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Composition of corn dry-grind ethanol by-products: DDGS, wet cake, and thin stillage

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Abstract

DDGS and wet distillers' grains are the major co-products of the dry grind ethanol facilities. As they are mainly used as animal feed, a typical compositional analysis of the DDGS and wet distillers' grains mainly focuses on defining the feedstock's nutritional characteristics. With an increasing demand for fuel ethanol, the DDGS and wet distillers' grains are viewed as a potential bridge feedstock for ethanol production from other cellulosic biomass. The introduction of DDGS or wet distillers' grains as an additional feed to the existing dry grind plants for increased ethanol yield requires a different approach to the compositional analysis of the material. Rather than focusing on its nutritional value, this new approach aims at determining more detailed chemical composition, especially on polymeric sugars such as cellulose, starch and xylan, which release fermentable sugars upon enzymatic hydrolysis. In this paper we present a detailed and complete compositional analysis procedure suggested for DDGS and wet distillers' grains, as well as the resulting compositions completed by three different research groups. Polymeric sugars, crude protein, crude oil and ash contents of DDGS and wet distillers' grains were accurately and reproducibly determined by the compositional analysis procedure described in this paper.

Keywords: Analysis of DDGS; Compositions; Ethanol; Dry grind process

1. Introduction

Fuel ethanol production from corn grain in the US exceeded 4.9 billion gallons in 2006. Of that production, 82% was from facilities employing some variation of the dry-grind process for ethanol production (Renewable Fuels Association, 2007). Dry-grind processes are characterized by a lack of a steeping step at the front end of the process, a hallmark of wet milling of corn, and little or no fractionation of the corn kernel components prior to saccharification of the starch and fermentation (Kwiatkowski et al., 2006). In dry-grind processes, the whole grain is ground by hammer mills into a course powder with a mean particle diameter of approximately 1 mm (Rausch et al., 2005). An aqueous slurry of yeast cells and residuals from the ground corn kernels remaining after fermentation pass through a stripper where the ethanol is recovered. The non-volatile components then leave this step as a product called whole stillage (Bothast and Schlicher, 2005). Whole stillage contains the fiber, oil, protein, other unfermented components of the grain, and yeast cells. Whole stillage is usually

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centrifuged to produce a liquid fraction (thin stillage) and a solids fraction (wet distillers' grains). A significant fraction (15% or more) of the thin stillage is recycled as backset to be used as process water at the front end of the process to slurry the ground grain (Kwiatkowski et al., 2006). The remaining thin stillage is concentrated through multiple effect evaporators to produce a syrup called condensed distillers' solubles (CDS) (Ganesan et al., 2006). While wet distillers' grains, syrup, or the combination of both (wet distillers' grains with solubles, WDGS) can be sold as an animal feed, the combination of wet distillers' grains and syrup is often dried to produce dried distillers' grains with solubles (DDGS) in order to greatly lengthen its shelf-life (McAloon et al., 2000).

Composition of DDGS has been of great interest to researchers in the area of animal science, ethanol producers, and especially to people in the feed industry as majority of it has been sold as a feed ingredient for livestock. A major consumer of the DDGS is the animal feed industry. The compositional analysis of DDGS has been focusing on nutritional value of DDGS, such as digestibility, total digestible nutrients, net energy, amino acid and mineral profiles. Compositional analysis of the corn-to-ethanol by-products have utilized established methods for determining the nutritional value of forages and grain animal feeds (Spiehs et al., 2002; Stein et al., 2006). An extensive compositional analysis of DDGS has been completed by several researchers. The averaged composition of 118 samples of DDGS (Spiehs et al., 2002) collected from 10 different dry grind facilities as well as composition of DDGS collected at one plant over a five-year period (Belyea et al., 2004) are summarized in Table 1. Major components of DDGS have been given as crude protein, crude fat and crude fiber. Since DDGS production has tripled in the past decade to an annual production of 12 million metric tons in 2006 (Renewable Fuels Association, 2007), additional efforts are underway to further develop and standardize these methods to insure feed quality as these ethanol byproducts become a larger share of the animal feed market (American Feed Industry Association, 2007). As a result of a lack of standardization the compositional analysis procedures and resulting composition of the same DDGS were slightly varied depending on the methods applied by each research group.

This paper reports averaged composition of a common lot of DDGS, wet distillers' grains, and thin stillage, measured by three research groups, as well as the detailed analysis procedures that have been applied by the groups. The composition includes not only the common compositions such as proteins, fat, and ash, but also cellulose, xylan, arabinan, and starch contents that are especially valuable for research on enzymatic hydrolysis and fermentation of DDGS. The carbohydrates present in the fiber component of DDGS (cellulose and hemicellulose) have potential value as a source of fermentable sugars for increased ethanol yield per bushel of corn. Additionally, these polysaccharides are indigestible in monogastric livestock (e.g. swine and poultry) and are of limited value as feed components for cattle. Therefore, the cellulose and hemicellulose in DDGS presents a potential opportunity for implementing cellulose conversion technologies into the current US ethanol industry. Evaluating DDGS as an additional source for fermentable sugars requires a different set of compositional analysis. This includes more detailed chemical analysis, especially on polymeric sugars that can release fermentable sugars upon enzymatic hydrolysis. Application of analytical methods developed for analyzing the composition of cellulosic biomass has been applied to corn-to-ethanol byproducts with varying success in closing the material balance (Mosier et al., 2005; Tucker et al., 2004).

Comparisons between the methods and suggestions for improved analytical techniques are presented. Research results on the DDGS utilization published in this special issue are based on the composition given in this paper. We also include terminology for several terms frequently used throughout this special issue to ease understanding and communication between researchers.

