave as their temperatures are adjusted during storage or processing: 25°C was chosen for a midpoint, because it is often considered average room temperature; 10°C was chosen to predict behavior when products are cooled during storage or during winter conditions; 40°C was chosen to predict behavior as products are heated during processing or during storage at late summer conditions.

Moisture Content and Water Activity. Moisture content was determined following AACC International Approved Method 44-19.01 with a forced-convection laboratory oven (Thelco Precision, Jovan, Winchester, VA, U.S.A.). Water activity was determined by placing a small quantity of each sample into a calibrated water activity meter (Aqua Lab 4TEV, Decagon Devices, Pullman, WA, U.S.A.) and tested at room temperature (22 ± 1°C).

Mass Density. A specific gravity cup (H-38000-12, Cole-Parmer Instrument, Barrington, IL, U.S.A.) was used to determine the mass density. Material was poured into the cup, which was of a known mass and volume; the lid was placed on the container; excess material, which exited through a 1 mm opening in the lid, was then removed; and the filled cup was weighed on a balance. Density was then calculated as the ratio of mass to unit volume.

Thermal Properties. Thermal properties (diffusivity and conductivity) were determined with a thermal meter (KD2, Decagon Devices) that utilized the line heat source probe technique (Baghe-Khandan et al 1981).

Rheological Characteristics. Apparent viscosity was measured with a rheometer (HAAKE Rheostress 1, Thermo Electron, New Castle, DE, U.S.A.) with a stainless steel cone and plate attachment (LO4079 C60/1 Ti). To maintain constant temperature during testing, water at a specific set temperature was pumped through the base plate from a water bath. The shear rate for each sample was initiated at 10 (sec^{-1}), and was increased to 100 (sec^{-1}) by increments of approximately 1 (sec^{-1}). The data points gathered for the three different temperatures were then pooled together to develop an overall regression equation to describe the general behavior of thin stillage and CDS as shear rate and temperature changed simultaneously.

Chemical Properties

Centrifugation (3,000 rpm [1,500 × g] for 30 min) was used (CL-2, Thermo Scientific, Milford, MA, U.S.A.) to separate the liquid fraction from the precipitate in both CDS and thin stillage to examine the distribution of chemical components in the coproducts.

Protein. Protein assays for CDS and thin stillage as well as the precipitates of each were determined through SDS-PAGE. Samples (of each material) were prepared by placing them into solution with a dilution of 16 µg of sample/500 µL of water. This dilution was chosen through preliminary experimentation, as it was the least dilute solution that would allow the proteins to appear as separate bands within the gels. The dilutions were then placed into a 1:1 solution with Laemmli and β-mercaptoethanol (BME) sample buffer solutions and heated at 95°C for 5 min to dye the proteins. Once the samples were prepared, they were loaded (15 µL of sample per column) into the polyacrylamide gels (Ready Gel Precast Gels, Bio-Rad Laboratories, Hercules, CA, U.S.A.).

For comparison purposes, in addition to the coproduct samples, each gel was loaded with a ground corn sample, a zein sample, and two standards. The ground corn sample was diluted in the same fashion as coproduct samples. The zein sample was a zein solution extracted with ethanol (Bioconversions Engineering & Consulting, Madison, WI, U.S.A.); 200 µL of this solution was air-dried under ambient conditions and then dissolved in 40 µL of the BME sample buffer solution. The standard was a Kaleidoscope Precision Plus protein standard (Bio-Rad Laboratories).

A reactor (Mini-PROTEAN Tetra Cell, Bio-Rad Laboratories) containing a running buffer consisting of 1.84% Tris-glyceride solution was used to process the gels. Coomassie brilliant blue staining solution (R-250 solution, Bio-Rad Laboratories) was used to dye the bands, and Coomassie destain solution (R-250 solution, Bio-Rad Laboratories) was used for destaining.

Images of the gel scans were then uploaded into analysis software (Quantity One, 4.6.3, Bio-Rad Laboratories) to identify the molecular weights and the intensity of the individual bands. Volume analysis by densitometry was used to quantify the protein bands. The volumes from the three gels were then averaged together to get an overall protein distribution for that sample.

Sugars and Organic Acids. Analysis of the liquid portion of the CDS and thin stillage samples was performed on an HPLC system (Agilent Technologies, Santa Clara, CA, U.S.A.) with an Aminex HPX-87H column (Bio-Rad Laboratories) with a mobile phase of 0.005M H_2SO_4 at a flow rate of 0.6 mL/min at 65°C and a sample volume of 20 µL. A refractive index detector at 35°C was used to identify the compounds.

The liquid portion was removed from each coproduct sample with centrifugation of 3,000 rpm (1,500 × g) for 30 min. Once the liquid portions were separated from the whole sample, they were filtered through a 2 µm filter into an HPLC vial. Three known concentrations of the standards, cellobiose, glucose, xylose, galactose, mannose, arabinose, succinic acid, lactic acid, glycerol, acetic acid, and ethanol (Fisher Scientific, Pittsburg, PA, U.S.A.)

were also run with the samples to develop standard curves for quantification. These standards were prepared by diluting them to the recommended dilution found in NREL LAP/TP-510-42623 (Sluiter et al 2006).

Proximate Analysis. Proximate analysis of thin stillage and CDS included the determination of dry matter, crude protein, neutral detergent fiber, ash, and fat. These analyses were conducted by Servi-Tech Laboratories, Hastings, NE, U.S.A.

Data Analysis

Data analysis was completed for each property with Excel 2010 (Microsoft, Redmond, WA, U.S.A.) software to determine mean

TABLE I
Moisture Content (MC) and Water Activity of Ethanol Coproducts[z]

		Individual Sample Means			Overall Means		
Coproduct	**Sample**	**MC (% wb)**	**MC (% db)**	**Water Activity (–)**	**MC (% wb)**	**MC (% db)**	**Water Activity (–)**
CDS	1	74.9a	3.0a	0.95a	73.6B	2.8B	0.96B
		(0.1)	(0.1)	(0.01)	(1.7)	(0.2)	(0.01)
	2	74.6b	2.9b	0.96b			
		(0.1)	(0.1)	(0.01)			
	3	71.4c	2.5c	0.96a			
		(0.1)	(0.1)	(0.01)			
Thin stillage	1	93.4a	14.1a	0.99a	93.3A	13.9A	0.99A
		(0.2)	(0.4)	(0.01)	(0.2)	(0.4)	(0.01)
	2	93.1a	13.5a	0.99b			
		(0.1)	(0.1)	(0.01)			
	3	93.3a	14.0a	0.98c			
		(0.1)	(0.2)	(0.01)			

[z] Values in parentheses are standard deviations. CDS = condensed distillers solubles. Values for a given dependent variable (in a given column), for a given coproduct, followed by the same lowercase letter (a, b, c) are not significantly different ($\alpha = 0.05$, least significant difference [LSD]) between sampling periods. Values for a given dependent variable (in a given column) followed by the same capital letter (A, B, C) are not significantly different ($\alpha = 0.05$, LSD) between coproducts.

