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REVIEW

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Chemical Composition of Distillers Grains, a Review

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ABSTRACT: In recent years, increasing demand for ethanol as a fuel additive and decreasing dependency on fossil fuels have resulted in a dramatic increase in the amount of grains used for ethanol production. Dry-grind is the major process, resulting in distillers dried grains with solubles (DDGS) as a major coproduct. Like fuel ethanol, DDGS has quickly become a global commodity. However, high compositional variation has been the main problem hindering its use as a feed ingredient. This review provides updated information on the chemical composition of distillers grains in terms of nutrient levels, changes during dry-grind processing, and causes for large variation. The occurrence in grain feedstock and the fate of mycotoxins during processing are also covered. During processing, starch is converted to glucose and then to ethanol and carbon dioxide. Most other components are relatively unchanged but concentrated in DDGS about 3-fold over the original feedstock. Mycotoxins, if present in the original feedstock, are also concentrated. Higher fold of increases in S, Na, and Ca are mostly due to exogenous addition during processing, whereas unusual changes in inorganic phosphorus (P) and phytate P indicate phytate hydrolysis by yeast phytase. Fermentation causes major changes, but other processing steps are also responsible. The causes for varying DDGS composition are multiple, including differences in feedstock species and composition, process methods and parameters, the amount of condensed solubles added to distiller wet grains, the effect of fermentation yeast, and analytical methodology. Most of them can be attributed to the complexity of the dry-grind process itself. It is hoped that information provided in this review will improve the understanding of the dry-grind process and aid in the development of strategies to control the compositional variation in DDGS.

KEYWORDS: Distillers grains, DDGS, chemical composition, mycotoxins, ethanol, renewable fuel, biofuel, coproduct, dry-grind, corn, yeast

INTRODUCTION

During the past decade, in the United States and elsewhere, the fuel-ethanol industry has experienced a phenomenal surge in growth (Figure 1). Global production of bioethanol increased from 17 billion liters in 2000 to >46 billion liters in 2007,[2] whereas in the United States alone, ethanol production increased from about 6.5 billion liters in 1999 to >39 billion liters in 2009.[1] This worldwide increase has been driven mainly by increasing demand for motor fuels as well as government mandates for alternate fuel oxygenates. Although cellulosic biomass is envisaged to provide a significant portion of the raw materials for bioethanol production in the medium- and long-term due to its low cost, high sustainability, and low competition with food, at present the global ethanol supply is produced mainly from sugary or starchy grain feedstock.[2] In North America, corn is the major feedstock, with other grains as minor ones. In Brazil, sugar cane is commonly used. As demand for transportation fuels continues to increase, it is anticipated that the fuel-ethanol industry in the United States and elsewhere will continue to grow.

By a broader definition, distillers grains (DG) are a cereal coproduct of the distillation process. There are two main sources of DG, the traditional source from brewers, where beverage ethanol is produced, and the growing source from fuel-ethanol plants. Like beverage ethanol, fuel ethanol is produced by yeast fermentation of sugars, so the process principle is the same. There are two major industrial methods for producing ethanol from grains, wet milling and dry-grind. DG is the coproduct of dry-grind processing, whereas wet milling produces gluten meal and gluten feed as major coproducts.

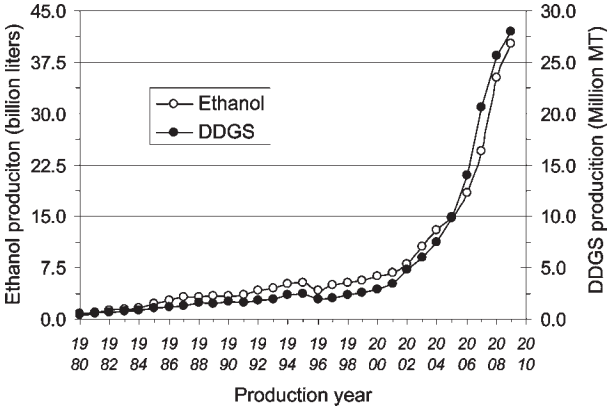

Figure 1. U.S. annual production of ethanol from grains and its main coproduct, distillers dried grains with solubles (DDGS), between 1980 and 2010. Adapted from RFA.[1]

The detailed principle and procedure of dry-grind processing are described in the literature.[3−5] Briefly, the dry-grind process is

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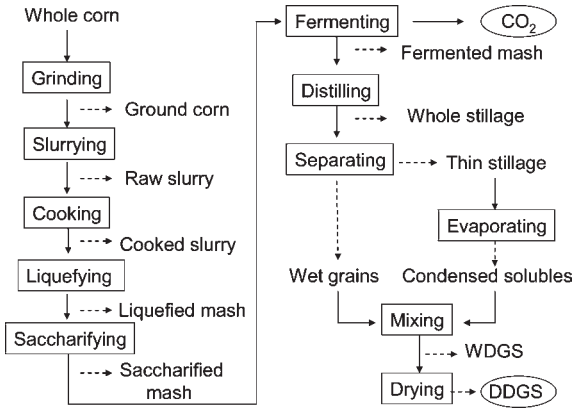

Figure 2. Schematic diagram of a conventional dry-grind ethanol production from corn.

designed to ferment as much of the grain kernel as possible. The whole grain is processed, and little is wasted in the production. The starch in grain flour is converted into ethanol and carbon dioxide. The rest of the grain constituents (protein, lipids, fiber, minerals, and vitamins) are relatively unchanged chemically, but concentrated. These residual components all end up in a coproduct, commonly known as distillers dried grains with solubles (DDGS), which is the major type of DG in the current market. The method consists of several major steps, including dry milling, liquefaction, saccharification, fermentation, distillation, and coproduct recovery (Figure 2). During coproduct recovery, the nonvolatile components following the distillation step, known as whole stillage, are usually centrifuged to produce a liquid fraction (thin stillage) and a solid fraction (distillers wet grains, or DWG). A significant portion (15% or more) of the thin stillage is recycled as backset to be used as processing water to slurry the ground grain. The remaining thin stillage is concentrated through evaporation into condensed distiller solubles (CDS), which are mixed with DWG to become distillers wet grains with solubles (DWGS) and then dried into DDGS.

Although a controversy exists regarding ethanol production from grains,[6,7] there is no question that millions of tons of nonfermentable residues are now available to the feed industry in the form of DDGS (Figure 1). Like fuel ethanol, DDGS has quickly become a global commodity for trade.[1] Because approximately two-thirds of the mass of the starting material (based on the starch content in corn) is converted into ethanol and carbon dioxide during dry-grind processing, it is normally expected that the concentrations of all unfermented nutrients, such as oil, protein, and minerals, will be increased about 3-fold over the original feedstock. Thus, DDGS is a rich source of significant amounts of protein, amino acids, phosphorus, and other nutrients for animal feed. However, the main problem hindering its use as animal feed is high variation in nutrient concentrations and nutritional quality among different sources.[8−11]

Although DG has been on the market for over a century,[12] its surge in global supply in recent years has stimulated many new investigations into this important coproduct of biofeul production. In particular, the chemical composition of DDGS has been of great interest to researchers in animal science, ethanol producers, and traders in feed industry. The literature on the subject is abundant, but a large portion, particularly that published before 1990, dealt with DG from beverage ethanol production. A comprehensive review with updated information about DG from fuel-ethanol production is lacking. Thus, the objective of this review is to provide in-depth and up-to-date coverage of chemical composition of DG in terms of concentrations (quantity) and composition (quality) of major and minor nutrients, changes during dry-grind processing, and the underlying causes for their higher variation in DG (as compared with other protein feeds, such as soy meal). In the final section, mycotoxins in DG are also discussed because there is an increasing concern with the issue. The ultimate goal is to develop strategies to control variation of DG quality, limit levels of mycotoxins, and thus solve the current major problems with use of DDGS as a feed ingredient. For recent reviews on fuel-ethanol production, fermentation technologies, policies, and trends, refer to refs 1, 2, 13, and 14. Reviews on the use of DG in poultry nutrition[15] and in swine diets[16] or constraints and opportunities of using DDGS[10] are also available in the literature.

CHEMICAL COMPOSITION OF DISTILLERS GRAINS

There are many reports on the general composition of DDGS and their variability. Some are in published literature.[8,9,11,16−20] Others are posted in various Web sites of state agricultural extension offices and trade or commodity organizations. Variation in chemical composition among CDS is also reported.[21]

Nutrient contents in DDGS vary with studies (Tables 1−3). Within the same paper, they differ not only among production plants but also between years of production from the same plant or even between batches. It is no surprise that nutrient composition of DDGS often differs from standard reference values as reported in NRC.[22,23] Thus, it has been recommended that a complete chemical analysis of each used source of DDGS be done on a regular basis.

Proximate Composition. In an early study, Cromwell et al.[17] evaluated the physical, chemical, and nutritional properties of DDGS from nine different sources (two from beverage and seven from fuel-alcohol production systems). They found that considerable variability in nutrient contents existed among DDGS samples. When converted to a dry matter basis, crude protein ranged from 26.0 to 31.7%, fat from 9.1 to 14.1%, ash from 3.7 to 8.1%, acid detergent fiber (ADF) from 11.4 to 20.8%, and neutral detergent fiber (NDF) from 33.1 to 43.9% (Table 1). The coefficient of variation (CV) for these nutrients ranged from 5.3 to 27.7%. The average values for protein, oil, ash, total carbohydrate, ADF, and NDF were 29.7, 10.7, 5.3, 54.3, 15.9, and 38.8%, respectively. The average dry matter content was 90.5% with a CV of 1.8%.

A decade later, Spiehs et al.[18] evaluated the nutrient content and variability of DDGS in a total of 118 samples from 10 fuel-ethanol plants during 1997, 1998, and 1999. They found that the average values (% dry matter) for protein, oil, ash, crude fiber, ADF, and NDF were 30.2, 10.9, 5.8, 53.1, 8.8, 16.2, and 42.1, respectively. The CV ranged from 6.4% for protein to 28.4% for ADF (Table 1). The average dry matter content was 88.9%, with a CV of 1.7%. These values are not substantially different from those of Cromwell et al.[17] Both showed higher variation in ash content and lower variation in dry matter content.

Belyea et al.[8] analyzed as many as 235 DDGS samples from a fuel-ethanol plant in Minnesota, and found that the average values (% dry matter) for protein, oil, ash, crude fiber, and ADF were 31.4, 12.0, 4.6, 10.2, and 16.8, respectively. They also reported the average content of residual starch as 5.3%. Thus, Belyea et al.[8] gave higher average values of protein, oil, and crude

Table 1. Gross Composition of DDGS from Different Plants, Years, and Sources Reported in Several Publications[a]

	Cromwell et al.[17]			Spiehs et al.[18]			Belyea et al.[8]			Liu[11]		
	mean	range	CV[b] (%)	mean	range[c]	CV (%)	mean	range[d]	CV (%)[d]	mean	range	CV (%)
no. of data points	9	9	9	118	10	118	235	5	5	6	6	6
dry matter	90.5	87.1−92.7	1.8	88.9	87.2−90.2	1.7						
protein	29.7	26.0−31.7	5.3	30.2	28.7−31.6	6.4	31.4	30.8−33.3	6.3	27.4	25.8−29.1	4.0
oil	10.7	9.1−14.1	6.3	10.9	10.2−11.4	7.8	12.0	10.9−12.6	5.6	11.7	11.0−12.2	4.0
ash	5.3	3.7−8.1	27.7	5.8	5.2−6.7	14.7	4.6	4.3−5.0	5.7	4.4	4.0−4.9	7.8
starch							5.3	4.7−5.9	9.7	4.9	3.2−5.7	25.7
total carbohydrate	54.3			53.1			52.1		5.2	56.5	55.7−57.9	1.6
crude fiber				8.8	8.3−9.7	8.7	10.2	9.6−10.6	3.7			
acid detergent fiber	15.9	11.4−20.8	21.1	16.2	13.8−18.5	28.4	16.8	15.4−19.3	9.3			
neutral detergent fiber	38.8	33.1−43.9	10.0	42.1	36.7−49.1	14.3						

[a] Nutrient values are all expressed in or converted to % dry matter basis. [b] CV, coefficient of variation, also known as relative standard deviation. [c] Range values for means of 10 sample origins (locations). [d] Range and CV (%) values for means of 5 sample groups (by year).