Table 1
Composition of distillers' dried grains with solubles (DDGS), previously reported by Spiehs et al. (2002) and Belyea et al. (2004)

	Spiehs et al., mean value, coefficients of variation	Belyea et al., mean (%)
Moisture content (% total)	11.1	Na
Dry matter content (% total)	88.9 (1.7)	Na
Total mass closure	100.0	
Crude protein	30.2% (6.4)	31.3
Crude fat	10.9% (7.8)	11.9
Crude fiber	8.8% (8.7)	10.2
Starch	Na	5.1
ADF	16.2% (28.4)	17.2
Ash	5.8% (14.7)	4.6

All values are % dry basis except where otherwise noted.

2. Methods

DDGS, wet distillers' grains (wet cake), and thin stillage were obtained from an operating dry-grind ethanol facility, Big River Resources, LLC (West Burlington, IA). Reagents and chemicals, unless otherwise noted, were purchased from Sigma–Aldrich (St. Louis, MO).

2.1. HPLC analysis

HPLC analysis of liquid samples was performed on a system consisting of a Varian 9010 Solvent Delivery System, Waters 717plus Autosampler, Aminex HPX-87H column (Biorad, Hercules, CA), Waters 2414 Refractive Index Detector, Waters 2487 Dual λ Absorbance Detector, and a Hewlett Packard HP3396G Integrator. The mobile phase

was 5 mM H_2SO_4 filtered through 0.2 μm nylon filter (Millipore) and degassed. The mobile phase flow rate was 0.6 mL/min and the column temperature was maintained at 60 °C by an Eppendorf CH-30 Column Heater controlled by an Eppendorf TC-50.

2.2. Forage/feed nutritional compositional analyses

These analyses were performed by the Experiment Station Chemical Laboratories, University of Missouri-Columbia. Samples were analyzed for crude protein (Method 990.03, AOAC 2000), moisture (Method 934.01), crude fiber (Method 978.10), crude fat (Method 920.39), ash (Method 942.05), acid detergent fiber (Method 973.18, A-D), cellulose (Method 973.18, A-D), and chloride (Method 9.15.01, 943.01). Hexose and pentose sugars were analyzed by HPLC. Minerals were analyzed by inductively coupled plasma atomic emission spectroscopy (ICP). A complete amino acid profile was determined by the AOAC (1995) official Method 45.3.05, 982.30 E. Total digestible nutrients (TDN%) were calculated using digestive factors of 0.78, 1.90, 0.57, and 0.85 for protein, fat, fiber, and nitrogen free extract, respectively, as follows: TDN% = [(% crude protein × 0.78) + (% crude fat × 1.90) + (% crude fiber × 0.57) + (% nitrogen free extract × 0.85)].

2.3. Cellulosic biomass compositional analyses

2.3.1. Moisture content

Moisture content and total solids were determined by either using convection oven or automated infrared moisture analyzer as described in the standard NREL Laboratory analytical procedure LAP 001. The moisture content of DDGS and wet distillers' grains was determined by using a Mettler Toledo halogen moisture analyzer (Model HB43, Columbus, OH), which used a quartz heater to dry 1 g samples of material at 105 °C until the mass varied less than ±0.001 g over a 30 s period. Selected samples were frozen at −20 °C and lyophilized for 48 h.

2.3.2. Starch

Purdue: The starch assay was performed on 2 g samples of ground material that passed 40 mesh sieves. Assay was done in triplicate. To each 2 g ground DDGS, 55 mL of Tris buffer at pH 7.2 was added and the mixture was autoclaved at 121 °C for 1 h. After cooling down the mixture at room temperature, 1.0 ml of 1 mg/ml of sodium azide, 50 mg of α-amylase (Sigma, Catalog # A4551) and 10 mg of amyloglucosidase (Sigma, Catalog # A7420) were added. The mixture was incubated at 37 °C, 200 rpm for 120 h. Released glucose concentration was measured by HPLC as described above. The starch content (%) was calculated as

$$\text{Starch}(\%) = \frac{\text{Weight of Glucose Measured by HPLC(g)} \times \frac{162}{180}}{\text{Sample Weight(dry, g)}} \times 100 \quad (1)$$

University of Illinois: The starch concentration of the samples was determined using a modified method of (Holm et al., 1986) as described previously (Ezeji et al., 2003), which uses heat-stable α-amylase and amyloglucosidase to release glucose from the sample. The amount of sample that was used for these studies was 1 g rather than 0.25 g.

USDA NCAUR: Starch content in ground corn was determined a measured amount of corn was placed in distilled water, boiled for 15 min. Amylase in a sodium acetate buffer was added and the starch was hydrolyzed for 120 min. Glucose was analyzed by the glucose oxidase/peroxidase method (Trinder, 1969).

2.3.3. Cellulose

Purdue: The total glucan content comprises starch and cellulose. Starch was differentiated from cellulose by using a mixture of amylase and amyloglucosidase to hydrolyze the starch in the sample. Glucose released from enzyme assay described above was labeled as "starch" and the difference between the glucose released by dilute acid hydrolysis and glucose from starch hydrolysis was labeled "cellulose". The ground sample of DDGS and wet distillers' grains was passed through 40 mesh screen and analyzed for total glucan by following the standard NREL laboratory analytical procedure LAP 002. The cellulose content was calculated by subtracting starch content from the glucan content as measured by LAP 002 procedure. Glucose content was analyzed by HPLC as described above.

University of Illinois: Cellulose was determined according to the procedure from the standard NREL Laboratory analytical procedure LAP 002. Glucose concentration was determined using a hexokinase and glucose-6-phosphate dehydrogenase (Sigma, St. Louis, MO) coupled enzymatic assay as described previously (Ezeji and Blaschek, 2005).

USDA NCAUR: Cellulose was determined using ASTM method E1758-95.

