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Changes in Composition and Amino Acid Profile during Dry Grind Ethanol Processing from Corn and Estimation of Yeast Contribution toward DDGS Proteins

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Three sets of samples, consisting of ground corn, yeast, intermediate products, and DDGS, were provided by three commercial dry grind ethanol plants in Iowa and freeze dried before chemical analysis. On average, ground corn contained 70.23% starch, 7.65% protein, 3.26% oil, 1.29% ash, 87.79% total carbohydrate (CHO), and 17.57% total nonstarch CHO, dry matter basis. Results from Plant 1 samples showed that compared to ground corn, there was a slight but significant increase in the contents of protein, amino acids (AA), oil, and ash before fermentation, although starch/dextrin decreased sharply upon saccharification. After fermentation, starch content further decreased to about 6.0%, while protein, oil, and ash contents increased over 3-fold. AA increased 2.0−3.5-fold. Total CHO content decreased by 40%, and the content of total nonstarch CHO increased over 2.5-fold. Concentrations of these attributes fluctuated slightly in the remaining downstream products, but oil and ash were concentrated in thin stillage, while protein was concentrated in distiller grains upon centrifugation. When AA composition is expressed in relative % (protein basis), its changes did not follow that of protein concentration, but the influence of yeast AA profiles on those of downstream products became apparent. Accordingly, a multiple linear regression model for the AA profile of a downstream product as a function of AA profiles of ground corn and yeast was proposed. Regression results indicated that, with an r^2 = 0.95, yeast contributed about 20% toward DDGS proteins, and the rest came from corn. Data from Plants 2 and 3 confirmed those found with Plant 1 samples.

KEYWORDS: DDGS; distiller grains; amino acid; yeast; proteins; composition; changes; dry grind process; ethanol; corn

INTRODUCTION

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. A major process for making ethanol from corn is the dry grind method. The basic steps of a dry grind process include grinding (dry milling), slurrying, cooking, liquefaction, saccharification, fermentation, distillation, and coproduct recovery, although in recent years simultaneous saccharification and fermentation is becoming popular (*1*, *2*). During coproduct recovery, the nonvolatile components following the distillation step are known as whole stillage, which is usually centrifuged to produce a liquid fraction (thin stillage) and a solid fraction, distiller grains (DG). A significant portion (15% or more) of the thin stillage is recycled as backset to be used as process water to slurry the ground grain (*1*, *2*). The remaining thin stillage is concentrated through evaporation into condensed distiller solubles (DS, also known as syrup). While DS, DG, or their combination known as wet distiller grains with solubles (WDGS) can each be sold as is for animal feeds, WDGS is often dried to produce distiller dried grains with solubles (DDGS) for easier handling (*2*). Production of DDGS has increased significantly in recent years, as the number of dry grind ethanol production facilities increases.

Upon conversion from corn to DDGS, where depletion of starch occurs, on average, protein is concentrated about 3.6 times; oil, 3.4 times; ash, 3.3 times; and total nonstarch carbohydrate (CHO), 2.9 times (*3*). Thus, DDGS has a valuble nutrient profile. Currently, most DDGS is being used for animal feed, but its oversupply is creating a need for exploration for alternative uses (*4*). Income from the marketing of DDGS is important to the economic viability of the dry grind industry because it partially offsets production costs.

Factors that affect the quality or marketability of DDGS can impact its market value. One of the major factors is variation in chemical composition (*5*−*8*). Among nutrients, protein is the most valuable component for animal diets. Variation in the protein content of DDGS can cause faulty formulation of feeds and thus affect animal productivity. Although reports on chemical composition and amino acid (AA) profile in corn and DDGS are readily available (*3*, *5*−*9*), data on their changes during the dry grind process of corn into ethanol and DDGS is lacking.

The causes for varying DDGS composition have been identified as varying chemical composition of DS (*10*), varying proportions

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of mixing DS and DG (*6*), and varying raw material composition and processing methods (*3*). Furthermore, since DDGS contains yeast biomass, another possible cause could be varying the contribution of yeast protein toward DDGS proteins. Yet, the proportion of yeast protein in DDGS proteins is not well documented in the literature. Belyea et al. (*6*) calculated the average ratio of essential AA concentrations of DDGS vs yeast and suggested that yeast protein may make up approximately half of the protein in DDGS. However, this approach is questionable because the average ratio merely reflected the ratio in protein concentrations of DDGS vs the yeast sample.