Table 2. Amino Acid Composition of DDGS Reported in Different Sources[a]

	Cromwell et al.[17]			Spiehs et al.[18]			Batal and Dale[24]		Kim et al.[19]	Han and Liu[25]		
	mean	range	CV[b] (%)	mean	range[c]	CV (%)	mean	CV (%)		mean	range	CV (%)
no. of data points	9	9	8	118	10	118	8	8	1	3	3	3
essential												
Arg	1.18	0.95−1.33	9.70	1.20	1.11−2.17	9.1	1.09	14.68	1.4	1.29	1.16−1.40	9.45
His	0.80	0.65−0.93	12.65	0.76	0.72−0.82	7.8	0.69	8.70	0.8	0.91	0.82−1.01	10.41
Ile	1.13	1.06−1.26	5.89	1.12	1.05−1.17	8.7	0.97	6.19	1.1	1.03	0.91−1.25	18.85
Leu	3.69	3.05−4.40	12.56	3.55	3.51−3.81	6.4	3.05	4.59	3.3	3.50	3.18−3.91	10.62
Lys	0.78	0.48−0.97	18.71	0.85	0.72−1.02	17.3	0.71	22.54	1.0	1.04	0.88−1.15	13.63
Met	0.57	0.49−0.61	6.65	0.55	0.49−0.69	13.6	0.54	11.11	0.6	0.72	0.65−0.76	8.45
Phe	1.61	1.39−1.91	9.48	1.47	1.41−1.57	6.6	1.31	3.05	1.4	1.50	1.37−1.76	14.79
Thr	1.13	0.99−1.28	10.00	1.13	1.07−1.21	6.4	0.96	6.25	1.1	1.17	1.06−1.26	8.67
Trp	0.22	0.18−0.25	11.09	0.25	0.21−0.27	6.7	0.20	25.00	0.2			
Val	1.49	1.30−1.64	7.18	1.50	1.43−1.56	7.2	1.33	5.26	1.5	1.56	1.40−1.80	13.72
nonessential												
Ala							1.78	3.93	1.9	2.07	1.86−2.27	9.91
Asp							1.75	11.43	1.7	1.97	1.77−2.16	9.91
Cys	0.59	0.49−0.66	9.52				0.56	7.14	0.5	0.57	0.53−0.60	6.33
Glu							3.49	6.88	3.3	5.48	4.94−6.01	9.76
Gly									1.1	1.19	1.11−1.31	8.72
Pro							1.99	5.03	2.0	2.19	1.94−2.63	17.32
Ser							1.09	6.42	1.2	1.45	1.32−1.58	9.00
Tyr							0.96	9.38	1.2	1.02	0.87−1.29	22.65

[a] Nutrient values are expressed in or converted to perent dry matter basis, except for data of Batal and Dale[24], which are expresssed as percent wet (as is) basis. [b] CV, coefficient of variation. [c] Range values for means of 10 sample origins (locations).

fiber and a lower value of ash compared to Spiehs et al.[18] Liu[11] showed average values of six DDGS samples for protein, oil, ash, and starch as 27.4, 11.7, 4.4, and 4.9%, respectively, dry matter basis. The lower estimate of the protein value as compared with the previous three studies might be due to the use of 5.75 as conversion factor from nitrogen instead of 6.25.

Amino Acid Composition. Cromwell et al.[17] reported that among nine different sources from beverage or fuel-alcohol production systems lysine varied from 0.48 to 0.97% (converted to dry matter basis), methionine from 0.49 to 0.61%, threonine from 0.99 to 1.28%, and tryptophan from 0.18 to 0.25% (Table 2). Lys was the most variable among the 11 amino acids (AA) measured, with a CV = 18.71%. In the Spiehs et al. (2002) study, 119 DDGS samples were analyzed for 10 essential amino acids. On a dry matter basis, the average lysine content was found to be 0.85%, ranging from 0.72 to 1.02%. Again, lysine was found to be the most variable among the 10 amino acids measured, with an average CV = 17.3% (Table 2). Methionine values ranged from 0.49 to 0.69%.

Table 3. Comparison of Mineral Concentration in Corn and DDGS among Several Studies[a]

source	parameter	K (mg/g)	P (mg/g)	Mg (mg/g)	S (mg/g)	Na (mg/g)	Ca (mg/g)	Fe (μg/g)	Zn (μg/g)	Mn (μg/g)	Cu (μg/g)
					Corn						
Belyea et al.[9]	no. of samples	9	9	9	9	9	9	9	9	9	9
	minimum	3.51	2.64	1.07	1.06	0.00	0.05	21	18	4.7	1.3
	maximum	3.87	3.09	1.21	1.26	0.01	0.09	31	21	5.6	1.6
	mean	3.61	2.87	1.16	1.11	0.00	0.06	25.33	19.67	5.26	1.42
	CV[b] (%)	3.24	5.25	3.59	5.77	55.98	26.17	11.34	5.08	5.22	8.45
Liu and Han[28]	no. of samples	3	3	3	3	3	3	10	3	3	3
	minimum	3.83	2.75	1.14	1.04	0.01	0.05	8.01	21.23	5.19	1.83
	maximum	4.10	3.01	1.31	1.08	0.01	0.09	10.25	23.42	5.36	2.01
	mean	3.92	2.89	1.21	1.07	0.01	0.07	9.02	22.34	5.32	1.92
	CV (%)	3.92	4.42	7.13	1.91	0.00	28.11	12.62	4.91	2.26	4.69
					DDGS						
Spiehs et al.[18]	no. of samples	118	118	118	118	118	118	118	118	118	118
	minimum	6.9	7.0	2.5	3.3	1.2	0.3	75.3	44.7	10.7	4.7
	maximum	10.6	9.9	3.7	7.4	5.1	1.3	156.4	312.0	21.3	7.6
	mean	9.4	8.9	3.3	4.7	2.4	0.6	119.8	97.5	15.8	5.9
	CV (%)	14.00	11.70	12.10	37.10	70.50	57.20	41.10	80.40	32.70	20.40
Batal and Dale[27]	no. of samples	12	12	12	12	12	12	12	12	12	12
	minimum	6.7	5	2.1	5.8	0.9	0.1	67	44	9	3
	maximum	9.9	7.7	3.3	11	4.4	7.1	325	88	48	18
	mean	9.1	6.8	2.8	8.4	2.5	2.9	149	61	22	10
	CV (%)	12.08	12.29	14.28	25.00	60.00	93.00	57.70	21.30	50.00	43.00
Belyea et al.[9]	no. of samples	9	9	9	9	9	9	9	9	9	9
	minimum	9.31	7.10	2.99	3.44	0.60	0.25	90.0	75.0	15.6	4.9
	maximum	12.40	9.43	3.79	8.27	2.30	0.34	109.0	170.0	19.3	6.8
	mean	11.22	8.52	3.48	5.76	1.30	0.28	98.7	113.7	17.0	5.6
	CV (%)	9.60	8.71	7.70	25.06	40.99	11.14	5.87	36.52	7.04	10.92
Liu and Han[28]	no. of samples	3	3	3	3	3	3	3	3	3	3
	minimum	10.72	8.35	3.24	6.03	2.16	0.31	17.52	63.36	14.57	5.01
	maximum	12.42	9.28	3.63	7.94	2.94	0.48	26.63	67.28	17.98	6.07
	mean	11.44	8.73	3.45	6.83	2.63	0.37	21.47	65.15	15.81	5.55
	CV (%)	7.66	5.60	5.79	14.56	15.56	26.02	21.75	3.04	11.93	9.52

[a] Values are expressed on dry matter basis. [b] CV, coefficient of variation, also known as relative standard deviation.

Average tryptophan and threonine values were 0.25 and 1.13%, respectively. The mean values for arginine, histidine, phenylalanine, isoleucine, leucine, and valine were 1.20, 0.76, 1.47, 1.12, 3.55, and 1.50%, respectively. Cromwell et al.[17] and Spiehs et al.[18] only analyzed essential AA, but others[19,24,25] looked at contents of both essential and nonessential AA. Like proximate composition, variation in contents of individual amino acids exists among papers and among sample sources.

Due to its high susceptibility to heat damage, lysine content and digestibility are the major concerns in the use of DDGS as a feed component. Cromwell et al.[17] reported that Lys concentration tended to be lowest in the darkest colored and highest in the lightest colored DDGS, and correlation between the Hunter *L*, *a*, and *b* scores and Lys content was also significant. This was later confirmed by Fastinger et al.,[26] who reported that Lys content in five sources ranged from 0.48 to 0.76% with the lowest Lys content in the darkest DDGS source. They also reported that apparent and true Lys digestibility evaluated in adult roosters was significantly lower in the dark-colored than in other DDGS samples. Differences in digestibility of other essential amino acids among sources were smaller but also significant. Concurrently, Batal and Dale[24] observed considerable differences in the true amino acid digestibility among samples, as well as significantly lower total and digestible Lys content in the darker DDGS samples, and attributed them to the Maillard reaction between reducing carbohydrates such as glucose and the ε-amino group of Lys. The reaction could result in destruction of a significant amount of Lys during excessive heating. They further suggested that color analysis might be a quick and reliable method of estimating the amino acid, particularly Lys, digestibility of DDGS for poultry.

On the basis of the above discussion, although the protein content in DDGS is increased 3-fold over that in a grain feedstock, the protein quality (in terms of amino acid composition

relative to total AA) is not substantially improved over that of the grain. Because corn is the primary grain used in ethanol production, the resulting DDGS has an amino acid (AA) profile similar to that of corn, although fermentation yeast has some effect (to be discussed later). Thus, the protein quality of DDGS is considered to be incomplete, relative to the amino acid requirements of animals.