2.3.4. Xylan, arabinan, and galactan

Purdue: Along with the glucan content, xylan and arabinan contents were determined by the NREL LAP 002 procedure.

University of Illinois: Sample was slurried in dilute acid (15% solid, w/v; 0.5% H_2SO_4, v/v; 121 °C; 1 h) according to the method described by Saha and Bothast (1999). Xylose, arabinose, and galactose were determined by HPLC using an Agilent 1050 system (Palo Alto, CA). The sugars were separated on a 5 μm Supelcosil LC-NH_2, 25 cm × 4.6 mm with 2 cm × 4.6 mm Supelcosil LC-NH_2 guard column at room temperature and detected using refractive index (Waters 410). Mobile phase for the HPLC

system was 85% (by volume) acetonitrile in deionized water at a flow rate of 2 ml/min.

USDA NCAUR: Arabinose and xylose were determined using trifluoacetic acid treatment as described previously (Dien et al., 1997).

2.3.5. Crude protein

Purdue: Protein content was determined by nitrogen analysis. Protein content can be determined from amino acid profile alone. However, the nitrogen analysis is an easier and faster way to determine crude protein content, once an appropriate nitrogen factor for a specific type of biomass is known. The nitrogen factor of 6.25, which is generally recommended for most of biomass except wheat grains (Hames and Scarlata, 2005), may not be suitable for the distillers' grains, as it is a processed product that underwent multiple heat treatments and drying processes. An appropriate nitrogen factor has to be determined to reduce errors.

The nitrogen content was analyzed by the Dumas combustion method which produces nitric oxide by combustion and then reduces it to molecular nitrogen. A detector then measures the nitrogen gas to determine total nitrogen content in the sample. The nitrogen gas was measured by the Perkin–Elmer 2400 series II CHNS/O analyzer in the microanalysis laboratory at Purdue University. Nitrogen content (N in %) was converted to an equivalent weight of proteins. To get a crude protein concentration (%) from nitrogen content, it was multiplied by a nitrogen factor (NF) which was calculated from amino acid profile of the wet distillers' grains. Using the amino acid profile of wet distillers' grains given in Table 3, the nitrogen factor was calculated to be 5.9 for DDGS and 5.4 for wet distillers' grains, based on their measured nitrogen contents. The total protein content was calculated by following:

$$\text{Crude Protein}(\%) = \text{Nitrogen Factor} \times \text{Nitrogen Content}(\%) \quad (2)$$

USDA NCAUR: Protein was measured by following AOAC method 976.06, which is based on measuring total nitrogen.

2.3.6. Ether extractives (crude oil)

Ether extraction of DDGS determines crude oil content. Ether extraction was done by AOAC official method 920.39 which is a standard method for crude fat measurement in animal feed.

2.3.7. Hot water extractives (water solubles)

DDGS was measured for hot water extractives by washing 10 g of dry DDGS, previously extracted with ether, using 200 mL of 80–90 °C hot DI water. The wash liquid was collected and lyophilized for 120 h. The weight of materials left after lyophilization was measured as water solubles in DDGS. The water extractives were re-solubilized in 50 mL of water and analyzed by NREL procedure LAP-014 to determine their chemical compositions.

2.3.8. Ash

Ash content was measured by following NREL procedure LAP 005. Three samples were analyzed and averaged. Accurately weighed samples in crucibles were ashed in a muffle furnace at 575 ± 25 °C for 24 ± 6 h until the weight of ash remains constant upon 1 h of re-heating the crucible. The ash content was calculated as following:

$$\text{Ash}(\%) = \frac{\text{Weight}_{\text{crucible+ash}}(g) - \text{Weight}_{\text{crucible}}(g)}{\text{Dry weight of sample(g)}} \times 100 \quad (3)$$

2.3.9. Thin stillage composition

Two different batches of thin stillage were analyzed for chemical composition by the analytical procedure LAP-014 "Dilute Acid Hydrolysis Procedure for Determination of Total Sugars in the Liquid Fraction of Process Samples" developed by NREL.

3. Analysis procedure

A flow diagram of cellulosic biomass compositional analyses is shown in Fig. 1. Although slight variation is possible, the order given in Fig. 1 was suggested to minimize any interfering effects between the components present in the DDGS and wet distillers' grains.

Compositional analysis of cellulosic feedstock is usually done by following analytical procedures developed by NREL. This series of analyses is referred to as NREL LAP procedures and can be applied to various cellulosic biomass such as corn stover, poplar, bagasse, etc. The LAP procedures for compositional analysis of cellulosic biomass is generally done in the following order: (1) determine total solids in biomass; (2) water extraction to remove dirt, fertilizer, non-structural sugars, etc.; (3) ethanol extraction to remove chlorophyll, waxes, other minor components; (4) acid hydrolysis of extractives-free biomass to determine sugar components. Lignin measurement is done co-currently with acid hydrolysis. Ash is determined separately. However, it was found that this sequence of LAP

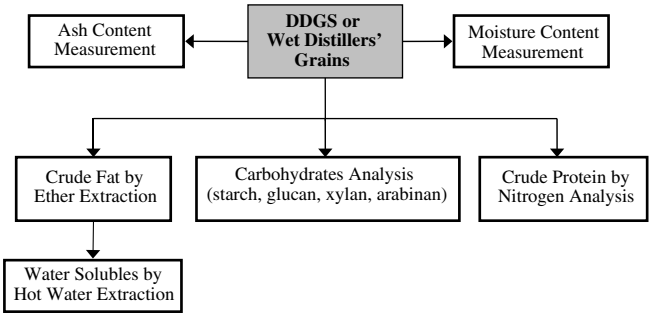

Fig. 1. Flow diagram of cellulosic biomass compositional analysis.

procedures can not be applied to DDGS and wet distillers' grains, due to their high content of oil and proteins which interfere with analysis of other components. DDGS contains 11–12% of crude oil as presented in Table 1. The oil (fat) can not be fully extracted by an ethanol extraction step of the standard LAP procedure. While the ethanol extraction works well for cellulosic feedstock which is intrinsically low of oils, it does not work for DDGS with high amounts of oil and lipids. Therefore, a more severe extraction method had to be applied, such as petroleum ether extraction. The ether extraction is a typical method for determining crude fat content in animal feed.