TABLE II
Physical Properties of Ethanol Coproducts at Various Temperatures[z]

			Individual Sample Means			Overall Means		
Coproduct	**Sample**	**Temperature**	**Mass Density (g/cm^3)**	**Thermal Diffusivity (mm^2/sec)**	**Thermal Conductivity (W/m°C)**	**Mass Density (g/cm^3)**	**Thermal Diffusivity (mm^2/sec)**	**Thermal Conductivity (W/m°C)**
CDS	1	10	1.09a	0.10a	0.47a	1.09A	0.10A	0.45A
			(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.03)
	2	10	1.08b	0.10a	0.43a			
			(0.01)	(0.01)	(0.03)			
	3	10	1.10c	0.10a	0.45a			
			(0.01)	(0.01)	(0.03)			
	1	25	1.09a	0.10a	0.49a	1.08A	0.11AB	0.49B
			(0.01)	(0.01)	(0.01)	(0.00)	(0.01)	(0.01)
	2	25	1.08b	0.10a	0.48a			
			(0.01)	(0.01)	(0.01)			
	3	25	1.08c	0.11a	0.50a			
			(0.01)	(0.01)	(0.01)			
	1	40	1.08a	0.11a	0.52a	1.08A	0.11B	0.54C
			(0.01)	(0.01)	(0.03)	(0.01)	(0.01)	(0.03)
	2	40	1.07b	0.11a	0.55a			
			(0.01)	(0.01)	(0.01)			
	3	40	1.09c	0.11a	0.54a			
			(0.01)	(0.01)	(0.05)			
Thin stillage	1	10	1.02a	0.11a	0.55a	1.02A	0.11A	0.54A
			(0.02)	(0.01)	(0.04)	(0.01)	(0.01)	(0.03)
	2	10	1.02a	0.11a	0.52a			
			(0.01)	(0.00)	(0.03)			
	3	10	1.03a	0.11a	0.55a			
			(0.01)	(0.01)	(0.01)			
	1	25	1.03a	0.11a	0.55a	1.03A	0.11A	0.55A
			(0.01)	(0.01)	(0.01)	(0.01)	(0.01)	(0.01)
	2	25	1.03a	0.11a	0.55a			
			(0.01)	(0.01)	(0.01)			
	3	25	1.03a	0.11a	0.55a			
			(0.01)	(0.01)	(0.01)			
	1	40	1.03a	0.14a	0.73a	1.03A	0.12B	0.66B
			(0.01)	(0.01)	(0.01)	(0.01)	(0.02)	(0.07)
	2	40	1.03a	0.11b	0.60b			
			(0.01)	(0.01)	(0.06)			
	3	40	1.03a	0.12b	0.64b			
			(0.01)	(0.01)	(0.02)			

[z] Values in parentheses are standard deviations. CDS = condensed distillers solubles. Values for a given dependent variable (in a given column), for a given coproduct, followed by the same lowercase letter (a, b, c) are not significantly different ($\alpha = 0.05$, least significant difference [LSD]) between sampling periods. Values for a given dependent variable (in a given column) followed by the same capital letter (A, B, C) are not significantly different ($\alpha = 0.05$, LSD) between temperatures.

values and standard distributions and then with two-way analysis of variance by general linear models using SAS version 8 (SAS Institute, Cary, NC, U.S.A.), using a type I error rate (α) of 0.05 to determine the main and interaction effects, and least significant differences between sample means. Rheological data were modeled with the PROC NLIN numbering regression procedure. Gel analysis was done with 1-D analysis software (Quantity One, 4.6.3).

RESULTS AND DISCUSSION

Physical Properties

Moisture Content and Water Activity. Moisture contents (both wet and dry basis) of both CDS and thin stillage can be found in Table I. The CDS was found to have a moisture content of nearly 74% (wb), about 20% less than that of thin stillage. Because thin stillage is the liquid fraction of whole stillage, it was expected to have a very high moisture content. In this study the moisture content of the three samplings of thin stillage was found to be around 93% (wb). The high moisture content of both thin stillage and CDS makes them susceptible to spoilage and microbial growth. To keep the products from spoiling and to reduce the cost associated with transportation, the moisture content would need to be reduced to approximately 12% wb, which is the recommended moisture content for feed products, as it allows them to be microbiologically safe and reduces the cost of transport (Beauchat 1981; Wang et al 1997). This reduction in moisture content is achieved by combining the products into DWG and then drying them to form DDGS.

Table I also contains the mean values of water activity for both CDS and thin stillage. Water activity is a measure of how much free (or unbound) water is available in the product; it is this water that is available for use by microorganisms and chemical reactions that can lead to deterioration. Water activity values are measured in comparison to the water activity of distilled water, which is defined as 1.0. In this study, it was found that the mean water activity of CDS was 0.96, whereas that of thin stillage was 0.99. Not surprisingly, these high water activity values indicate that both products are at risk for quick spoilage by yeast, bacteria, and mold. According to Barbosa-Canovas and Vega-Mercado (1996), materials become safe from yeast at water activities below approximately 0.9, safe from bacterial growth below about 0.8, and safe from mold growth below 0.6. Therefore, to extend the life of both CDS and thin stillage, they must be processed to reduce their water activity and moisture contents. Of course, combining with DWG and drying to produce DDGS is the current practice. Another possibility may include blending with other feed ingredients and then extruding them into new feed products.

Mass Density. Mass density is defined as the mass per unit volume of a material; it is generally a function of temperature. For a given unit of mass, an increase in temperature generally causes an increase in volume and thus a decrease in density. The mean values for mass density are given in Table II. The mass density of the thin stillage was determined to be approximately 1.02, 1.03, and 1.03 g/cm^3 at 10, 25, and 40°C, respectively. Temperature did not have a significant effect on the density, at least for the temperature range in this study. Overall, thin stillage had a mean mass density of 1.03 g/cm^3, which was only slightly higher than that of water, 1.0 g/cm^3. This result was expected because thin stillage has such a high water content.