Minerals. Many studies have also documented mineral composition in DDGS.[9,18,27,28] As in other biological materials, major minerals in DDGS are Ca, P, K, Mg, S, and Na. Mean concentrations ranged from 0.05% for Ca to 1.15% for K (dry matter). Concentrations of the other four fall in between (Table 3). Minor minerals in DDGS include Zn, Mn, Cu, Fe, Al, and Se. Their concentrations range between 6 ppm for Cu and 149 ppm for Fe, among studies. Variation in mineral contents is much larger than the composition of other nutrients (Tables 1 and 2). For some minerals (such as S, Na, and Ca), the CV value can be unusually high within a single study. Exogenous addition of some mineral compounds during processing may be an explanation. For example, ethanol plants may use sodium hydroxide to sanitize equipment. They may also use it, along with sulfuric acid, to adjust the pH of mashes for optimum enzyme activity during liquefaction and/or to meet yeast requirements during fermentation.[9]

High concentrations and high variation of minerals affect the value and end use of DDGS as animal feed.[9,18,27,28] High concentration can lead to not only nutritional disorders but also excessive minerals in wastes, whereas high variation in mineral contents makes accurate diet formulation difficult because assumed concentrations could be different from actual concentrations. Among all of the minerals in DDGS, phosphorus (P) is of greatest interest for all types of feed because it is the third most expensive nutrient in the diet and has significant implications in not only animal nutrition but also the environment. Although the mean level of P varies among papers (Table 3), a concentration range of 0.5−1.0% is generally agreeable. Such a concentration range is much higher than that in common grains and exceeds the requirements of most ruminants.[29] Thus, the high P concentration of DDGS has become an emerging issue. When ruminants consume diets containing elevated concentrations of P, such as diets with high DDGS inclusion, the amount of P excreted in wastes is increased.[30]

Lipids. The lipid in DDGS originates from the feedstock for ethanol production. In North America, the major feedstock is yellow dent corn, although sorghum and other grains are also used to a limited extent. Therefore, lipid profiles in DDGS mostly resemble those in corn, except for an about 3-fold increase in concentrations. The major lipid in DG is triglyceride, and minor ones include phytosterols, tocopherols, tocotrienols, and carotenoids.[31−36] Yet, unlike the original feedstock, DG is found to contain unusually high amounts of free fatty acids (6−8 vs 1−2% in corn, based on extracted oil weight).[33,34,36] Oil extracted from solubles was also found to contain higher levels of free fatty acids (7.92−12.18 vs 2.28%, oil mass basis).[36]

Because the crude oil content in DG is around 10%, there is a renewed interest in removing oil either before (front-end or upstream processing)[37] or after fermentation (back-end or downstream processing).[38,39] Unlike oil removed at the front end, oil removed at the back end is no longer edible but can be used as a feedstock of biodiesel production.

Carbohydrates and Low Molecular Weight Organics. During dry-grind processing, starch is converted to simple sugars, which are then fermented to ethanol and carbon dioxide. However, other carbohydrates (CHO), such as cell wall CHO, remain relatively unchanged chemically. DDGS also contain low molecular weight organic compounds, which are present in the original feedstock or produced during the process. Because starch conversion cannot lead to completion under normal processing conditions, there are also some residual starches and sugars in the coproduct.[8,11]

Dowd et al.[40] reported that the low molecular weight organics in distillers solubles made from corn included lactic acid (10.40 g/L), glycerol (5.8 g/L), and alanine (free amino acid, 4.08 g/L), as well as smaller amounts of ethanol, and various nonnitrogenous and nitrogenous acids, polyhydroxy alcohols, sugars, and glucosides. Kim et al.[19] reported that thin stillage also contained lactic acid (16.8 g/L), glycerol (14.4 g/L), and other lower molecular weight organics. When adjusted for moisture content, the values for these constituents were higher than those of Dowd et al.[40]

Wu[41] measured various types of sugars in DDGS, distillers dried grains (DDG), and distillers dried solubles (DDS) after hydrolyzing samples with trifluoroacetic acid (TFA) followed by high-performance liquid chromatography (HPLC) analysis and found that these ethanol coproducts contained many sugars after TFA hydrolysis, including glycerol, arabinose, xylose, mannose, glucose, and galactose. The carbohydrates of DDS had the highest percentages of neutral sugars (38.7% total), followed by DDGS (38.0%) and DDG (35.8%). The sugar composition also differed among the three coproducts. For example, among the sugars in DDGS, the highest amount was glucose (11.9%), followed by xylose (8.5%), glycerol (7.8%), arabinose (6.4%), galactose (1.9%), and mannose (1.6%). Note that these sugars were not present in free form, but rather as a complex carbohydrate, commonly seen in cell walls. They became measurable after TFA hydrolysis.

Traditionally, DG is mainly used as animal feed. Compositional analysis is thus centered at such key nutrients as protein, oil, minerals, etc. Yet, with an increasing demand for fuel ethanol, DG is viewed as a potential feedstock for cellulosic ethanol production. Thus, Kim et al.[19] developed a new analytical approach, which aimed at determining more detailed chemical composition, especially of polymeric sugars, such as cellulose, starch, and xylan, which release fermentable sugars upon action by cellulosic enzymes. Not surprisingly, DDGS had higher water extractives than DDG because the solubles, as a part of DDGS, contained more simple sugars (Table 4). Here, the ether extractives can be considered as crude oil content, whereas water extractives can be considered as soluble carbohydrates. When studying this table, assuming that the complex carbohydrate consists of glucan (which includes residual starch and cellulose), xylan, and arabinan, by adding all four constituents plus water extractives, we can have a total CHO as measured amount. This measured amount (59.4% dry matter) is very close to the calculated amount of total CHO (59.0%) by subtracting the sum of protein, ether extractives, and ash from 100%. In either case, the total CHO content in DDGS was higher than those reported in Table 1.

CHANGES IN CHEMICAL COMPOSITION DURING DRY-GRIND PROCESSING

Some workers reported compositional differences between the raw material (mostly corn) and the end product (DDGS) of the dry-grind process.[8] Others investigated compositional

Table 4. Cellulosic Biomass Compositional Analysis of Distillers Wet Grains (DWG) and DDGS[a]

contituent	DWG	DDGS
dry matter (% wet basis)	35.3	88.8
ether extractives	9.6	11.6
crude protein	36.6	24.9
ash	2.0	4.5
total CHO[b] by calculation	51.8	59.0
water extractives	8.8	24.7
glucan	18.5	21.2
cellulose	12.6	16.0
starch	5.9	5.2
xylan and arabinan	20.9	13.5
xylan	14.9	8.2
arabinan	5.5	5.3
total CHO[b] measured	48.2	59.4

[a] Adapted from Kim et al.[19]. All values are expressed on dry matter basis except where otherwise noted. [b] CHO, carbohydrate.

changes in a laboratory setting by comparing different versions of the dry-grind process, that is, traditional versus modified methods.[37,42] These studies provided some information about changes that occur during the process, but lacked step-by-step details. Belyea et al.[9] investigated mineral concentrations of primary process streams from the dry-grind process, the first of its kind, although the documented changes were limited to minerals only. Recently, Liu and his colleagues conducted a comprehensive study, with an objective to monitor changes in concentrations and composition of various nutrients during the entire dry-grind process, from corn to the final product. The study, documented in several papers,[25,28,36] used sets of samples that were provided from three commercial dry-grind ethanol plants in Iowa. Each set consisted of ground corn, yeast, intermediate masses, and DDGS (Figure 2). Intermediate masses included raw slurry, cooked slurry, liquefied mash, saccharified mash, fermentation mash (beer), whole stillage, thin stillage, CDS (syrup), DWG (wet cake), and DWGS, although the total number of intermediate masses varied slightly among the plants. The results of these reports are covered in detail in the following subsections.

Changes in Proximate Composition. Protein, oil, and ash contents in the dry mass of processing streams increase slightly at the beginning of the process, up to the saccharification step (Figure 3a). The increase of these components in cooked slurry as compared with ground corn is most likely due to the use of a portion of thin stillage as backset to slurry ground corn; the contents of protein, oil, and ash in thin stillage are much higher than those in ground corn. After fermentation, these nutrients are concentrated dramatically, >3-fold over corn. The increase is mainly due to depletion of starch as it is fermented into ethanol and carbon dioxide. Distillation causes little change in composition but centrifugation does; thin stillage is higher in oil and ash content but lower in protein content than DWG (dry matter basis). This implies that in whole stillage a larger portion of oil is

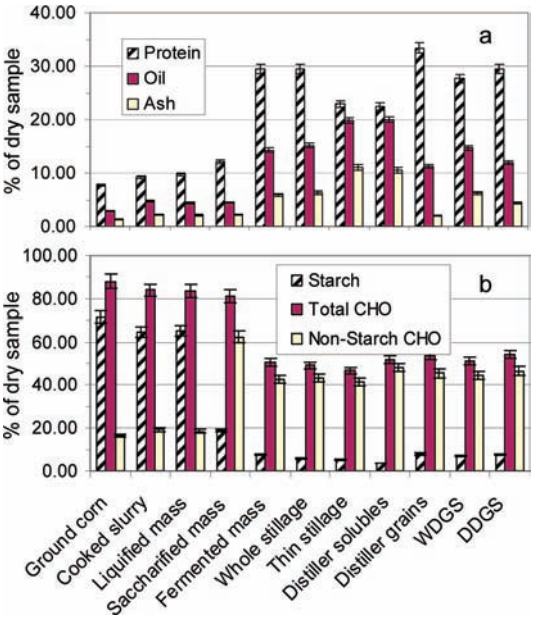

Figure 3. Changes in proximate composition during dry-grind ethanol processing from corn at a commercial plant in Iowa: (a) contents of protein, oil, and ash; (b) contents of starch/dextrin, total CHO (carbohydrates), and total nonstarch CHO. Adapted from Han and Liu.[25]

in emulsion and the majority of ash is soluble, so that more goes into the liquid fraction than into the solid fraction during centrifugation. Among all of the downstream samples, on a dry matter basis, oil and ash are highest in thin stillage and its condensed form (CDS), whereas protein is highest in DWG. In addition, the ash content is so greatly reduced in DWG upon centrifugation that it is only slightly higher than that in ground corn. When the two are mixed together to become DWGS, the composition is averaged and becomes similar to that of the whole stillage. There is a slight but significant ($P < 0.05$) difference in contents of protein, oil, and ash between DWGS and DDGS. This difference is most likely due to the dynamics of drying, because a portion of the DDGS output is recycled and mixed with DWG and CDS for improving operation performance.[43]

Changes in starch/dextrin and total carbohydrate during the dry-grind process of corn (Figure 3b) are opposite to those of protein, oil, and ash (Figure 3a). At the beginning of the process, starch and dextrin are relatively unchanged, although a decrease from corn to cooked slurry is noticeable. This decrease is apparently due to an increase in protein, oil, and ash contents discussed earlier. Upon saccharification, starch/dextrin decreases substantially and further to about 6% after fermentation. It remains unchanged in the rest of the processing streams. Enzymatic action and fermentation convert most of the starch to ethanol, but apparently cannot reach a complete conversion. Residual starch in coproducts was also reported elsewhere.[8,11,44] Concomitantly with starch/dextrin change, the total CHO is relatively stable at about 83% until fermentation, when it decreases substantially to about 51%. This value fluctuates slightly in the rest of the processing streams (Figure 3b).