Hot water extraction is also essential to close the total material balance of DDGS. Syrup, which is evaporated thin stillage containing various soluble components, is sprayed onto the wet distillers' grains and dried to produce the distillers' dried grains with solubles (DDGS). These water soluble components are mostly fermentation by-products, soluble sugars, soluble proteins, and organic acids. These components are easily solubilized in warm water. As oils can interfere with hot water extraction step, the ether extraction is done before the hot water extraction to minimize the oils being recovered during hot water extraction.

The standard NREL procedure determines carbohydrate contents in biomass by two-step acid hydrolysis and measures the amount of released sugars. The acid hydrolysis procedure is typically done with biomass that is previously extracted with water and ethanol. This is because the extractives can interfere with lignin measurement that is carried out simultaneously with the acid hydrolysis.

The same procedure is applied to measure polymeric sugars in the DDGS and wet distillers' grains, except that the material is not extracted beforehand, unless lignin content of the distillers' grains is expected to be abnormally high and needs to be measured. Soluble sugars are mostly recovered during hot water extraction and not identified as sugars unless additional compositional analysis is done with the water extractives. Therefore, the total amount of carbohydrates in DDGS or wet distillers' grains is best measured by acid hydrolysis of the starting material, rather than previously extracted material. Although the soluble sugars may be doubly counted in both water extractives and acid hydrolyzate, this does not affect the total mass balance significantly, as the soluble sugars recovered as water extractives were found to be less than 10% (by dry weight) of the total water extractives (data not shown).

This approach was taken to reduce the number of steps needed to complete the analysis, as well as to minimize errors in the determined sugar composition of the feedstock. The same reasoning applies for protein measurement in distillers' grains. Some of the proteins in the initial material are extracted during hot water extraction. However, the protein recovered in hot water extractives was not significant. To determine the total proteins in the distillers' grains, the nitrogen analysis was carried out on a starting material, rather than an extractives free material.

4. Results

A summary of the average composition (dry basis) of DDGS determined by three consortium partners (Purdue, USDA NCAUR, and U. of Illinois), forage/feed analysis results and amino acid profile of DDGS are given in

Table 2
Composition of DDGS by (A) cellulosic biomass compositional analysis (average from three research groups, Purdue, University of Illinois and USDA NCAUR); (B) forage/feed nutritional analyses

	Average	Relative deviation
(A) Cellulosic biomass compositional analysis		
Dry matter	88.8	0.0
Water extractives	24.7	0.0
Ether extractives	11.6	0.1
Crude protein	24.9	0.1
Glucan (total)	21.2	0.2
Cellulose	(16)	(0.1)
Starch	(5.2)	(0.1)
Xylan and arabinan	13.5	0.2
Xylan	(8.2)	(0.1)
Arabinan	(5.3)	(0.0)
Ash	4.5	NA
Total dry matter mass closure	100.4	
(B) Forage/feed nutritional compositional analyses carried out by University of Missouri, Columbia Agricultural Extension Chemical Laboratories		
Compositional analysis		
Dry matter		88.9
Crude protein		27.3
Crude fat		14.5
Carbohydrates		53.5
Ash		4.7
Total		100
Amino acid analysis		
Arginine		1.4
Histidine		0.8
Isoleucine		1.1
Leucine		3.3
Lysine		1.0
Methionine		0.6
Cystine		0.5
Phenylalanine		1.4
Threonine		1.1
Tryptophan		0.2
Valine		1.5
Hydroxyproline		0.1
Aspartic acid		1.7
Serine		1.2
Glutamic acid		3.3
Proline		2.0
Lanthionine		0.0
Glycine		1.1
Alanine		1.9
Tyrosine		1.2
Ornithine		0.1
Total		25.7

All values are % dry basis except where otherwise noted.

Table 3
Composition of wet distillers' grains (wet cake, DG) by (A) cellulosic biomass compositional analysis (average from three research groups, Purdue, University of Illinois and USDA NCAUR); (B) forage/feed nutritional analyses

(A) Cellulosic biomass compositional analysis	
Dry matter	35.3
Water extractives	8.8
Ether extractives	9.6
Crude protein	36.6
Glucan (total)	18.5
Cellulose	(12.6)
Starch	(5.9)
Xylan and arabinan	20.9
Xylan	(14.9)
Arabinan	(5.5)
Ash	2.0
Total dry matter mass closure	96.4
(B) Forage/feed nutritional compositional analyses	
Compositional analysis	
Dry matter	44.1
Crude protein	34.4
Crude fat	10.9
Carbohydrates	52.7
Ash	2.0
Total	100%
Forage analysis	
Total digestible nutrients	90.0
Gross calories (kcal/kg)	446
ADF (acid detergent fiber)	17.2
Cellulose	13.6
Starch	4.2
Mineral analysis	
Calcium (ppm)	113.0
Phosphorous	0.5
Potassium	0.4
Magnesium	0.2
Sulfur	0.6
Sodium	0.1
Chloride	0.8
Iron (ppm)	61.0
Manganese (ppm)	8.0
Amino acid analysis	
Arginine	1.5
Histidine	0.9
Isoleucine	1.4
Leucine	4.4
Lysine	1.1
Methionine	0.8
Cystine	0.8
Phenylalanine	1.8
Threonine	1.3
Tryptophan	0.3
Valine	1.8
Hydroxyproline	0.0
Aspartic acid	2.2
Serine	1.5
Glutamic acid	5.5
Proline	2.8
Lanthionine	0.2
Glycine	1.3
Alanine	2.53
Tyrosine	1.40
Ornithine	0.03
Total	33.39

All values are % dry basis except where otherwise noted.