The mass density of CDS was slightly higher, with an overall mean of 1.08 g/cm^3. Temperature did not appear to have a significant effect on the mass density of CDS either, as the mass density of CDS was found to be approximately 1.09, 1.08, and 1.08 g/cm^3 at 10, 25, and 40°C, respectively. Based on these results, it can be concluded that the volume of both thin stillage and CDS was fairly constant within the range of temperatures examined in this study.

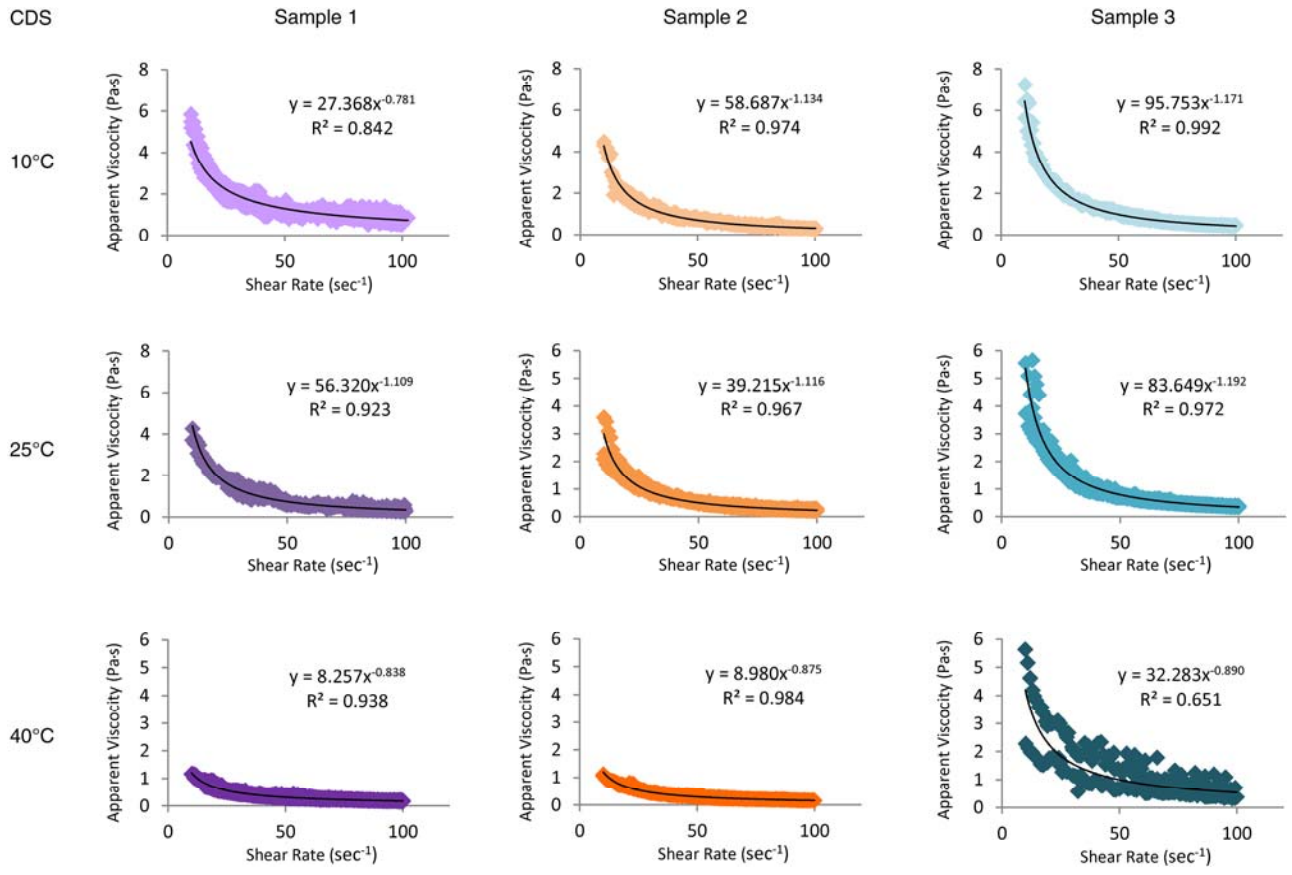

Fig. 1. Relationship between apparent viscosity and shear rate as a function of temperature for condensed distillers solubles (CDS) samples.

Thermal Properties. Thermal diffusivity measures a substance's ability to conduct heat relative to its ability to store heat (Stroshine 2001). The thermal conductivity of a substance governs the ability of that substance to transfer heat via conduction and serves as a way of predicting the rate of energy loss by the material (Stroshine 2001). Understanding how a material's thermal properties behave is vital in the design and utilization of unit operations requiring heating or cooling of products, especially when they contain temperature-sensitive components. Water content significantly influences thermal properties such as diffusivity and conductivity, but it does not completely govern the behavior of the material. The thermal diffusivity and thermal conductivity values are found in Table II.

The thermal diffusivity of CDS at 10°C was determined to be about 0.10 mm^2/sec, whereas it was approximately 0.11 mm^2/sec at both 25 and 40°C. The average thermal diffusivity for thin stillage at both 10 and 25°C was determined to be 0.11 mm^2/sec, whereas that at 40°C was determined to be about 0.12 mm^2/sec. These values are lower than that of water, 0.15 mm^2/sec, which means that CDS and thin stillage conduct heat more slowly than water does and would adsorb heat from surroundings at a slower rate. Thus, within a heating operation, the processing parameters used for water would not be adequate for these products (i.e., residence time would need to be increased to heat either the thin stillage or CDS to the same temperature as water).

As shown, the thermal conductivity of CDS was determined to be about 0.45, 0.49, and 0.54 W/m°C at 10, 25, and 40°C, respectively. Thermal conductivity of thin stillage was determined to be about 0.54, 0.55, and 0.66 W/m°C at 10, 25, and 40°C, respectively. The thermal conductivity of water at 0°C is 0.56 W/m°C, and it increases to 0.60 W/m°C at 20°C. Comparing these values with the experimental data collected for CDS and thin stillage, it can be concluded that both CDS and thin stillage had a lower thermal conductivity than water, as a result of the dissolved solubles, but similarly to water, their thermal conductivities increased as the temperature increased. Because the thermal conductivity of thin stillage and CDS were lower than that of water, longer residence times would be required when cooling CDS and thin stillage compared with pure water.