Total nonstarch carbohydrate refers to all carbohydrates excluding starch and dextrin. It includes cellulose, hemicellulose, and lignin, which are all cell wall components. It also includes soluble sugars and other low molecular weight organics. In

Table 5. Changes in Amino Acid Concentration (Percent Dry Weight) during Dry-Grind Ethanol Processing from Corn at Plant 1 (Adapted from Han and Liu[25])

amino acid (AA)	milled corn[a]	cooked slurry	liquefied mass	saccharified mass	fermented mass	whole stillage	thin stillage	distiller solubles	distiller grains	WDGS	DDGS[a]	yeast[a]
essential												
Arg	0.36 ± 0.03	0.37	0.34	0.39	1.28	1.22	1.03	0.98	1.34	1.24	1.32 ± 0.02	1.59 ± 0.06
His	0.32 ± 0.01	0.36	0.31	0.39	0.81	0.87	0.65	0.60	1.00	0.74	1.01 ± 0.00	0.87 ± 0.01
Ile	0.37 ± 0.03	0.29	0.35	0.30	1.05	0.77	0.48	0.69	0.99	0.96	0.91 ± 0.01	1.38 ± 0.01
Leu	1.24 ± 0.01	1.03	1.14	1.12	3.21	2.75	1.35	1.62	3.97	2.83	3.42 ± 0.04	2.37 ± 0.03
Lys	0.32 ± 0.00	0.32	0.32	0.31	1.13	1.01	0.81	0.97	1.17	1.05	1.09 ± 0.03	2.57 ± 0.01
Met	0.34 ± 0.08	0.45	0.33	0.37	0.67	0.69	0.60	0.56	0.68	0.48	0.76 ± 0.04	0.66 ± 0.03
Phe	0.66 ± 0.05	0.49	0.68	0.58	1.53	1.22	0.76	0.97	1.59	1.26	1.38 ± 0.07	1.44 ± 0.05
Thr	0.40 ± 0.01	0.40	0.38	0.42	1.15	1.05	0.77	0.83	1.29	1.05	1.19 ± 0.02	1.84 ± 0.02
Val	0.76 ± 0.01	0.69	0.75	0.70	1.57	1.31	1.03	1.23	1.55	1.40	1.47 ± 0.02	1.68 ± 0.00
nonessential												
Ala	0.66 ± 0.01	0.66	0.66	0.66	2.05	1.81	1.29	1.45	2.20	1.87	2.09 ± 0.03	1.79 ± 0.02
Asp	0.60 ± 0.02	0.63	0.64	0.65	1.97	1.76	1.26	1.42	2.11	1.78	1.99 ± 0.03	3.35 ± 0.05
Cys	0.30 ± 0.05	0.34	0.30	0.35	0.59	0.55	0.49	0.46	0.64	0.48	0.58 ± 0.02	0.43 ± 0.03
Glu	1.80 ± 0.02	1.68	1.78	1.73	5.30	4.74	3.22	3.51	5.93	4.69	5.50 ± 0.06	6.33 ± 0.02
Gly	0.35 ± 0.02	0.34	0.36	0.37	1.20	1.08	0.93	1.03	1.14	1.13	1.16 ± 0.03	1.44 ± 0.01
Pro	0.68 ± 0.04	0.45	0.73	0.54	2.21	1.56	0.84	1.30	2.17	1.91	1.94 ± 0.03	0.68 ± 0.08
Ser	0.51 ± 0.01	0.49	0.48	0.53	1.41	1.26	0.87	0.92	1.60	1.27	1.44 ± 0.02	1.71 ± 0.03
Tyr	0.49 ± 0.17	0.38	0.50	0.35	1.16	0.78	0.56	0.76	1.00	0.74	0.91 ± 0.02	0.81 ± 0.15
total AA	10.13 ± 0.28	9.35	10.04	9.73	28.28	24.46	16.93	19.32	30.38	24.88	28.15 ± 0.46	30.91 ± 0.31
protein	7.70 ± 0.22	9.27 ± 0.13	9.77 ± 0.04	12.08 ± 0.02	29.43 ± 0.03	29.50 ± 0.48	22.90 ± 0.63	21.31 ± 0.24	33.40 ± 0.09	27.67 ± 0.37	29.47 ± 0.04	36.90 ± 0.28

[a] Mean ± standard deviation. The rest are values of single measurement.

ground corn, starch is a major portion of total CHO, and the total nonstarch CHO was around 17% of dry matter (Figure 3b). This value remains relatively unchanged until saccharification, when it increases significantly due to conversion of starch and dextrose to simple sugars. Upon fermentation, depletion of soluble sugars causes some decrease in total nonstarch CHO, but the value is still about 43%, more than double the value in ground corn. This value fluctuated slightly in the remaining streams.

Changes in Amino Acid Composition. Amino acid composition is a major nutritional index of a protein ingredient. It is typically expressed as concentration (% of sample weight, dry or fresh weight basis) or relative percent (based on weight of total amino acids or protein in a given sample). DDGS proteins, like other proteins, contain essential and nonessential amino acids. In general, changes in AA concentrations (Table 5), either essential or nonessential, follow the pattern of protein changes during the dry-grind process (Figure 3a). Before fermentation, there is a slight change. Upon fermentation, concentrations of all AA increase 2.0−3.5-fold, resulting from starch depletion. When whole stillage is separated into thin stillage and DWG, AA concentrations, just like protein content, are higher in DWG than in thin stillage. When thin stillage is concentrated to CDS and mixed with DWG into DWGS, the concentration of each AA becomes close to that in whole stillage. There is some minor change upon drying into DDGS. The content of total amino acids is close to the protein content in each sample, but the difference between the two fluctuates between positive and negative values, depending on sample type and ethanol plant. This difference is presumably attributed to the difference in nonprotein nitrogen content among samples and the variation of two separate analytical methods. Note that Table 6 also includes yeast AA concentration.

Although the general trend in AA concentration follows that of protein content, the extent of change for each AA of a given downstream product as compared with that of ground corn varies with individual AA (Table 5). For example, upon fermentation, some amino acids increase in concentration significantly more rapidly than others. Furthermore, when the AA profile is expressed as relative percent (based on total AA), it describes more protein quality than quantity. Unlike AA concentrations, the change in AA composition in terms of relative percent (Table 6, converted from Table 5) does not follow the trend of protein change. Upon fermentation, some AA increase, others decrease, and still others remain unchanged.

Yu et al.[45] had limited comparison of protein molecular structures and fraction profiles between feedstock grains (corn and wheat) and resulting DDGS and found that proteins from original grains had a significantly higher ratio of α-helix to β-sheet than those of coproducts produced from bioethanol processing (1.38 vs 1.03, $P < 0.05$). There were significant differences between wheat and corn (1.47 vs 1.29, $P < 0.05$) but no difference between wheat DDGS and corn DDGS (1.04 vs 1.03, $P > 0.05$).

Changes in Fatty Acid Composition. When the fatty acid composition of DDGS oil is expressed in relative percent, linoleic acid is the major one (53.96−56.53%), followed by oleic acid (25.25−27.15%) and then palmitic acid (13.25−16.41%), with low levels of stearic (1.80−2.34%) and linolenic (1.15−1.40%) acids.[35,36] Although some minor yet significant differences exist in mean values of individual fatty acid among steps (fractions), all major fatty acids generally remain constant from corn to DDDS during dry-grind processing.[36]

Table 6. Changes in Amino Acid Composition (Relative Percent of Individual Amino Acids in Each Sample) during the Dry-Grind Ethanol Process from Corn at Plant 1 (Adapted from Liu and Han[25])

amino acid (AA)	milled corn[a]	cooked slurry	liquefied mass	saccharified mass	fermented mass	whole stillage	thin stillage	distiller solubles	distiller grains	WDGS	DDGS[a]	yeast[a]
essential												
Arg	3.55 ± 0.43	3.96	3.40	4.05	4.51	4.98	6.11	5.09	4.42	5.00	4.68 ± 0.13	5.15 ± 0.15
His	3.13 ± 0.17	3.85	3.09	4.05	2.86	3.57	3.84	3.13	3.28	2.98	3.59 ± 0.06	2.81 ± 0.07
Ile	3.61 ± 0.35	3.08	3.50	3.04	3.71	3.15	2.84	3.56	3.25	3.85	3.25 ± 0.02	4.46 ± 0.00
Leu	12.23 ± 0.25	11.00	11.32	11.49	11.36	11.23	7.95	8.40	13.07	11.38	12.16 ± 0.06	7.67 ± 0.18
Lys	3.19 ± 0.09	3.41	3.19	3.15	3.99	4.13	4.76	5.03	3.84	4.22	3.87 ± 0.05	8.31 ± 0.04
Met	3.30 ± 0.74	4.84	3.29	3.83	2.38	2.82	3.55	2.88	2.25	1.93	2.68 ± 0.10	2.14 ± 0.08
Phe	6.49 ± 0.32	5.28	6.79	5.97	5.40	4.98	4.47	5.03	5.22	5.05	4.90 ± 0.18	4.66 ± 0.20
Thr	3.96 ± 0.03	4.29	3.81	4.28	4.07	4.27	4.55	4.29	4.25	4.22	4.23 ± 0.01	5.95 ± 0.00
Val	7.50 ± 0.12	7.37	7.51	7.21	5.56	5.35	6.11	6.38	5.12	5.64	5.22 ± 0.00	5.43 ± 0.07
nonessential												
Ala	6.56 ± 0.26	7.04	6.58	6.76	7.25	7.42	7.60	7.48	7.26	7.52	7.42 ± 0.01	5.79 ± 0.00
Asp	5.91 ± 0.33	6.71	6.38	6.64	6.97	7.19	7.46	7.36	6.95	7.16	7.06 ± 0.00	10.83 ± 0.06
Cys	2.95 ± 0.42	3.63	2.98	3.60	2.09	2.25	2.91	2.39	2.11	1.93	2.06 ± 0.02	1.38 ± 0.12
Glu	17.73 ± 0.66	17.93	17.70	17.79	18.73	19.40	19.03	18.15	19.53	18.85	19.52 ± 0.09	20.47 ±
Gly	3.43 ± 0.26	3.63	3.60	3.83	4.23	4.42	5.47	5.33	3.77	4.54	4.11 ± 0.05	4.66 ± 0.01
Pro	6.67 ± 0.23	4.84	7.30	5.52	7.81	6.39	4.97	6.74	7.16	7.66	6.90 ± 0.00	2.18 ± 0.25
Ser	5.02 ± 0.05	5.28	4.73	5.41	4.99	5.17	5.11	4.78	5.25	5.09	5.12 ± 0.03	5.52 ± 0.14
Tyr	4.82 ± 1.54	4.07	4.94	3.60	4.11	3.19	3.34	3.92	3.28	2.98	3.23 ± 0.00	2.60 ± 0.46

[a] Mean ± standard deviation. The rest are values of single measurement.

Changes in Functional Lipids. According to Moreau et al.,[36] the major phytosterols in ground corn are sitosterol > campesterol > sitostanol > campestanol. Ten other minor phytosterols (stigmasterol, avenasterol, and others) and squalene are also detected, but their total proportions ranged from 12 to 15% (based on total phytosterols mass). Because ergosterol, the major sterol in yeast,[46] is not detected in any of the postfermentation samples, the contribution of yeast sterols to the total phytosterol pool is considered to be negligible. There are some differences in the sterol levels among samples collected at each step, but no obvious trends are observed. The proportions of the various phytosterols remain relatively constant among the nine fractions. Total phytosterol content in oil extracted from DDGS samples ranges from 1.5 to 2.5% and averages around 2%. These data indicate that phytosterol content and composition remain relatively constant throughout the dry-grind process.