Table 2. The DDGS is at 11.2% moisture. Total glucan was determined to be 21.2%, of which 16.0% is cellulose and 5.2% is starch on a dry matter basis. Xylan and arabinan account for 13.5% of the dry mass. The remaining dry masses are crude protein (24.9%), crude oil (11.6%), water extractives (24.7%) and ash (4.5%). Forage/feed analysis gave similar results for protein, oil and ash contents. Total carbohydrate content is 53.5% by forage/feed analysis, which is significantly higher than the total carbohydrates (34.7%) determined by cellulosic biomass analysis. In the forage/feed analysis, crude protein, fat and ash are determined and whatever is left to make up 100% mass balance is considered to be total carbohydrates. Glycerol, acetic acid and other fermentation by-products are counted as a part of the total carbohydrates by the forage/feed analysis, therefore, resulting in an over-estimate of the total carbohydrates.

The water extractives were analyzed for their composition and found to be mostly residual mono- and oligo-saccharides, organic acids, such as succinic acid and lactic acid, and fermentation by-products like glycerol and butanediol. About 10% (equivalent to 2.7% of the total mass balance) of the total protein and 7% (equivalent to 2.4% of the total mass balance) of the total carbohydrates were recovered during hot water extraction. If we subtract the double counted protein and sugar contents, the total mass closure becomes 95.3%.

Wet distillers' grain (wet cake) was also analyzed by following the methods described in this paper. Wet distillers' grains gave similar results for glucan composition, but the xylan and arabinan contents were twice of that found in DDGS. The wet distillers' grains differed from the DDGS in that it was obtained wet, and before evaporated stillage was sprayed onto it. The wet cake had a moisture content of 63.9% (by Mettler Toledo balance). Lyophilization gave a similar value at 64.7% moisture.

The chemical composition of the wet distillers' grains was determined by both cellulosic biomass method and forage/feed nutritional analysis on a dry weight basis (Table 3). While the forage/feed analysis gives a total carbohydrate content of the material, the cellulosic biomass method breaks it down into constituent polymeric sugars: cellulose, starch, xylan and arabinan. The total glucan percentage, which combines cellulose and starch, is 18.5% as determined by the cellulosic biomass method. Xylan and arabinan comprises 20.4% of the total dry mass of the wet distillers' grains. The feed analysis also over-predicted the total carbohydrates content (38.9% by cellulosic biomass analysis, 52.7% by forage/feed nutritional analysis). Total glucan,

Table 4
Composition of thin stillage by (A) cellulosic biomass compositional analysis (average of two batches); (B) forage/feed nutritional analyses

(A) Cellulosic biomass compositional analysis	
Dry matter	7.7
Glucose (g/L)	0.9
Glucan (oligosaccharide, g/L)	12.4
Xylose (g/L)	0.7
Xylan (oligosaccharide, g/L)	3.7
Arabinose (g/L)	0.4
Arabinan (oligosaccharide, g/L)	0.5
Lactic acid (g/L)	16.8
Glycerol (g/L)	14.4
Acetic acid (g/L)	0.3
Butanediol (g/L)	1.9
Ethanol (g/L)	0.6
(B) Forage/feed nutritional compositional analyses	
Compositional analysis	
Dry matter	6.2
Crude protein	1.3
Crude fat	1.3
Carbohydrates	2.8
Ash	0.8
Total	100
Forage analysis	
Gross calories (kcal/kg)	28
ADF (Acid detergent fiber)	0.1
Cellulose	0.1
Starch	0.5
Mineral analysis	
Calcium (ppm)	31.0
Phosphorous	0.1
Potassium	0.2
Magnesium	0.1
Sulfur	0.1
Sodium	0.1
Chloride	0.0
Iron (ppm)	8.0
Manganese (ppm)	2.0
Amino acid analysis	
Arginine	0.1
Histidine	0.0
Isoleucine	0.1
Leucine	0.1
Lysine	0.1
Methionine	0.0
Cystine	0.0
Phenylalanine	0.1
Threonine	0.1
Tryptophan	0.0
Valine	0.1
Hydroxyproline	0.0
Aspartic acid	0.1
Serine	0.1
Glutamic acid	0.1
Proline	0.1
Lanthionine	0.0
Glycine	0.1
Alanine	0.1
Tyrosine	0.0
Ornithine	0.0
Total	1.1

All values are % dry basis except where otherwise noted.

combining cellulose and starch, was determined to be 17.8% of the total dry matter by the forage/feed analysis.

Crude protein composes 36.6% of the total dry mass of wet distillers' grains as measured by nitrogen analysis. The nitrogen factor to determine the protein content of DDGS or wet distillers' grains may be calculated from the complete amino acid profile provided in Tables 2 and 3 and the measured nitrogen content (w/w%) of the feedstock. An average of 6.8% of total dry matter of the wet distillers' grains is nitrogen as measured by combustion method. The nitrogen factor for wet distillers' grains was determined to be 5.4, with a maximum upper limit of 6.0 and lower limit of 4.8. The nitrogen content of DDGS was measured to be 4.0%. Assuming that amino acid profile of DDGS is similar to that of wet distillers' grains, the nitrogen factor for DDGS is estimated to be 5.9, with maximum upper and lower limits, at an average of 6.2 and 5.7. Wet distillers' grains were found to contain more proteins than DDGS on a dry mass basis. This is expected since the added components from the condensed distillers' solubles are sprayed onto the dried distillers' grains, resulting in relatively lower protein per dry mass in DDGS than in wet distillers' grains. Unlike the protein content, crude fat (ether extractives) was determined to be similar for both DDGS and wet distillers' grains. It was 11.6% for DDGS and 9.6% for wet distillers' grains.