Rheological Characteristics. Rheology viscosity is defined as a fluid's resistance to flow (Stroshine 2001). For many fluids (i.e., non-Newtonian ones) it is expected that as either the shear rate or temperature increase, the apparent viscosity would decrease. This behavior was observed in both the CDS and thin stillage. Figures 1 (CDS) and 2 (thin stillage) depict this behavior for apparent viscosity (Pa·sec) as a function of shear rate for each temperature. As expected, the relationship followed a decreasing nonlinear power function that could best be represented by

$$\eta = K\dot{\gamma}^{n}$$

where η = apparent viscosity (Pa·sec); K = empirical regression constant (Pa·sec·sec^n); $\dot{\gamma}$ = shear rate (sec^{-1}); and n = empirical exponential constant (–). Based on this relationship, it can be concluded that both CDS and thin stillage behaved as pseudoplastic fluids or shear-thinning fluids (viscosity decreased as shear rate increased).

Understanding a fluid's viscous properties is key to designing process parameters for the material. A fluid's viscosity greatly affects the fluid's ability to move through processing pumps and piping, as it governs the pressure drop that can occur. Viscosity also interacts with a fluid's heat-transfer coefficients, which are required for designing heat exchangers and other heat-transfer processes. For example, as with other shear-thinning materials, the use of pumps and mixers in the initial processing steps can reduce the energy costs associated with processing, as lower viscosities reduce the amount of work or stress required to move or mix the materials in later steps.

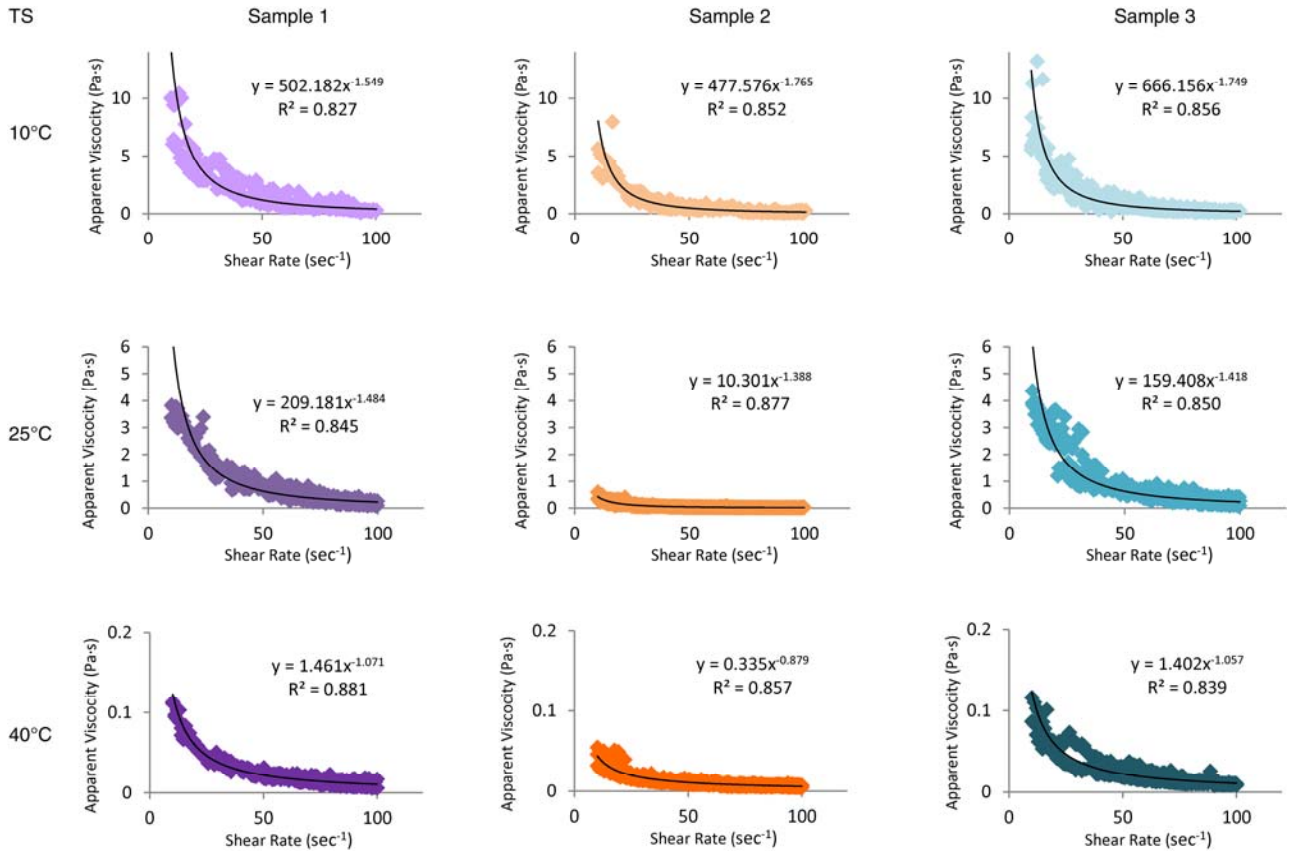

Fig. 2. Relationship between apparent viscosity and shear rate as a function of temperature for thin stillage (TS) samples.

As a material is processed, it changes temperature (e.g., frictional heating or applied temperature gradients); temperature changes can greatly affect the viscosity of the material, in turn affecting the energy required to mix it or move it through piping. For this reason, the viscosity of thin stillage and CDS was determined at three different temperatures. In Figures 1 and 2, the three replications for each sample were combined into a single regression line for each temperature. Small variations existed between the samples' initial apparent viscosities; however, all apparent viscosities decreased as both the shear rate and temperature were increased. It can also be observed that the magnitude of the apparent viscosity decreased (i.e., became less viscous) as the temperature of the sample increased, which is typical fluid behavior.

Because viscosity is affected by both the shear rate and the temperature, predictions of thin stillage and CDS viscous behavior must be conducted using both. Therefore, the three temperatures at all shear rates must be pooled to form one comprehensive prediction equation (Table III). Figure 3 pools all of the CDS data and the thin stillage data, and it provides a single regression relationship that simultaneously accounts for shear rate and temperature for each coproduct. Based on the overall regression equations, it was determined that thin stillage exhibited viscosity changes at a greater rate when the temperature was increased. This behavior was expected, because of the higher water content in the thin stillage.

Chemical Properties

Protein. DDGS is a valuable animal feed because of its moderately high protein content. This protein could potentially be extracted from either the DDGS or its upstream components and could potentially serve purposes other than animal feed.