Moreau et al.[36] also made quantitative analyses of the tocotrienols and tocopherols in the various fractions (Table 7) and confirmed previous reports that γ-tocopherol is the major tocopherol and γ-tocotrienol is the major tocotrienol in ground corn[47] and in DDGS,[31] with small amounts of α- and δ-tocopherols and trace amounts of α- and δ-tocotrienols. Yet, in the HPLC chromatograms, of all samples, there is an unknown peak that eluted between α-tocopherol and α-tocotrienol, which prompted Moreau et al.[35] to suggest that the unknown peak might be "α-tocomonoenol". Overall, levels of tocols and the proportions of the homologues remain relatively stable throughout the dry-grind operation. However, there is an exception. Both δ-tocopherol and the unknown peak that was named α-tocopherol* show significant increases upon fermentation and remained relatively high thereafter. Because the total tocols include the values of the two compounds, they show significantly higher values for all fractions after fermentation. Thus, dry-grind processing causes little change to the majority of the tocols and some minor increases of the rest. The preservation of these important antioxidants may help maintain the oxidative stability of corn oil extracted from DDGS.

Changes in Mineral Composition. Following the study to monitor P concentrations and flow in corn wet milling streams,[48] for the first time, Belyea et al.[9] studied element concentrations in primary process streams from nine dry-grind plants. Samples included corn, ground corn, fermented mash (beer), syrup, wet cake, and DDGS. They found that there were differences among streams for mean concentrations of many elements. The concentrations of most elements in beer were 3 times those in corn. CDS (syrup) had the highest element concentrations. However, the study did not include samples of cooked slurry, liquefied mash, and thin stillage. Liu and Han[28] conducted a similar study but included all possible streams of dry-grind processing, from corn to DDGS, and found that the changes in individual mineral content followed the changing pattern of protein, oil, and ash shown in Figure 3a. Fermentation caused the most dramatic increase in mineral content mainly due to depletion of starch. Upon centrifugation, more minerals went to the liquid fraction (thin stillage) than to the solid fraction (DWG). They also showed that among processing streams, thin stillage (not CDS) had the highest levels of all minerals, whereas DWG grains had the lowest.

More importantly, several studies reported larger fold (>3) increases in Na, S, and Ca concentrations in processing streams (including DDGS) over corn, as compared to other minerals. Batal and Dale[27] noted that the content of most minerals in DDGS appeared generally consistent with a 3-fold concentration

Table 7. Tocopherol and Tocotrienol Composition of Nine Fractions from Three Dry-Grind Ethanol Plants[a] (Adapted from Moreau et al.[36])

plant	fraction	α T	α T*	γ T	δ T	α T3	γ T3	δ T3	total tocols
8	1	12.74	46.22	109.93	16.19	12.08	24.90	1.35	223.42
9	1	19.80	32.13	84.92	9.96	11.09	19.48	0.98	178.37
10	1	23.16	31.35	85.80	9.65	13.04	25.94	1.48	190.41
mean[b]		18.57 b	36.56 e	93.55 b	11.94 e	12.07 b	23.44 e	1.27 cde	197.4 f
8	2	9.88	10.01	93.48	11.05	7.23	19.18	1.20	152.04
9	2	16.70	9.47	74.02	10.31	6.84	15.65	0.87	133.85
10	2	22.27	8.88	76.60	6.41	8.55	18.75	0.99	142.45
mean[b]		16.28 d	9.45 g	81.36 d	9.25 f	7.54 e	17.86 f	1.02 e	142.78 h
8	3	9.95	19.58	89.86	13.74	9.06	21.87	1.23	165.29
9	3	18.86	12.01	80.48	10.85	9.82	20.80	1.05	153.86
10	3	22.27	18.88	73.50	9.84	11.65	24.67	1.28	162.07
mean[b]		17.03 cd	16.82 f	81.28 d	11.48 e	10.17 cd	22.45 e	1.19 de	160.41 g
8	4	11.79	126.05	95.75	64.82	10.20	25.52	1.89	336.03
9	4	16.50	103.34	72.41	70.23	9.90	22.62	1.17	296.17
10	4	22.47	103.79	73.26	49.72	10.33	23.04	1.59	284.20
mean[b]		16.92 cd	111.06 d	80.47 d	61.59 a	10.14 cd	23.73 de	1.55 bcd	305.47 c
8	5	13.76	154.29	100.79	69.44	10.90	28.04	2.07	379.29
9	5	18.21	90.28	77.16	61.07	11.08	24.59	1.45	283.83
10	5	18.69	126.79	70.76	48.31	10.67	23.61	1.67	300.49
mean[b]		16.88 cd	123.79 c	82.9 cd	59.60 b	10.88 c	25.42 c	1.73 bc	321.2 b
8	6	9.33	188.08	91.09	70.66	12.74	33.29	2.48	407.68
9	6	15.98	113.45	77.01	50.41	15.37	32.63	2.64	307.49
10	6	17.80	201.42	67.74	54.01	13.30	30.70	3.16	388.14
mean[b]		14.37 e	167.65 a	78.61 d	58.36 b	13.80 a	32.21 a	2.76 a	367.77 a
8	7	9.62	93.63	88.05	12.55	12.09	30.72	2.15	248.81
9	7	15.95	123.11	68.84	34.86	13.40	28.28	2.76	287.19
10	7	17.25	155.53	59.94	23.70	13.61	29.12	2.41	301.55
mean[b]		14.27 e	124.09 c	72.28 e	23.7 d	13.03 a	29.37 b	2.44 a	279.18 d
8	8	14.06	40.29	112.53	27.93	6.71	18.25	1.06	220.82
9	8	21.52	30.79	89.95	28.51	7.31	17.25	0.88	196.21
10	8	28.82	50.84	92.90	27.55	8.01	19.20	0.96	228.29
mean[b]		21.47 a	40.64 e	98.46 a	28.00 c	7.34 e	18.23 f	0.97 e	215.10 e
8	9	13.21	112.21	104.00	22.14	9.62	25.29	1.91	288.37
9	9	16.18	87.01	73.87	28.32	7.52	20.30	1.41	234.61
10	9	24.05	197.89	84.52	24.39	12.79	29.33	2.35	375.32
mean[b]		17.81 bc	132.37 b	87.46 c	24.95 d	9.98 d	24.97 cd	1.89 b	299.43 c
8	mean[c]	11.59 c	87.82 b	98.39 a	34.28 a	10.07 b	25.23 a	1.71 a	269.08 a
9	mean[c]	17.74 b	66.84 c	77.63 b	33.83 a	10.26 b	22.4 b	1.47 b	230.18 c
10	mean[c]	21.86 a	99.49 a	76.11 b	28.17 b	11.33 a	24.93 a	1.76 a	263.66 b

[a] Means of duplicate results, expressed as mg/100 g extracted oil. T, tocopherol; T3, tocotrienol; T*, an unknown tocopherol. [b] Column means of three plants for each of nine fractions bearing different letters differ significantly at $P < 0.05$. [c] Column means of nine fractions for each of three plants bearing different letters differ significantly at $P < 0.05$.

increase, but unusually larger range of values were noted for Na and Ca. Belyea et al.[9] reported that in the beer stream, both Na and S had unusually higher increases in concentrations than other minerals. They attributed this to addition of Na and S

compounds during the dry-grind process. Liu and Han[28] showed that Na, S, and Ca in DDGS had much higher fold increases over corn than other minerals, presumably due to exogenous addition of compounds containing these minerals.

Changes in Various Types of Phosphorus. Besides concentration, bioavailability of P in animal feed is another important factor that affects retention of P in ingested feeds by animals and the amount of P excreted in wastes. P bioavailability is determined by its chemical forms. Grains and their byproducts contain different types of P, including inorganic P, phytate P, and other P. Phytate or inositol hexaphosphate is found in most cereal seeds. It is the main storage form of P in grains.[49] Inorganic P (also known as phosphate P or free P) has higher bioavailability than phytate P. The other P represents the sum of all P-containing compounds in a sample other than phytate P and inorganic P. It includes, for example, P found in DNA, RNA, proteins, lipids, and starch. Because phytate P is not well utilized by monogastric animals, it contributes to increased P discharge into the environment.[50] In addition, phytate has been shown to interact directly and indirectly with various dietary components, and in particular certain minerals such as Ca and Zn, and thus reduces their availability to humans and animals.[51]

Although reports on total P content of both corn and DDGS are readily available,[18,27] data on various types of P as well as their changes during dry-grind processing of corn into DDGS are limited. Belyea et al.[9] studied changes in element concentration in primary process streams from dry-grind plants, which included total P content, but other types of P were not included. Noureddini et al.[52] measured total P in several stream products of the dry-grind process, including corn, milled corn, whole stillage, CDS (syrup), DWG, DWGS, and DDGS, and found that syrup contained the highest total P concentration (1.34%, dry matter). They further showed that about 59% of total P in whole stillage was phosphate P and attributed all the remaining P in whole stillage as phytate P. However, their HPLC analysis of this stream and its two centrifuged fractions did not reveal the presence of phytate.

Besides mineral concentration, Liu and Han[28] also determined levels of different types of P in all possible streams of the dry-grind process, from corn to DDGS, and found that syrup contained very high concentrations of total P (a mean value of 1.88%, dry matter basis), but thin stillage had the highest total P concentration (1.98%). About 48% of total P in whole stillage was phytate P, whereas the remaining were phosphate P (25.2%) and other P (26.8%). When the sum of contribution by both phosphate P and other P is collectively considered as non-phytate P contribution toward total P, the present study showed that in DDGS the non-phytate P was 56.30%. This value matches well with 54% reported by the NRC.[22] They further showed that the increase in DDGS over corn was 1.8-fold for phytate P but 10.8-fold for inorganic P. Furthermore, during fermentation, percent phytate P in total P decreased significantly, whereas percent inorganic P in total P increased. These observations suggest that phytate underwent degradation, presumably due to activity of yeast phytase, and are consistent with an observation that the bioavailability of P in DDGS is significantly higher than that in corn.[44]

CAUSES FOR VARYING CHEMICAL COMPOSITIONS

The nutrient composition of all feed ingredients varies, but using ingredients (such as DDGS) that are highly variable can reduce profitability of livestock operations because of increased feed costs and/or reduced production. Increased feed costs occur when diets are over supplemented to avoid reduced production. Reduced production occurs when a diet does not contain adequate concentrations of a particular nutrient because a feed has less than anticipated concentrations of that nutrient.

The causes for varying DDGS composition have been a subject of many studies. They include, but are not limited to, differences in feedstock and composition, process methods, and parameters, the amount of CDS added to DWG, effect of fermentation yeast, and analytical methods, etc. Understanding of many causes for nutrient variation will help us to develop strategies to control quality variation and thus improve value-added utilization of DDGS.

Effect of Raw Materials. For the dry-grind process, variation in raw materials includes grain species, varieties, and blends. Even with the same species and same variety, there is variation in production condition and year, which can lead to compositional differences of feedstock. With regard to species, corn is by far the most common cereal grain used for ethanol production in the United States. However, in other parts of the world, other grains such as sorghum,[53] wheat,[54,55] pearl millets,[56] and barley[57] are also used. Due to differences in composition among grains the resulting DDGS are expected to be different in composition and feed value. Ortin and Yu[58] compared wheat DDGS, corn DDGS, and blended DDGS from bioethanol plants and found great variation in chemical composition and nutritional values among them.