The objectives of this study were (1) to determine the chemical composition and AA profile in streams of the dry grind process, from corn to DDGS, and in yeast as well and to monitor their changes during ethanol production, and (2) to develop a better model to estimate the contribution of yeast toward DDGS proteins. Such information can help us better understand the causes for nutrient variation in DDGS and develop strategies to modify processing steps for maximum balance of nutrients in DDGS.

MATERIALS AND METHODS

Materials. Three sets of samples, consisting of ground corn, intermediate products, DDGS, and yeast, were provided by three selected dry grind ethanol plants located in the state of Iowa. The three plants were numbered as Plants 1, 2, and 3, according to the order of the samples received. The samples from the processing streams (intermediate products) included raw slurry, cooked slurry, liquefied mass, saccharified mass, fermented mass, whole stillage, thin stillage, condensed distiller solubles, wet distiller grain, and wet distiller grains with solubles (WDGS) (**Figure 1**). There was some minor variation with regard to the type and number of stream samples collected among the three plants since Plants 2 and 3 apparently used simultaneous saccharification and fermentation, and no saccarified mass was provided by these two plants. All samples were frozen after collection for transportation and storage, and freeze-dried in our laboratory just before chemical analysis.

Chemical Analysis. Samples were analyzed for contents of moisture, protein, fat, starch, ash, and amino acids. All attributes were measured in duplicate except for the AA profile for which only certain types of samples were measured in duplicate, and the rest had a single measurement (for cost savings). Moisture and ash contents were determined according to official methods (*11*). The moisture content was used to convert concentrations of other components into a dry matter basis. The total nitrogen/protein content in samples was measured by a combustion method (*11*), using a protein analyzer (Model FT528, Leco Crop. St. Joseph, MI). The protein content was calculated with a conversion factor of 5.75. The oil content was determined by an AOCS official procedure (*12*), using a fat analyzer (Model XT 10, Ankom Technology, Macedon, NY). However, instead of using petroleum ether, hexane was used as the extracting solvent.

Starch was measured according to an enzymatic method using a starch test kit (R-Biopharm, Inc., Marshall, MI). Samples were treated with dimethylsulfoxide and HCl to solubilize starch, which was then hydrolyzed to D-glucose in the presence of amyloglucosidase. The resulting D-glucose reacted with hexokinase and glucose-6-phosphate dehydrogenase. The amount of NADPH (reduced nicotinamide−adenine dinucleotide phosphate) formed in the reaction was determined colorimetrically, which was stoichiometric to the amount of D-glucose. The total carbohydrate (CHO) was calculated on the basis of the contents of protein, oil, and ash, on a dry matter basis, while the total nonstarch CHO was calculated on the basis of the difference between the total carbohydrate and starch content, also on a dry matter basis.

Amino acids were analyzed according to an AOAC official method (*11*). Briefly, after hydrolysis in 6 N HCl for 24 h at 110 °C, samples were analyzed for AA concentrations, using an amino acid analyzer (model L-8500A, Hitachi, Chyoudaku, Japan). Analysis for methionine and cysteine concentrations was performed separately after performic acid oxidation. Tryptophan was not analyzed.

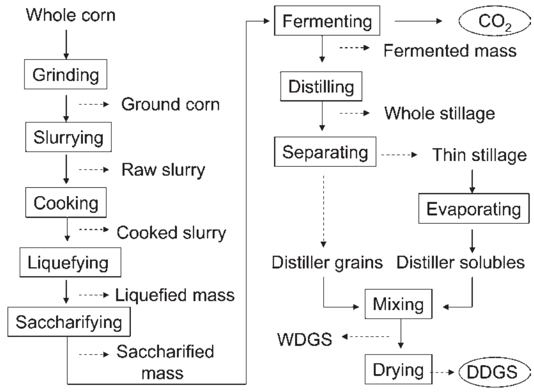

Figure 1. General flow diagram of a dry grind ethanol process from corn, showing various downstream products collected at three Iowa plants. In recent years, simultaneous saccharification and fermentation has become popular.