Thin stillage was analyzed by acid hydrolysis (NREL LAP 014 procedure). Its composition is presented in Table 4. The moisture content is 92.3%. The majority of the remaining solids are glucan oligomers and glycerol. In the current dry grind process, the thin stillage is evaporated to make a condensed syrup which is later sprayed onto the wet distillers' grains to produce DDGS. It is also used as backset for liquefaction of ground corn. Its high water content and buffering effect suggest that it can be recycled and used as a media for liquid hot water pretreatment of the corn-to-ethanol byproducts, since one of the conditions of liquid hot water pretreatment is maintaining pH between 4 and 7. The recycle of thin stillage as a pretreatment media has the added advantage of reducing the need for additional water input for the pretreatment step. Pretreatment and hydrolysis results of the distillers' grains mixed with thin stillage are discussed in a separate paper (Kim et al., 2008) in this special issue of the journal.

5. Conclusions

Chemical compositions of DDGS, wet distillers' grains and thin stillage were determined by a series of analyses described in this paper. A complete set of analyses to quantify the major components of DDGS and wet distillers' grains was established by modifying the procedure of the standard cellulosic biomass compositional analysis. The compositional analysis of DDGS and wet distillers' grains presented in this paper produced reproducible and accurate results, with a close to 100% mass closure. DDGS and wet distillers' grains are rich in glucan, xylan and arabinan, the

source of fermentable sugars for ethanol production. Total available sugars (glucan and xylan) of DDGS and wet distillers' grains for producing ethanol were measured to be 29.4% and 33.4%, respectively, based on a total dry mass basis. Crude protein comprises 25% of the total dry mass of DDGS. Crude oil measured as ether extractives is 11.6%.

Acknowledgements

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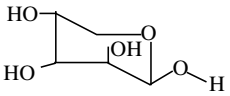
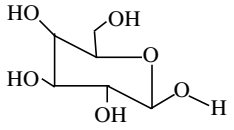
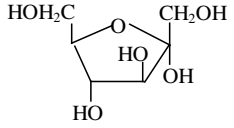
Appendix

Terminology

To ease understanding and prevent confusion on the terms used in this special issue, the glossary is provided below.

Acetic Acid	A carboxylic acid, CH_3COOH, molecular weight = 60 g per g-mol. Acetic acid is found naturally substituted to hemicellulose through covalent ester bonds that may be hydrolyzed at processing temperatures as the hemicellulose is hydrolyzed.
Acetyl	Refers to the anhydrous form of acetic acid as found substituted to hemicellulose though an ester linkage. CH_3COOH, molecular weight = 42 g per g-mol. Measurement of acetyl is performed by hydrolyzing the biomass with sulfuric acid, measuring the resulting acetic acid by HPLC, and finally correcting the final mass for the water added during hydrolysis (18 g/mol). See also *material balance* and *feed analysis*.
ADF	Acid detergent fiber (ADF) is a *feed analysis* method which measures the least digestible cell wall components (recalcitrant *cellulose* and *lignin*). ADF values are inversely related to digestibility, so forages with low ADF concentrations are usually higher in energy. Calculated feed energy values usually include consideration of ADF content. ADF is measured by refluxing feed material in acidified (1 N sulfuric acid) quaternary detergent solution to dissolve cell solubles, hemicellulose and soluble minerals leaving a residue of cellulose, lignin, and heat damaged protein and a portion of cell wall protein and minerals (ash). ADF is determined gravimetrically as the residue remaining after extraction. Near infrared (NIR) spectroscopy methods for determining ADF have been developed and are used by some commercial laboratories for measurements. This method shares some similarities to the methods associated with *material balance* determination, but the values may be slightly different between methods. See also *feed analysis* and *NDF*.
Arabinan	Refers to the anhydrous form of arabinose as found within largely hemicellulose that has 1 molecule of water (18 g/mol) less mass due to the condensation reaction forming the polymer, $C_5H_8O_4$, molecular weight = 132 g per g-mol. This is a term of convenience for mass balance considerations that does not reflect the complexity of highly substituted hemicellulose. Measurement of arabinan is performed by hydrolyzing the biomass with sulfuric acid, measuring the resulting arabinose by HPLC, and finally correcting the final mass for the water added during hydrolysis. See also *material balance* and *feed analysis*.
Arabinose	Refers to the monomeric form of arabinose, $C_5H_{10}O_5$, molecular weight = 150 g per g-mol: 
Ash	Mineral components of biomass material. *Material balance* and *feed analysis* methods are slightly different in measuring this component of biomass. See also *material balance* and *feed analysis*.
Dextrose	An older name for *glucose* sometimes still used in the food industry. See also *glucose*.
Distillers' dried grains with solubles (DDGS)	Distillers' dried grains with solubles (DDGS) is one of the products of the *Dry grind* ethanol process. DDGS is composed of unhydrolyzed, unfermented grain components such as the seed hull (pericarp), germ, protein, and oil. DDGS is produced by mixing *wet cake* with evaporated *light stillage* and drying. When feed markets for DDGS are close to the ethanol facility, the drying step may be eliminated, which significantly lowers the energy cost for the facility.