Even with the same grain species, different varieties are sometimes used. For example, when wheat is used as a feedstock for ethanol production, soft wheat, either soft white or soft red class, is preferred to hard wheat because soft wheat generally contains higher starch content.[54] Therefore, inconsistencies in the feedstock, ranging from variability in grain species and variety to variability in the blend of different grains (corn, wheat, barley, etc.), are expected to have an effect on the nutritional characteristics of the DDGS produced.

In the United States, the majority of ethanol producers use yellow dent corn as the feedstock. Thus, variation is limited only to production condition and year. Oftentimes, by intuition and common reasoning, fuel-ethanol processors believe that (1) particle size distribution of ground corn affects that of DDGS and (2) variation in the composition of corn is a major cause of variation in composition of DDGS. Yet, Rausch et al.[59] compared particle size distribution between ground corn and DDGS from dry-grind processing and concluded that the two were not significantly different from each other, in terms of particle size. Belyea et al.[8] compared the chemical compositions of corn and DDGS produced in multiple years from a single plant and found no significant correlations (r ranged from -0.21 to 0.16) between nutrient contents (fat, protein, starch, crude fiber, and ADF) in corn and DDGS. They concluded that variation in the chemical composition of DDGS was not related to the composition of corn used in fermentation but rather to variations in processing techniques. Later, the same group[9] studied element concentrations in primary process streams from nine dry-grind plants and found that the concentrations of most elements in corn samples from these plants were not significantly different, but the mineral content in process streams varied with plants as well as streams. Because the nine plants used similar processing equipment to convert corn into ethanol and DDGS, they concluded that variations in element contents of DDGS and

parent streams were due to processing conditions and not corn. Stein et al.[60] found that energy and nutrient digestibility vary among sources of DDGS even when the DDGS is produced from ethanol plants that use corn grown within a narrow geographical region. Thus, factors other than corn-growing region are also responsible for the variability of these parameters in DDGS.

Yet, Liu[20] measured particle size distribution and proximate composition of ground corn and corresponding DDGS samples from six processing plants in the whole sample as well as sieved fractions and showed that in terms of particle size distribution, the two had a highly positive correlation ($r = 0.807$). There also were some positive correlations in contents of protein and nonstarch CHO between corn and DDGS. Thus, results of Liu[20] partially support the common belief expressed by processors regarding relationships in quality parameters between corn and DDGS. Here, the support is partial because the study showed that raw material affected DDGS quality only to some extent and that other factors, such as processing method and fermentation yeast, were also considered to be responsible. The major reason causing the disagreement is that in both the Belyea et al.[8] and Rauch et al.[59] studies correlation was performed only in the whole sample between ground corn and DDGS, but in the Liu[20] study linear regression was also made for attributes measured in all sieved fractions between the two types of samples.

Effect of Processing Methods. In a conventional dry-grind process, corn is ground using a hammer mill; water is added to produce slurry, which is to be fermented. Because nothing is removed from the corn, the feedstock mainly consists of starch, protein, germ, and fiber fractions. Of these four fractions, only starch is fermentable; the other three fractions remain relatively unchanged and end up in the DDGS. Yet, even with the same conventional method, variation in processing parameters exists. Such variation can cause significant changes in chemical composition and nutritional properties of DDGS.[8,9,43] As mentioned before, during dry-grind processing, some plants use sodium hydroxide for sanitation of processing equipment and sulfuric acid for pH adjustment. This not only causes larger variation for the content of Na and/or S in DDGS but also creates nutritional challenges when included in some animal diets.[9]

Much of the variation in nutrient contents is related to the drying step of the process.[43] Uneven mixing and variability in the quantity and quality of CDS added back to DWG during the drying process will certainly affect the nutrient content of resulting DDGS since CDS has a rather different composition from DWG.[25,28] Fluctuation in the ratio of CDS to DWG entering the dryer occurs as the rates are often adjusted to improve the drying characteristics. The ratio of CDS to DWG may vary from batch to batch and from plant to plant.[8] Aggregation and lumping often occur if the ratio of CDS to DWG is too high. Also, during drying, DDGS is subject to high temperature conditions, which may result in reduced protein quality despite the high overall crude protein content.

Besides the conventional method, over the years, several modified methods have been developed, featured by fractionation either before (front-end) or after (back-end) the fermentation step to remove one or more nonfermentable fractions. As a result, not only is the ethanol production efficiency improved, but also the chemical composition of DDGS is significantly altered.[37,42,61] The modified DDGS generally feature higher protein, lower oil, and/or lower fiber contents than the conventional ones. For example, in one study,[37] a modified process in a laboratory setting was found to reduce the fiber content of DDGS from 11 to 2% and increase the protein content of DDGS from 28 to 58%.

The front-end fractionation is further divided into wet and dry methods. The representatives of wet fractionation methods include a "quick germ" method in which germ can be recovered from the mash by using a corn wet-milling degermination process[62] and a "quick germ and quick fiber" method, in which corn is soaked in water and both germ and pericarp fiber are removed before fermentation.[63,64]

In addition, Wang et al.[42] reported modifications based on front-end fractionation, which included treatment of corn slurry with enzymes and/or using a new granular starch hydrolyzing enzyme STARGEN 001. STARGEN 001 can convert starch into dextrins at low temperatures as well as hydrolyze dextrins into fermentable sugars. Robinson et al.[65] evaluated the nutritional composition of four types of distillers grains, resulting from different processing methods, including conventional DDGS, DDGS using BPX technology (raw starch hydrolysis), high-protein distiller grains (with most fiber and germ removed prior to fermentation), and dehydrated dry-milled corn germ. They found significant differences in many of the nutrients measured among the four samples.

For dry front-end fractionation, Murthy et al.[66] reported a method for processing corn into ethanol as follows: The grain is tempered, degermed, and passed through a roller mill. Ground corn is sieved to separate germ and fiber fractions, and the remaining endosperm is processed by conventional dry-grind ethanol methods to produce ethanol. Corredor et al.[53] used eight sorghum hybrids with 0, 10, and 20% of their outer layers removed as raw materials for ethanol production and found that ethanol yields increased as the percentage of decortication increased and that the decortication process resulted in DDGS with higher protein content and lower fiber content. Wang et al.[67] carried out similar work with rye and triticale as feedstock for ethanol production and found that partial removal of outer grain solids by pearling in an alcohol plant would improve plant efficiency and decrease energy requirements for mash heating and cooling and ethanol distillation. They did not look at the effect of pearling on DDGS quality.

Back-end fractionation refers to removal of oil, fiber, or other valuable components from ethanol coproducts at any stage after the fermentation. It not only modifies DDGS composition but also results in value-added products. The methods can also be divided into dry and wet ones. The back-end dry fractionation is limited to removing fiber from DDGS by sieving,[68] sieving and elutriation,[69] or sieving followed by winnowing,[70] whereas studies on wet methods of back-end fractionation focus mostly on removing oil from various types of coproducts. They include removing oil from DDGS through ethanol extraction,[71] from thin stillage through centrifugation,[38] and from CDS through heating to a higher temperature and centrifugation with a disk stack centrifuge.[39] Conventional DDGS contain about 12% oil on a dry matter basis. Although the presence of oil increases the energy density of DDGS as livestock feed, it may interfere with normal milk production by dairy cattle. Therefore, partial removal of oil from DDGS will improve its feed quality. More importantly, the oil recovered can be used as a feedstock for biodiesel production, although it is not suitable for food use. The back-end recovery process costs less in equipment and operation than the front-end method. In a new development, several recent studies attempted the use of wet solvents in a laboratory setting to extract proteins for producing protein-rich products and leave the residue enriched with carbohydrates.[72−76]

Effect of Fermentation Yeast. Yeast contributes insignificantly toward the cost of raw materials during dry-grind processing of grains into ethanol, yet it is an important ingredient for fuel-ethanol production. A strain of healthy and well-selected yeast is needed for an efficient fermentation. It can also potentially affect the final product quality. As early as 1944, Bauernfiend et al.[77] defined corn distillers dried solubles as a grain-yeast concentrate comprising the water-soluble nutrients derived from the original grains and from the grain−yeast fermentation. Thus, there is no doubt that DDGS proteins come from corn and yeast, yet the effect of fermentation yeast on DDGS protein quantity and quality (AA profile) has not been well documented.

In the literuate, there are at least four described methods for estimating yeast contribution toward distillers grains. As discussed below, the estimate results vary greatly with methods. Because major factors affecting DDGS quality and market values include protein quantity (concentration) and quality (amino acid composition) and because the yeast AA profile is better than that of corn, investigation into yeast effect and accurate estimation of yeast contribution will have a positive impact on feed and ethanol industries and at the same time increase our basic understanding of the processing system.

Bauernfiend et al.[77] suggested that yeast cell (all dead) content can be estimated by hemacytometer counts of thin stillage, condensed solubles, or dried solubles. They reported that dried distillers solubles contained about $(4 \pm 0.5) \times 10^9$ cells/g. When this figure was compared to that of dried yeast, the approximation was reached that 20% by weight of dried solubles is dried yeast. The method may not be applicable directly to DDGS unless a separation of solubles is carried out or a portion of solubles is first estimated or assumed, it also needs an estimated number of cells per 1 g dry yeast.

Ingledew[78] estimated the amount of yeast contribution toward DDGS based on assumptions and calculation of protein and mass in a known amount of fermentation mash. He stated that in the late 1990s the annual fuel-ethanol production in North America was 7×10^9 L. Assuming that the fermentation mash contains 12% alcohol, the total mash would be 6×10^{10} L. At the peak of fermentation, the yeast count in a fermentor is assumed to be 1.9×10^{11} cells/L of mash, so total yeast cells in total annual mash production would be $(6 \times 10^{10}) \times (1.9 \times 10^{11}) = 1.14 \times 10^{22}$. Assuming that 1 g dry yeast contains 4.87×10^{10} cells, the total annual mash would contain $(1.14 \times 10^{22})/(4.87 \times 10^{10}) = 2.34 \times 10^{11}$ g = 234,000 tons of yeast biomass. Then, estimating that the average yeast cell contains 38% protein, the total annual mass would contain 234,000 × 38% = 88,920 tons yeast protein. If 1000 L of ethanol leads to 860 kg of DDGS, then total annual DDGS in this scenario would be $(7 \times 10^9$ L)/1000 L × 860 kg = 6×10^6 tons. Assuming that average protein content in DDGS is 28%, the total annual DDGS protein was $6 \times 10^6 \times 28\% = 1.68 \times 10^6$ tons. Therefore, yeast contribution by mass would be 234,000/6,000,000 = 3.9%, and yeast contribution by protein 88,920/1,680,000 = 5.3%. Because the approach is based on many assumptions, the accuracy of the final estimation is uncertain.

Belyea et al.[8] calculated the average ratio of essential AA concentrations (dry sample weight basis) of DDGS versus yeast and suggested that yeast contributes up to 50% of DDGS protein. This approach has some shortcomings: (1) it disregards corn protein contribution, (2) it does not include nonessential AA, and (3) the average ratio in AA concentrations actually reflects the ratio of protein concentration of DDGS versus yeast.