Before extraction techniques can be explored, the type of proteins present must first be identified. SDS-PAGE was used to separate the proteins within the thin stillage and CDS to verify the presence of zein and to determine what other proteins were present in these streams. Figure 4 illustrates examples of gels after electrophoresis, staining, and destaining. The molecular weight distribution for the zein and corn flour can be seen in Figure 5. Based on these distributions, zein appeared in two different locations, a primary band at 15,000–20,000 and a secondary band at 20,000–25,000. The band at 20,000–25,000 can further be identified as α-zein, based on the observations by Cookman and Glatz (2009) that α-zein falls between 20,000 and 24,000, whereas β-zein falls around 17,000–18,000. Thus, the zein band at 15,000–20,000 was most likely β-zein. Because γ-zein appears at a higher molecular weight, approximately 25,000–30,000 (Wolf and Lawton 1997), it was not present in this standard.

The molecular weight distribution of corn flour, on the other hand, showed that 70% of the corn protein fell in the ranges 10,000–15,000, 15,000–20,000, and 50,000–75,000. The bands existing in the 20,000–37,000 range made up another 10% of the protein, and the remaining 20% was distributed among 0–10,000, 37,000–50,000, 75,000–100,000, and 100,000–150,000. The protein in the 15,000–20,000 range (28%) was most likely β-zein,

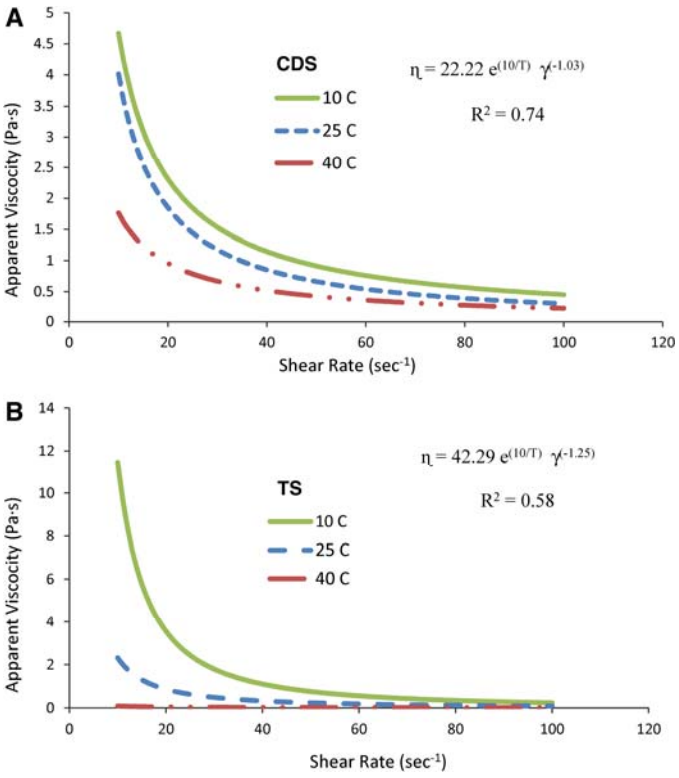

Fig. 3. Overall relationships between apparent viscosity and shear rate as a function of temperature, using all sample data simultaneously. **A,** condensed distillers solubles (CDS); **B,** thin stillage (TS). T = temperature (°C); γ = shear rate (sec^{-1}).

TABLE III
Best-Fit Parameter Estimates for Relationships Between Apparent Viscosity and Shear Rate as a Function of Temperature[z]

		Overall Means								
		10°C			25°C			40°C		
Coproduct	Sample	K (Pa·sec·sec^n)	n (–)	R^2	K (Pa·sec·sec^n)	n (–)	R^2	K (Pa·sec·sec^n)	n (–)	R^2
CDS	1	27.37aA	–0.78aA	0.84	56.60aA	–1.11aA	0.92	8.26aB	–0.84aA	0.94
		(25.55)	(0.19)	(0.10)	(60.16)	(0.26)	(0.02)	(1.24)	(0.02)	(0.01)
	2	58.10ab	–1.31ab	0.97	39.22a	–1.12a	0.97	8.98a	–0.88a	0.98
		(11.80)	(0.05)	(0.01)	(34.66)	(0.19)	(0.01)	(1.08)	(0.02)	(0.01)
	3	95.75b	–1.17ab	0.84	83.65a	–1.19a	0.97	31.82a	–0.89a	0.65
		(17.54)	(1.34)	(0.01)	(44.87)	(0.11)	(0.01)	(18.69)	(0.02)	(0.01)
Thin stillage	1	502.18aA	–1.55aA	0.82	209.18aB	–1.48aB	0.84	1.46aB	–1.07aC	0.88
		(282.84)	(0.05)	(0.08)	(276.83)	(0.39)	(0.02)	(1.39)	(0.29)	(0.00)
	2	477.58a	–1.77a	0.85	10.30a	–1.39a	0.88	0.34a	–0.88a	0.96
		(93.30)	(0.11)	(0.05)	(6.56)	(0.08)	(0.02)	(0.26)	(0.23)	(0.02)
	3	666.16a	–1.75a	0.86	159.41a	–1.42a	0.85	1.40a	–1.06a	0.84
		(478.87)	(0.20)	(0.07)	(222.73)	(0.38)	(0.03)	(0.65)	(0.04)	(0.02)

[z] η (Pa·sec) = [K (Pa·sec·sec^n)][γ (1/sec)]^n. See Figure 3. CDS = condensed distillers solubles. Values in parentheses are standard deviations. Values for a given dependent variable (in a given column) for a given coproduct, at a given temperature, followed by the same lowercase letter (a, b, c) are not significantly different (α = 0.05, least significant difference [LSD]) between sampling periods. Values for a given dependent variable (in a given column) followed by the same capital letter (A, B, C) are not significantly different (α = 0.05, LSD) between temperatures. Each regression consisted of three replications, each containing 100 data points.

whereas that in the 20,000–37,000 range (10%) was most likely α-zein, based upon the zein standard distribution.