Appendix (*continued*)

Dry grind	The dry grind ethanol process is one of two processes for commercial fuel ethanol production from grain. The dry grind process, unlike the *wet milling* process, does not include a steeping step or a separation of the starch from the other grain components before saccharification and fermentation. In the dry grind process, whole grain in milled, cooked, hydrolyzed, and then fermented. The unfermentable components (seed pericarp, germ, oil, etc) pass through the entire process and are separated from the ethanol and soluble components at the end by distillation (removes ethanol) and centrifugation (removes water and dissolved solids).
Galactan	Refers to the anhydrous form of galactose as found within largely hemicellulose that has 1 molecule of water (18 g/mol) less mass due to the condensation reaction forming the polymer, $C_6H_{10}O_5$, molecular weight = 162 g per g-mol. This is a term of convenience for mass balance considerations. Measurement of galactan is performed by hydrolyzing the biomass with sulfuric acid, measuring the resulting galactose by HPLC, and finally correcting the final mass for the water added during hydrolysis. Galactose and xylose co-elute from the type of HPLC column commonly used for this analysis, so galactan may be reported combined with xylan. See also *material balance* and *feed analysis*.
Galactose	Refers to the monomeric form of D-galactose, $C_6H_{12}O_6$, molecular weight = 180 g per g-mol:  See also *Galactan*.
Cellulose	A straight chain polymer of glucose β1-4 glucose found in plant cell walls and the cell walls of some algae and bacteria. Cellulose is a straight, ribbon-like polymer that naturally forms crystals through van Der Waals interactions and hydrogen bonding between the flat faces of multiple cellulose chains. This macromolecular structure gives cellulose its tensile strength and resistance to hydrolysis (water is unable to easily penetrate the crystalline structure). In biomass feedstocks containing starch (such as corn fiber), cellulose content is determined by difference between total glucan as measured by acid hydrolysis (see *Glucan*) and starch as measured by enzymatic hydrolysis (see *Starch*).
Fat, crude	Crude fat is a *feed analysis* measurement based upon mass extracted by petroleum ether. Similar to ethanol extractives as measured by *material balance* methods developed by NREL. See also *feed analysis* and *material balance*.
Feed analysis	Since DDGS is sold as an animal feed, feed quality and compositional analyses have been adapted from grain and forage testing methods to assess the value of DDGS as an animal feed. These methods, unlike *material balance* methods, asses the nutritional value of the material by simulating digestion in the target animal or by proxy methods that have been correlated to digestion behavior and nutrient value. See also *ADF, crude fat, crude protein*, and *NDF*.
Fructose	Refers to the monomeric form of D-fructose, $C_6H_{12}O_6$, molecular weight = 180 g per g-mol. Fructose is an isomer of *glucose*.  See also *sucrose*.
Glucan	Refers to the anhydrous form of D-glucose as found within a polysaccharide such as starch or cellulose that has 1 molecule of water (18 g/mol) less mass due to the condensation reaction forming the polymer, $C_6H_{10}O_5$, molecular weight = 162 g per g-mol. This is a term of convenience for mass balance considerations since it does not distinguish between the possible sources anhydro-glucose (e.g. cellulose, starch, etc.). Measurement of glucan is performed by hydrolyzing the biomass with sulfuric acid, measuring the resulting glucose by HPLC, and finally correcting the final mass for the water added during hydrolysis. See also *material balance* and *feed analysis*.

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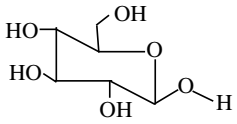
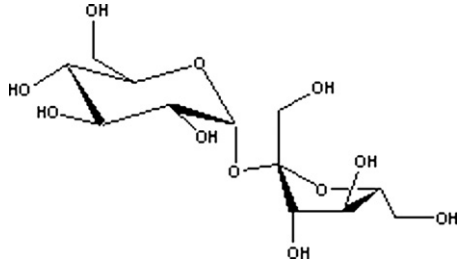
Glucose	Refers to the monomeric form of D-glucose, $C_6H_{12}O_6$, molecular weight = 180 g per g-mol:  Also known as corn sugar or *dextrose*.
Hemicellulose	A highly branched, highly substituted polymer comprised largely of xylose or arabinose, with minor amounts of galactose and glucose that is found in plant cell walls. The exact carbohydrates and their ratios vary between plant types. Corn (and other grasses) has hemicellulose that is largely xylose with minor amounts of glucose. Hemicellulose is substituted with acetic and glucoronic acid esters. Hemicellulose largely acts as a "glue" in plant cell walls that hold crystalline microfibers of cellulose in place. Hemicellulose hydrolyzes relatively easily compared to cellulose largely due to its amorphous highly branched structure.
Hexose	A carbohydrate containing 6 carbon atoms (e.g. glucose, galactose), $C_6H_{12}O_6$, molecular weight = 180 g per g-mol.
Hydrolysis	The breaking of a glycosidic bond (formed through a condensation polymerization reaction) within a polysaccharide chain through the addition of water.
Invertase	An enzyme that hydrolyzes *sucrose* to *glucose* and *fructose*. The official name for invertase is beta-fructofuranosidase (EC3.2.1.26). Commercially available invertase is derived from yeast.
Lignin	Lignin is a highly complex and branched polymer of phenylpropanoid units. Lignin is found in the secondary plant cell wall and acts as a seal to prevent microbial attack of the cells and to increase the resiliency and strength of the plant cell wall. Lignin is deposited during the last stages of plant cell growth. The resiliency of the lignin seal prevents any additional cell elongation after deposit. Highly lignified plant cells are found in mature portions of the plant largely responsible for structural support. Plant cell walls in corn fiber have relatively low amounts of lignin that is largely in an immature form. The methods for determining lignin content are slightly different between the standard methods for determining *material balance* and *feed analysis*. See also *material balance* and *feed analysis*.
NDF	Neutral detergent fiber (NDF) is a *feed analysis* measurement. A neutral detergent solution is used to dissolve the easily digested pectins and plant cell contents (proteins, sugars and fats), leaving a fibrous residue that is primarily cell wall components of plants (*cellulose*, *hemicellulose* and *lignin*). Detergent is used to solubilize the proteins and sodium sulfite also helps remove some nitrogenous matter; EDTA is used to chelate calcium and remove pectins at boiling temperatures; triethylene glycol helps to remove some nonfibrous matter from concentrate feeds; and heat-stable amylase is used to remove starch. However, since the methods for obtaining *feed analysis* measurements and *material balance* measurements differ, there may be some differences in the two measurements. See also *ADF* and *feed analysis*.
Material balance	A material balance measures the chemical components in each processing stream and tracks the flows and transformations of these chemicals throughout the process. In cellulosic ethanol research, the chemical compounds of particular interest are carbohydrates, specifically monomeric (simple) sugars such as *arabinose, galactose, glucose*, and *xylose*. Within the plant cell wall, these simple sugars exist as subunits in polysaccharides (*cellulose, hemicellulose*, and *starch*). Since the processing goal is to hydrolyze these polymers into simple sugars for fermentation, analytical methods have been developed by the National Renewable Energy Laboratory and others which measure these sugars for the purpose of developing material balances for these processes. These methods hydrolyze the polysaccharides into simple sugars using sulfuric acid, which are then measured by HPLC. Since the hydrolysis products are measured, and not the polysaccharides directly, material balances developed using these methods usually treat the polysaccharides as classes of anhyro-sugars (*arabinan*, *galactan*, *glucan*, and *xylan*), rather than by physiological structure/function (*cellulose*, *hemicellulose*, or *starch*). Thus, as simple sugars are liberated during the process, yields and material balances are easily calculated by measuring the conversion of anhydro-sugars to free sugars.
Pentose	A carbohydrate containing 5 carbon atoms (e.g. xylose, arabinose), $C_5H_{10}O_5$, molecular weight = 150 g per g-mol.