Recently, Han and Liu[25] proposed a multiple linear regression model based on changes in amino acid profile in terms of relative percent during the entire process of dry-grind ethanol production (Table 6). As discussed earlier, when amino acid composition is expressed on a dry sample weight basis, the change in AA concentrations, either essential or nonessential, follows the pattern of protein changes during dry-grind processing (Table 5). However, when expressed as relative percent (based on total AA), the change of AA profile (Table 6) does not follow the trend of protein change. Upon fermentation, concentrations of some AA increase, others decrease, and still others remain unchanged. This is because the expression of AA in terms of relative percent of total AA focuses on the quality of protein, not the quantity. More importantly, when amino acid composition is expressed as concentration in dry samples, there is little information about the influence of yeast AA composition on up- and downstream products (including DDGS) (Table 5). However, when AA is expressed as relative percent (protein based), the influence of yeast AA on stream products of processing becomes clear (Table 6). For example, Arg in corn was 3.55% and in yeast, 5.15%, so there is an increasing trend of Arg in postfermentation samples. Met in corn is 3.32% and in yeast, 2.14%, so the trend is decreasing. Although most changing trends in AA composition depend on the difference between yeast and corn AA compositions, there are some exceptions. For example, for Pro, there is no clear pattern of change during processing, but yeast has a much lower value than corn (2.18 vs 6.67%).

Thus, on the basis of observed data from samples of three plants, Han and Liu[25] proposed that AA composition of a downstream product (response variable) is a function of AA composition of corn (independent variable 1) and AA composition of yeast (independent variable 2), based on a multiple linear regression model:

$$Y = AX_1 + BX_2 + C$$

Y = relative percent of an amino acid in a stream product, X_1 = relative percent of the AA in ground corn, X_2 = relative percent of the AA in yeast, A is a fixed-value parameter indicating the extent of contribution by corn AA, B is a fixed-value parameter showing the extent of influence by yeast AA, and C is a fixed-value parameter showing the intercept on the Y-axis.

According to the proposed model, regression results show that parameters A, B, and C varied greatly with the type of stream products. When regression was conducted on the combined data set of three plants, results show that before fermentation, the value of parameter A was about 0.92, and that of B was around 0.05 (Table 8). After fermentation, the value of A was reduced to about 0.84 and the value of B was increased to about 0.20. This implies that the average AA composition for fermentation mash from all three plants would increase by a factor of 0.84 if AA composition of ground corn increased by 1% and AA composition of yeast remained fixed. Similarly, a 1% increase in yeast AA composition, with corn AA held fixed, would now increase mean AA of fermentation mash by a factor of 0.20. Furthermore, upon centrifugation, the B value increased in thin stillage and its condensed form (syrup), but decreased in wet distiller grains. The A value changed accordingly, decreasing in thin stillage and syrup but increasing in DWG. The two parameters in both DWGS and DDGS became similar to those found in whole stillage and fermented mass.

On the basis of the multiple linear regression of amino acid composition (% relative) of DDGS protein with those of corn and yeast proteins as two independent variables, the fermentation yeast accounts for about 20% of the effect on DDGS amino acid profile. Corn accounts for the remaining 80%. The method is

Table 8. Multiple Linear Regression for Amino Acid Composition (Relative Percent of Individual Amino Acids in Each Sample) of Intermediate and End Products of Dry-Grind Processing from Three Commercial Plants, with Amino Acid Compositions of Milled Corn and Yeast as Variants X_1 and X_2, Respectively[a] (Adapted from Han and Liu[25])

parameter	cooked slurry	liquefied mass	fermented mass	whole stillage	thin stillage	distiller solubles	distiller grains	WDGS	DDGS	average
A	0.918	0.926	0.835	0.802	0.513	0.488	0.950	0.843	0.889	0.796
B	0.046	0.060	0.196	0.238	0.390	0.407	0.165	0.214	0.195	0.212
C	0.208	0.080	−0.180	0.240	0.571	0.622	−0.680	−0.343	−0.511	0.001
r^2	0.977	0.978	0.954	0.963	0.936	0.940	0.949	0.955	0.951	0.956

[a] Regression was based on a multiple linear model $Y = AX_1 + BX_2 + C$, where Y = relative % of amino acid in a downstream product, X_1 = relative % of AA in milled corn, and X_2 = relative % of AA in yeast.

also useful to estimate the effect of yeast on AA profile of intermediate products. For example, before fermentation, yeast accounts for about 5% (due to recycle of thin stillage), but after fermentation its contribution increased to about 20%. This value was higher in thin stillage and CDS, but lower in DWG. Because yeast has a better AA profile than corn (particularly with regard to Lys), the higher the yeast effect, the better the DDGS AA profile as compared with that of corn.

Among the four methods described, the proposed multiple-regression model is believed to be the most accurate estimation for the effect of yeast on the AA profile of DDGS. First, it links DDGS AA profile as a function of both corn and yeast AA profiles. Second, it includes all amino acids. Third, it is based on relative percent rather than absolute concentrations of AA. The latter is affected by the protein content in the sample. Fourth, it can estimate the yeast effect on not only DDGS but also any intermediate products. Finally, unlike the previous three methods that focus on how much protein in DDGS is of yeast origin, the regression approach focuses on the impact of yeast and corn AA profiles on that of DDGS and intermediate products.

Effect of Analytical Methodology. The use of various analytical methods for DDGS has in part led to significant variation in reported compositional values among laboratories and has therefore created confusion for producers, marketers, nutritionists, regulatory bodies, and end users. For example, Ileleji et al.[79] reported that the various methods that have been used for moisture determination of DDGS do not give identical results, and therefore caution should be exercised when selecting a method for determining moisture in DDGS. One key factor leading to the situation of using various methods is lack of standardized protocols for characterizing DDGS quality. To address the issue, Thiex[80] conducted a study to evaluate analytical methods for DDGS. The study was commissioned by the American Feed Industry Association (AFIA) and Renewable Fuels Association (RFA), because with increasing production of distillers dried grains with solubles (DDGS), both fuel-ethanol and animal feed industries are demanding standardized protocols for analytical methodology.

Recently, Liu[81] used an AOCS Approved Procedure (Am 5-04)[82] for measuring crude oil content in both milled corn and resulting DDGS and found that for the crude oil analysis by the AOCS method, particle size had no effect for ground corn but had the most significant effect for DDGS among other factors. DDGS with larger particle size (such as those with original matrix) tend to have significantly lower measured values of crude oil content than samples with reduced particle sizes, when other analytical conditions are kept the same. On average, the measured oil content in DDGS ranged from 11.11% (original matrix) to 12.12% (for <0.71 mm particle size) and to 12.55% (for <0.50 mm particle size). It is commonly believed that there is a strong relationship between surface area and solvent extraction efficiency. The smaller the size of the particles, the greater the surface area and, thus, the greater the extraction efficiency would be. However, the effect of particle size on crude oil analysis cannot be fully explained by the increase in surface area of particles, because the same study showed that for ground corn, particle size had no significant effect. It is presumably attributed to the differences in chemical composition and physical matrix between raw corn and DDGS.

During the chemical analysis of DDGS, improper sampling can be another factor for causing variation. Aggregation and inconsistent physical characteristics of the DDGS make it challenging to obtain a truly representative sample from such a small quantity of material.[83] Thus, sampling of the material must include a large pooled sample composed of multiple samples per batch throughout the production process.

MYCOTOXINS IN DISTILLERS GRAINS

Mycotoxins are defined as toxic or carcinogenic chemicals that are secondary metabolites of fungi that colonize crops. When ingested, these substances can cause a number of adverse health effects in humans and domestic animals. Mycotoxins are unavoidable contaminants in crops and thus occur in commodities entering the marketing chain, including the grain feedstock for ethanol production. During dry-grind processing of grains into ethanol, mycotoxins, like other nonfermentable components, remain relatively unchanged and are concentrated in the DDGS due to depletion of starch.[84−86] Furthermore, mycotoxins can stress the yeast during fermentation and result in lower ethanol yields.[84] Therefore, there has been a great concern with mycotoxins in the original feedstock and resulting DDGS. In this final section, mycotoxins and their occurrence in DDGS are briefly discussed. A detailed review of the subject is beyond the scope of this paper. However, general information on mycotoxins can be found elsewhere.[87−90]

Types of Mcotoxins. Several mycotoxins can potentially be found on grains and resulting DG, including aflatoxins, fumonisins, deoxynivalenol (DON), T-2 toxin, and zearalenone (ZON). Most of these toxins can occur in preharvested grains and persist in harvested grains. Such occurrence, however, is dependent upon the unique environmental conditions that are conducive to the growth of specific molds that produce mycotoxins during crop development. Mycotoxins also accumulate as a result of fungal growth during improper handling and storage of grains. Each fungal species thrives in specific growing, harvesting, or storage conditions, and more than one type of mycotoxin is often found in a contaminated product. Also, mycotoxin contamination of grains does not necessarily occur every year because the appropriate environmental conditions for the growth of the

specific responsible fungi are often lacking. The presence of fungi in grains is not a confirmatory test for the presence of mycotoxins.[88,89]

Aflatoxins are a group of mycotoxins produced by some *Aspergillus* species, such as *Aspergillus flavus* or *Aspergillus parasiticus*. The primary aflatoxins are B_1, B_2, G_1, and G_2 with aflatoxin B_1 as the most frequently occurring. These primary aflatoxins are the most toxic and carcinogenic of the known mycotoxins. Corn becomes susceptible to aflatoxin formation during growth under high temperature/drought condition or in high moisture/humid storage. Insect injury may also contribute to increased aflatoxin contamination in corn.[87,89]

The fumonisins are a new group of mycotoxins that were discovered in 1988[91] and are produced primarily by *Fusarium verticillioides* and *Fusarium proliferatum*, although a few other *Fusarium* species may also produce them. Infection is increased if kernels are physically damaged, especially by insect feeding, and drought stress followed by warm, wet weather also favors fungal growth. There are at least 28 different forms of fumonisins, most designated as A-series, B-series, C-series, and P-series. Fumonisin B_1 is the most common and economically important form, followed by B_2 and B_3. Corn is the most commonly contaminated crop.[87−89,91]

The trichothecenes are the largest group of mycotoxins known to date, consisting of more than 200 chemically related toxic compounds that have a strong impact on the health of animals and humans due to their immunosuppressive effects. These mycotoxins are produced by several fungal species, including *Fusarium*, *Stachybotrys*, *Trichoderma*, and *Trichothecium*. The most important structural features causing the biological activities of the trichothecences are the 12,13-epoxy ring, the presence of hydroxyl or acetyl groups, and the structure and position of the side chains.[88,89]

Deoxynivalenol (DON) is the most important trichothecene mycotoxin.[88] It is a common contaminant of wheat, barley, and corn, produced by *Fusarium graminearum*. In corn, the mold usually develops during cool damp weather, resulting in a white or reddish fungus. DON is also known as vomitoxin because of its deleterious effects on the digestive system of monogastric animals. T-2 is another member of trichothecenes. *Fusarium sporotrichioides* is the principal fungus responsible for the production of T-2. The production of T-2 is greatest with increased humidity and temperatures of 6−24 °C.[88] Zearalenone is a mycotoxin that mimics the reproductive hormone estrogen and hence affects reproduction. It is produced primarily by the fungus *F. graminearum*, the same fungus that produces deoxynivalenol in corn and small grains. Zearalenone contamination is economically important in corn, and high humidity and low temperatures favor the production of zearalenone by *F. graminearum*.[89]