Figure 6 shows the protein distributions for the whole thin stillage and CDS samples as well as for the precipitates. For the thin stillage, more protein bands were visible in the precipitate than in the whole sample, even though both had the same dilutions. From this, it can be surmised that the majority of the proteins found in the whole samples were more water insoluble, as the dilution of the whole sample did not allow for more than three bands to present themselves. The α-zein only presented itself in the precipitate sample and represented approximately 6–17% of the protein present. The β-zein presented itself in only one of the thin stillage whole samples, representing 50% of the protein in that sample. It was, however, present in all three samples of the precipitate, representing 10–20% of the total protein. Similarly, both the whole sample and precipitate sample showed protein at 0–10,000, a band in common with that of the corn flour. This band represented about 4% of the corn flour, 1–9% of the thin stillage precipitate, and approximately 22–23% of the protein in the whole thin stillage sample. These samples also had a similar band at 10,000–15,000; this band represented about 20% of the protein in the corn flour, 18–43% of the protein in the precipitate, and 24–50% of the protein in the whole sample. Bands at 50,000–75,000 also occurred in the corn sample, the precipitate sample, and the whole sample. This band represented around 24% of the protein found in the corn flour, 7–12% of the protein in the precipitate, and 25–43% of the protein in the whole thin stillage sample. The precipitate sample had one band in common with the corn flour that did not present itself in the whole sample: this band existed at 100,000–150,000 and made up 9–25% of the precipitate protein and 4% of the corn flour protein.

With CDS, unlike with thin stillage, there were few differences in the number of bands present in the whole sample and the precipitate. This similarity was likely because the precipitate made up such a large percentage of the whole sample. The precipitate sample had 8–16% β-zein, whereas the whole sample showed 9–25% β-zein. The α-zein was about 10–20% in the precipitate and 7–13% in the whole sample. The two dominating bands found in the corn flour were also found in the CDS. The band present from 10,000–15,000 made up about 16–26% of the precipitate and 26–44% of the whole CDS. The band at 50,000–75,000 represented 6–13% of the protein in the precipitate and 15–20% of the protein in the whole sample. As with the precipitate from the thin stillage, the precipitate from CDS showed a band that did not exist in the corn flour, at 150,000–300,000. It represented 10–26% of the protein present.

As expected, and as verified by the SDS-PAGE analysis, zein was a predominant protein found within these ethanol coproducts. Zein is a water-insoluble protein only found within corn. It can serve as coatings, biodegradable plastics, textiles, and adhesives. α-Zein can be extracted with aqueous alcohol, whereas β-zein requires a reducing agent within the alcohol (Anderson 2011). Recently, studies have focused on the presence and extraction of zein protein from corn before fermentation, and POET (Sioux Falls, SD) commercially markets the zein-based product Inviz, which is extracted from POET's Dakota Gold HP distillers grains. These extractions are very costly, and the zein produced has been sold from $10–40/kg (depending on purity), compared with the price of synthetic plastics at approximately $2/kg (Anderson 2011).

To make zein a more competitive product, its price must be drastically reduced. This requires finding new extraction methods

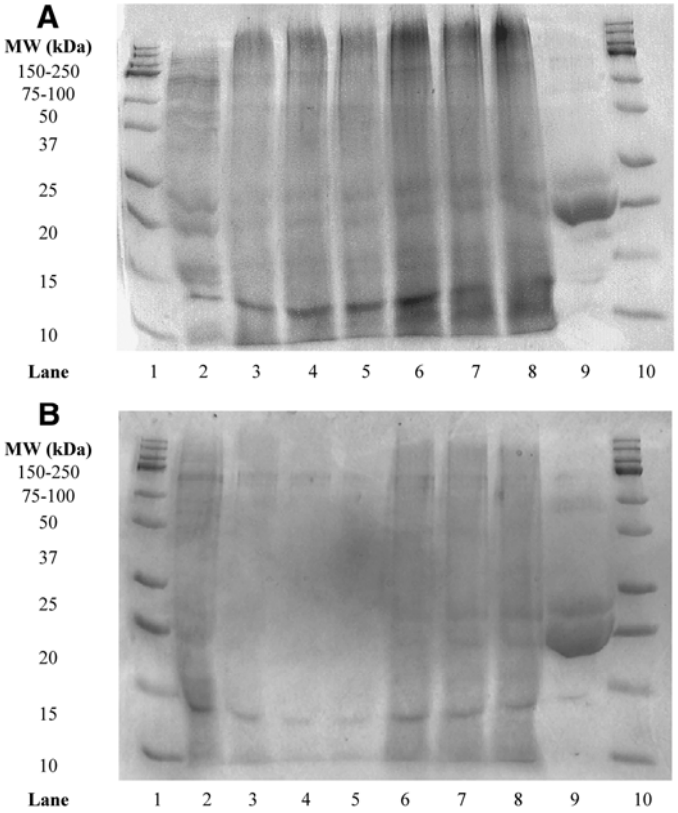

Fig. 4. Gel electrophoresis of condensed distillers solubles (CDS) and thin stillage (TS). Lanes 1 and 10: molecular weight standard; lane 2: corn flour; lane 3: TS1; lane 4: TS2; lane 5: TS3; lane 6: CDS1; lane 7: CDS2; lane 8: CDS3; and lane 9: zein. **A,** Precipitate removed from CDS and TS samples through centrifugation; **B,** whole (i.e., uncentrifuged) CDS and TS.

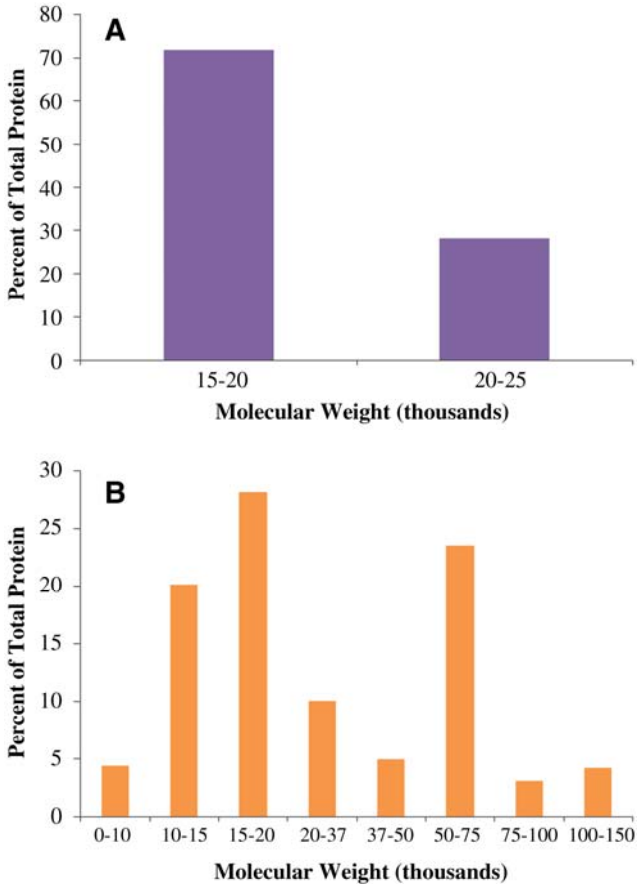

Fig. 5. Molecular weight distributions of corn proteins for comparison to condensed distillers solubles and thin stillage proteins (shown in Fig. 6). **A,** zein; **B,** corn flour (i.e., ground corn) proteins.

that increase the yield without increasing the processing costs. It is believed that the drying of DDGS can degrade zein because high drying temperatures can induce cross-linking of zein proteins in the protein bodies (Anderson 2011). This potential degradation could be eliminated if the zein were extracted from the upstream liquid products (i.e., thin stillage or CDS) before they are dried to produce DDGS.