Appendix (*continued*)

Pretreatment	Hydrolysis of the polysaccharides in plant cell walls is hindered by the macromolecular/nano-scale structure of the plant cell wall. Pretreatment refers to processes performed prior to hydrolysis that act to disrupt these nano-scale structures and partially hydrolysis some of the polysaccharides to produce a substrate that is hydrolysable by enzymes at increased rates and increased ultimate yields of fermentable sugars.
Protein, crude	Crude protein estimates protein content based upon a measurement of nitrogen, usually obtained by some variant of the Kjeldahl method. This method measures total nitrogen in the sample, which includes protein, damaged protein (shift bases with carbohydrates), and other nitrogen containing compounds. See also *feed analysis*.
Starch	Straight chain (amylose) and branched chain (amylopectin) polymers of glucose α1-4 glucose found in plant seeds (e.g. corn kernels). Starch naturally forms a helical structure through hydrogen bonding between constituent glucan moieties. The disruption of this helical, insoluble structure into a more hydrolysable form can be achieved by cooking in hot water (gelatinization). Measurement of starch is performed by enzymatic hydrolysis of the biomass using amylase enzymes, measuring the resulting glucose by HPLC, and finally correcting the final mass for the water added during hydrolysis.
Stillage	Stillage is the liquid stream from the distillation bottoms. Stillage contains residual oligosaccharides, organic acids, and non-volatile metabolic by-products of the fermentation. See also *stillage*, *heavy* and *stillage*, *light*.
Stillage, heavy	Heavy stillage is the whole slurry stream that exits the bottom of the beer column (or stripper) in the distillation system. Heavy stillage is largely water containing both dissolved and undissolved solids (pericarp, germ, protein, oil).
Stillage, Light	Light stillage, also known as thin stillage, is the liquid stream resulting from the centrifugation of *heavy stillage* to remove the majority of the undissolved solids. Light stillage is concentrated by evaporation to produce *syrup* that is usually mixed with *wet cake* and dried to produce *DDGS*.
Sucrose	Refers to the disaccharide, α-D-glucopyranosyl-(1->2)-β-D-fructofuranoside, $C_{12}H_{22}O_{11}$, molecular weight = 342 g per g-mol. Sucrose can be hydrolyzed by acids, heat, or *invertase* into one *glucose* and one *fructose* molecule per molecule of sucrose. Yeast (*Saccharomyces cerevisiae*) are able to ferment sucrose to ethanol because they express high levels of *invertase*. Sucrose is also known as table sugar, cane sugar, beet sugar, or confectioner's sugar. 
Syrup	Light (thin) stillage that has been processed by evaporators to remove water and concentrate the dissolved solids. Syrup is mixed with *wet cake* and dried to produce *DDGS*.
Wet Cake	Wet cake refers to the solid material exiting the centrifugation of *heavy stillage*. Wet cake is largely composed of fiber from the grain pericarp, germ, and insoluble protein. Wet cake usually has a 60–80% moisture content. In the *dry grind* ethanol plant, wet cake is mixed with evaporated *light stillage* and dried to produce *DDGS*.
Xylan	Refers to the anhydrous form of xylose as found within largely hemicellulose that has 1 molecule of water (18 g/mol) less mass due to the condensation reaction forming the polymer, $C_5H_8O_4$, molecular weight = 132 g per g-mol. This is a term of convenience for mass balance considerations that does not reflect the complexity of highly substituted hemicellulose. Measurement of xylan is performed by hydrolyzing the biomass with sulfuric acid, measuring the resulting xylose by HPLC, and finally correcting the final mass for the water added during hydrolysis. Xylose and galactose co-elute from the type of HPLC column commonly used for this analysis, so *galactan* may be reported combined with xylan. See also *material balance* and *feed analysis*.

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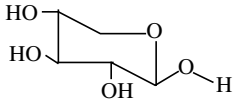
Xylose	Refers to the monomeric form of xylose, $C_5H_{10}O_5$, molecular weight = 150 g per g-mol:  See also *material balance* and *xylan*.

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