Regulations and Guidance. On the basis of the wealth of available information on the adverse animal health effects associated with certain groups of mycotoxins, over the years, the U.S. Food Drug and Administration (FDA) has established regulatory levels for certain groups of mycotoxins in feed ingredients (Table 9). "Action levels" for aflatoxins in animal feeds have been established for different animal species and at different production stages. The FDA action level represents the minimum limit at which the FDA can take legal action to remove feed ingredients from the market.[92] The recommended (or guidance) maximum levels for fumonisins[93] and the advisory (maximum) levels for deoxynivalenol[94] in animal feeds were also set by the FDA. No action levels, advisory levels, or guidance levels for T-2 toxin and zearalenone are available from the FDA at this time. For food, the maximum levels are set even lower than those for animal feed.[92−94]

Occurrence in DG. The occurrence, levels, and ultimately the safety risk of mycotoxinis in DDGS and other ethanol coproducts depend on (1) the levels in the original grain feedstock and (2) the fate of mycotoxins during dry-grind processing. Over the years, a number of studies have been carried out to determine the fate of mycotoxins during ethanol produciton, using either naturally contaminated grain or grain artificially contaminated by the addition of known quantities of pure mycotoxins.[84−86,95,96] These studies showed that during ethanol production from grains, there is very little degradation of mycotoxins. They also showed that mycotoxins are not found in distilled ethanol and that the original mycotoxins remain largely intact in the other fractions, including DWG, CDS, and DDGS. More importantly, due to nearly complete depletion of starch and little degradation of mycotoxins during ethanol production from grains, the resulting coproducts typically have a much higher concentration of mycotoxins than in the original grain, about a 3-fold increase.[86]

The presence of mycotoxins in the grain feedstock may present another problem for the fuel-ethanol industry. Studies showed that mycotoxins stress fermentation yeast, resulting in lower ethanol yields.[86,96] However, another paper indicated no effect of aflatoxins on dry-grind ethanol process.[97]

The observation that mycotoxins remain relatively unchanged during ethanol production and are thus concentrated in the coproduct presents a serious problem because DG are often used for animal feed. In one study, based on an economic model, it was estimated that current losses to the swine industry from weight gain reduction due to fumonisins in added DDGS were at an average of $9 million annually.[98] If there is complete market penetration of DDGS in swine feed with 20% DDGS inclusion and fumonisins are not controlled, losses may increase to an estimated average of $147 million annually. The authors further stated that these values represent only those losses attributable to one mycotoxin on one adverse outcome on one species. The total loss due to mycotoxins in DDGS could be significantly higher due to additive or multiplicative effects of multiple mycotoxins on animal health.

Despite the potential for high levels and thus safety concern of mycotoxins in DDGS, Zhang et al.[99] measured aflatoxins, deoxynivalenol, fumonisins, T-2 toxin, and zearalenone in 235 DDGS samples collected from 20 ethanol plants in the Midwestern United States and 23 export shipping containers from 2006 to 2008 and found that (1) none of the samples contained aflatoxins or deoxynivalenol at levels higher than the FDA guidelines for use in animal feed; (2) no more than 10% of the samples contained fumonisins at levels higher than the recommendation for feeding equids and rabbits, and the rest of the samples contained fumonisins lower than FDA guidelines for use in animal feed; (3) none of the samples contained T-2 toxins higher than the detection limit; and (4) most of the samples contained zearalenone levels lower than the detection limit. This study provided a comprehensive assessment of the occurrence and levels of mycotoxins in DDGS from the U.S. ethanol industry.

Prevention and Detoxification. As DG makes up an increasing proportion of animal feed, it is important to consider how much DDGS can be included in animal diets to meet the U.S. FDA guidelines and action levels on mycotoxins. For this reason, it is important to ensure that grains entering ethanol

Table 9. FDA Action Levels for Total Alfatoxins, Guidance Levels for Total Fumonisins, and Advisory Levels for Total Vomitoxin in Feeds for Livestock and Pet Animals[a]

Aflatoxins		
class of animals	feed type or commodity	action levels (ppb)
finishing beef cattle	corn and peanut products	300
beef cattle, swine or poultry	cottonseed meal	300
finishing swine over 100 lb	corn and peanut products	200
breeding cattle, breeding swine and mature poultry	corn and peanut products	100
immature animals	animal feeds and ingredients, excluding cottonseed meal	20
dairy animals, animals not listed above, or unknown use	animal feeds and ingredients	20
Fumonisins		
class of animals	feed ingredients (max % in the diet, dry weight basis)	guidance levels, ppm (levels in finished diets, ppm)
poultry being raised for slaughter	corn and corn byproduct (50)	100 (50)
ruminants ≥3 months old being raised for slaughter and mink being raised for pelt production	corn and corn byproduct (50)	60 (30)
breeding ruminants, breeding poultry, and breeding mink	corn and corn byproduct (50)	30 (15)
swine and catfish	corn and corn byproduct (50)	20 (10)
equids and rabbits	corn and corn byproduct (20)	5 (1)
all other species or classes of livestock and pet animals	corn and corn byproduct (50)	10 (5)
Deoxynivalenol (DON, Vomitoxin)		
class of animals	feed ingredients (maximum % in the diet, dry weight basis)	advisory levels, ppm (levels in finished diets, ppm)
ruminating beef and feedlot cattle >4 months	grain and grain byproduct (50)	10 (5)
chickens	grain and grain byproduct (50)	10 (5)
swine	grain and grain byproduct (20)	5 (1)
all other animals	grain and grain byproduct (40)	5 (2)

[a] Data adapted from FDA.[92−94].

facilities have acceptable mycotoxin levels. An effective way is to have integrated methods to prevent and reduce mycotoxin contamination in grains at both pre- and postharvest stages. It is also crucial to monitor mycotoxins in DG.[100]

Once mycotoxins are present in grain feedstock and ethanol coproducts, practical detoxification procedures are essential. Several approaches to detoxification by physical, chemical, and biological mechanisms have been developed. In undamaged kernels, mycotoxin-producing fungi and their mycotoxins are found primarily in the pericarp and germ portions of the kernel.[101] Therefore, separation of these components (kernel fractionation) prior to fermentation could be advantageous for increasing the value of coproducts. For example, dehulling grain kernels can effectively reduce mycotoxins concentrations.[102,103] Also, broken kernels are known to be higher in aflatoxins, and they can be removed by sieving due to smaller particle size.[104]

For ethanol coproducts, Lillehoj et al.[95] detoxified aflatoxin B_1 in whole stillage with sodium hydroxide, ammonium hydroxide, sodium hypochlorite, and hydrogen peroxide. Bennett et al.[105] reduced zearalenone in fermentation solids with formaldehyde. An integrated process was also developed for ammonia inactivation of aflatoxin-contaminated corn and ethanol fermentation.[106] Stepanik et al.[107] used electron beam irradiation to reduce the content of DON in the original feedstock and resulting DDGS and found that electron beam treatment produced a 17.6% reduction in the DON level of wheat at the highest dose used, but had no effect on DON in DDGS.

Analytical Methodology. Analysis for mycotoxins in DDGS involves obtaining an adequate sample, preparing it for analysis, and choosing an assay method for a specific mycotoxin of interest. Every step of the process is important to obtain results that accurately reflect the mycotoxin concentration in the original lot.[88] In particular, sampling is usually the largest source of variability associated with the mycotoxin test procedure. This is because a small percentage of kernels are contaminated and the level of contamination on a single seed can be very large.[108] It is therefore necessary to collect and composite more and larger subsamples for testing and to adhere to established sampling protocols, where they are established.

For the past five decades, many analytical methods have been developed to test mycotoxins in human food and animal feeds due to health concerns.[109] Among the developed methods, thin-layer chromatography, enzyme-linked immunosorbent assay (ELISA), and immunosensor-based methods have been widely used for rapid screening, whereas high-performance liquid chromatography with fluorescence detection and mass spectrometry detection have been used as confirmatory and reference methods.[110] Rapid testing kits, approved by the Grain Inspection, Packers and Stockyards Administration of the U.S. Department of Agriculture, are also available for semiquantitative or quantitative tests.[111] They are mostly based on the immunology

principle. Yearly review and updates on mycotoxin analysis are available in annual reports of the AOAC International Committee on Natural Toxins and Food Allergens.[112,113]

CONCLUDING REMARKS; CURRENT AND FUTURE RESEARCH NEEDS

Marketability and suitable uses of distillers dried grains with solubles (DDGS) are keys to the economic viability of fuel-ethanol production. As the industry has grown, the importance of the distillers grains (DG) has also increased. In this review, several topics, which are crucial to the use of DDGS, have been discussed with updated information, ranging from nutrient levels in DDGS to their variations among studies and within the same study, and from compositional changes during the entire dry-grind process to analysis of several key causes for higher compositional variation.

One key challenge for using DDGS as a feed ingredient is its larger variation in nutrient levels as compared to some other feed ingredients, such as soy meal. The main cause for the large variation is the dry-grind process itself, because it is more complex than processes of other feed protein ingredients (such as oilseed) and entails more steps and more variables in processing parameters within a method. There are also different versions of the process (in terms of front-end and back-end fractionation). Different grain species or their blends as feedstock add another factor. For better utilization of DDGS, scientific communities, DDGS producers, and end users all need to find strategies to manage the variation. Toward this objective and on a broader basis, at the present and in the near future, several efforts are undertaken or need to be undertaken, including (1) development of high-efficiency enzyme systems for converting starch to glucose, (2) development of high-efficiency yeast and fermentation system for converting glucose to ethanol, (3) development of alternative methods of coproduct recovery so that variables are better controlled, (4) development of front-end and/or back-end fractionation methods to capture values of unfermentable components, (5) development and standardization of analytical methodology, (6) investigation into levels and certain toxicants (such as mycotoxins) in DG and their effects on animal health, and (7) exploration of new ways of using DDGS as animal feed, industrial materials, and even food ingredients.

Another major issue with use of DDGS as animal feed is the potential for high levels of mycotoxins. During ethanol production, mycotoxins, if present in the original grain feedstock, remain relatively unchanged and are thus concentrated in the coproducts. Several approaches are effective to address this issue: (1) ensuring that grains entering ethanol facilities have acceptable mycotoxin levels, (2) developing effective methods to prevent mycotoxin occurrence during pre- and postharvest of grains, (3) developing effective detoxification methods for grains and resulting DG once mycotoxins are present, and (4) monitoring mycotoxin levels in DG.

Although a controversy exists, grain-based ethanol production will continue to play an important role in the growing biofuel industry, and DG will continue to be a major feed commodity in the global market. Even when cellulosic ethanol and biodiesel industries become predominant, DG will not disappear completely from the market. It is hoped that information provided so far helps stimulate the interest of the scientific commodity, fuel, and feed industries toward DDGS quality and end uses. It is also hoped that this review not only provides timely information about DG but also serves as a benchmark for coproducts from other fuel industries, such as cellulosic ethanol and algae biofuel.

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ABBREVIATIONS USED

AA, amino acid(s); ADF, acid detergent fiber; CDS, condensed distillers solubles (also known as syrup); CHO, carbohydrate; CV, coefficient of variation; DG, distillers grains; DDG, distillers dried grains; DDGS, distillers dried grains with solubles; DWG, distillers wet grains (also known as wet cake); DDWS, distillers wet grains with solubles; DON, deoxynivalenol; HPLC, high-performance liquid chromatography; NDF, neutral detergent fiber; NRC, National Research Council; RFA, Renewable Fuel Association; TFA, trifluoroacetic acid; U.S., United States; ZON, zearalenone

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