Sugars and Organic Acids. Ethanol is produced by converting the starches in corn into sugars, which are then transformed through glycolysis to form pyruvate, which can then be fermented. The fermentation of the pyruvate determines the end products of fermentation. If it is pure alcoholic fermentation (i.e., with yeasts), the end products are simply ethanol and CO_2, but if it is a heterolactic fermentation (including bacteria and fungi), the products could also include organic acids and other forms of alcohols (Todar 2011). Thus, in traditional industrial production of ethanol, the products of fermentation can include ethanol, CO_2, organic acids, residual sugars, and other nonfermentable materials (i.e., proteins, lipids, fibers, and ash). Therefore, it is no surprise that various sugars, organic acids, and even alcohols were found in the liquid fractions of the CDS and the thin stillage (Table IV). Trace sugars were left behind when conditions became unfavorable for the yeast, causing it to stop metabolizing the sugars; an increase in pH or an increase in alcohol content are among such unfavorable conditions. Traces of cellobiose, glucose, arabinose, xylose, galactose, and mannose were also found. Xylose, galactose, and mannose have the same retention time, so their individual concentrations could not be determined specifically; however, their combined mean concentrations were determined to be approximately 11.07 g/L in the CDS and 2.79 g/L in the thin stillage. Cellobiose was determined to be the second most concentrated compound found in both liquid fractions, at levels of 34.84 g/L in CDS and 9.61 g/L in thin stillage.

Organic acids are produced by contaminant organisms in the fermentation, including wild yeast and bacteria (Ingledew et al 2009). The total concentration of lactic acid present during fermentation must remain lower than 0.8% w/v, and that of acetic acid must remain lower than 0.05% w/v (Ingledew et al 2009). When both of these acids are present, however, their concentrations must be even lower to prevent significant changes to the fermentation resulting from decrease in yeast growth and yeast loss. The mean concentrations of lactic acid in the CDS and thin stillage were determined to be 8.75 and 4.19 g/L, respectively. The acetic acid concentration was found to be 10.82 g/L in CDS and 3.22 g/L in thin stillage. Lactic acid and acetic acid concentrations of this magnitude could cause significant problems within the fermentation broth, as they are much greater than the allowable concentrations (0.08% w/v lactic acid and 0.05% w/v acetic acid). However, these are not the actual concentrations present within the fermentation broth; rather, these acids were concentrated in the thin stillage and the CDS after the ethanol was removed. The concentrated acids could then serve as a form of preservative, inhibiting the growth of certain forms of bacteria; they may also limit the number of potential uses for thin stillage and CDS. For example, these concentrations may need to be reduced to use the products in feeds.

Organic acids are responsible for changes in pH—which can cause incomplete fermentation, leaving residual sugars—and for protein accumulation in cells, causing dihydroxyacetone phosphate to form glycerol (Ingledew et al 2009). In fact, glycerol was found to be the most abundant compound in both CDS and thin stillage; it was found at a concentration of 63.18 g/L in the liquid portion of the CDS and 18.81 g/L in the liquid portion of the thin stillage.

Proximate Composition. The proximate compositions for both thin stillage and CDS can be seen in Table V. As was expected based on the moisture contents of the products, the CDS had

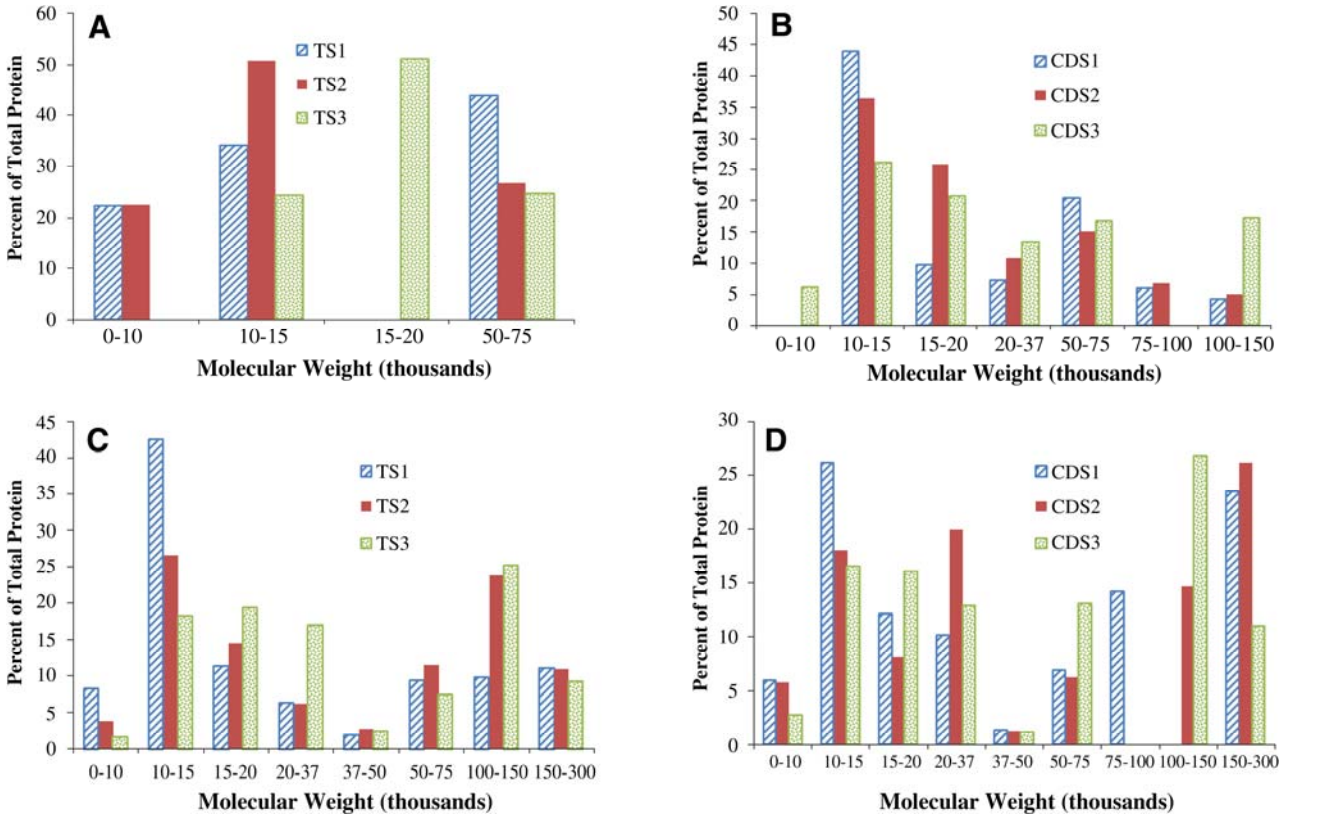

Fig. 6. Molecular weight distributions of protein for thin stillage (TS) and condensed distillers solubles (CDS). **A** and **B** show the molecular weight distributions for the whole samples (i.e., uncentrifuged); **C** and **D** show the molecular weight distributions for the precipitate removed from the whole samples (i.e., centrifuged).

higher concentrations of all the compounds measured and had around three times as much dry matter as the thin stillage. The quantities of protein and neutral detergent fiber (NDF) found in the CDS and thin stillage were very low (all less than 5%) when compared with the quantities typically found in DDGS (26–33% protein, 25–51% NDF) (Rosentrater and Muthukumarappan 2006). This reduction implied that most of these components were concentrated in the DWG stream. The concentrations of ash (2.83%) and fat (5.27%) found within the CDS stream fell within the known range of ash (2–10%) and fat (3–13%) typically observed within DDGS (Rosentrater and Muthukumarappan 2006). For thin stillage, the fat content was determined to be low, at 1.80%, and the ash content was even lower, at 0.90%.

Implications

Based on this examination of physical and chemical properties of CDS and thin stillage, it can be concluded that there may be opportunities for further exploration and development of value-added uses for these coproducts or the individual components within them. In fact, value can be extracted from CDS in the form of corn oil. Many U.S. ethanol plants do so through the use of centrifugation (Nouredinni et al 2009; Majoni et al 2010; Moreau et al 2010), and it can potentially be extracted with solvents (Majoni and Wang 2010).

Value might be extracted from CDS and thin stillage in the form of glycerol. Currently, large-scale biodiesel producers often refine crude glycerol through filtration, chemical addition, and fractional vacuum distillation so that it can be moved to markets in other industries (Pachauri and He 2006). Depending on the industry that is utilizing the glycerol (food, cosmetics, and pharmaceutical), it will need even further refining, such as bleaching, deodorizing, and removal of trace properties (Pachauri and He 2006). Livestock feed uses, on the other hand, typically do not entail additional processing. Various studies have explored ways to utilize the crude glycerol, including poly fabrics, synthesizing of carbon onions, and dielectric mediums (Yang et al 2004; Du et al 2005; Brown et al 1999).

The presence of zein also provides another opportunity for potential value-added product development. Zein has been used in the manufacture of buttons, fibers, adhesives, coatings, and binders (Lawton 2002). Extraction of zein from CDS and thin stillage has not yet been explored, but various techniques have been used to extract zein from corn, corn gluten meal, and DDGS (Lawton 2002).

TABLE IV
Chemical Composition of Ethanol Coproducts[z]

Coproduct/ Compound	Compound	Average Concentration (g/L)	
CDS			
Sugars	Cellobiose	34.84a	(4.83)
	Glucose	11.65a	(7.32)
	Xylose/galactose/mannose	11.07a	(1.57)
	Arabinose	6.17a	(0.54)
Organic acids	Succinic acid	5.71a	(0.67)
	Lactic acid	8.75a	(2.31)
	Acetic acid	10.82a	(1.31)
Glycerol		63.18a	(7.88)
Ethanol		0a	(0.01)
Thin stillage			
Sugars	Cellobiose	9.61b	(0.23)
	Glucose	3.49a	(1.30)
	Xylose/galactose/mannose	2.79b	(0.11)
	Arabinose	1.29b	(0.47)
Organic acids	Succinic acid	1.29b	(0.19)
	Lactic acid	4.19b	(0.32)
	Acetic acid	3.22b	(0.54)
Glycerol		18.81b	(0.33)
Ethanol		2.18b	(0.25)

[z] Values in parentheses are standard deviations. CDS = condensed distillers solubles. Values for a given dependent variable (i.e., compound) followed by the same letter are not significantly different ($\alpha = 0.05$, least significant difference) between coproducts.

TABLE V
Proximate Composition of Ethanol Coproducts[z]

Coproduct/ Compound	Average (%)			
	As Received		100% Dry Matter	
CDS				
Dry matter	28.90	(3.08)	…	…
Crude protein	4.70	(0.10)	16.33	(1.44)
NDF	1.50	(0.46)	5.17	(1.11)
Ash	2.83	(0.23)	9.80	(0.35)
Fat	5.27	(0.40)	18.30	(0.56)
Thin stillage				
Dry matter	9.73	(2.61)	…	…
Crude protein	1.23	(0.12)	13.13	(2.54)
NDF	1.20	(0.20)	13.13	(5.25)
Ash	0.90	(0.01)	9.73	(2.61)
Fat	1.80	(0.10)	19.10	(4.63)

[z] Values in parentheses are standard deviations. CDS = condensed distillers solubles; NDF = neutral detergent fiber.

CONCLUSIONS

Process streams such as CDS and thin stillage are currently manufactured into DDGS and then used as animal feeds, but there may be other potentially higher value opportunities available. This study explored protein, organic acid, and sugar profiles in the coproducts. It also examined various physical properties including density, thermal conductivity, thermal diffusivity, moisture, and water activity. This study is just one step toward pursuing opportunities for utilizing valuable components in these materials. Follow-up research should subject these materials to various types of separation processes and should also determine the quantity and yield of the target components. Finding new uses for such components could provide additional outlets for ethanol coproducts and potentially improve the sustainability of the industry.

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