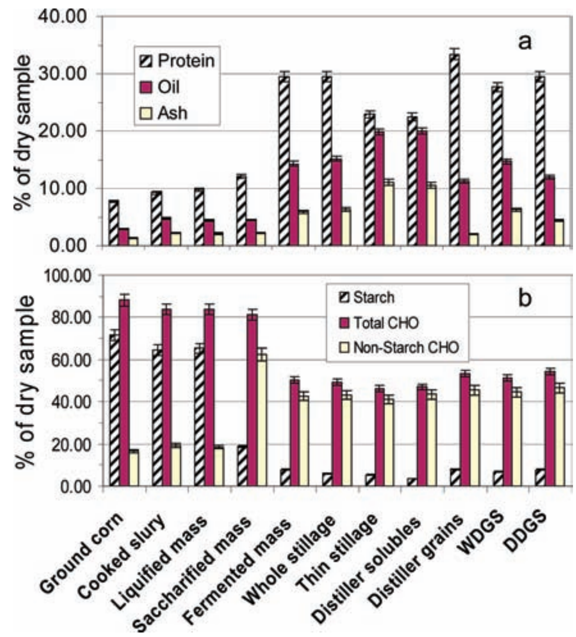

Figure 2. Changes in chemical composition during dry grind ethanol processing from corn at Plant 1. (**a**) Contents of protein, oil and ash and (**b**) contents of starch/dextrin, total CHO (carbohydrates), and total nonstarch CHO. Error bars represent standard deviations.

Statistical Treatment. Data from each plant were treated with the JMP software, version 5 (JMP, a business unit of SAS, Cary, NC) for calculating means and standard deviation and for analysis of variance (ANOVA) in order to determine the effect of processing steps. Tukey's HSD (honestly significant difference) test was conducted for pairwise comparisons when ANOVA showed a significant effect at $p < 0.05$. Multiple linear regression was also conducted for the amino acid profile of downstream products.

RESULTS AND DISCUSSION

Changes in Chemical Composition of Processing Streams from Plant 1. Protein, oil, and ash contents in the dry mass of processing streams from Plant 1 increased slightly but significantly ($p < 0.05$) at the beginning of the process, up to the saccharification step (**Figure 2a**). The increase of these components in the cooked slurry as compared with that of ground corn was most likely due to the use of backset for slurrying ground corn; the contents of protein, oil, and ash in thin stillage were much higher than those of ground corn. After fermentation, these

Table 1. Changes in Amino Acid Concentration (% Dry Weight) during Dry Grind Ethanol Processing from Corn at Plant 1[a]

amino acid (AA)	ground corn	cooked slurry	liquefied mass	sacchari- fied mass	fermented mass	whole stillage	thin stillage	distiller solubles	distiller grains	WDGS	DDGS	Yeast
						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] Means ± standard deviation. The rest are values of single measurement.

nutrients increased dramatically, over 3-fold. These increases were mainly due to the depletion of starch as it was fermented into ethanol and carbon dioxide. Distillation caused little changes in composition. Upon centrifugation, the whole stillage was divided into thin stillage and DG. Thin stillage was higher in oil and ash content but lower in protein content than DG. This implies that in whole stillage a larger portion of oil was in emulsion and that the majority of ash was soluble so that they went with the liquid fraction and were retained less in the solid grains during centrifugation. After evaporation of thin stillage, there was no significant change ($p < 0.05$) in contents of these attributes. More importantly, among all of the downstream samples, oil and ash were highest in thin stillage and its condensed form (DS), while protein was highest in DG. In addition, the ash content was so reduced in DG upon centrifugation that it was just slightly higher than that in ground corn. When the two were mixed together to become WDGS, the composition was averaged out and became similar to that of the whole stillage. There was a slight but significant ($p < 0.05$) difference in the contents of protein, oil, and ash between WDGS and DDGS. This difference was most likely due to the dynamics of drying since part of the DDGS output was recycled and mixed with DS and DG for feed conditioning to improve operation performance (*13*).

Since during the starch assay dextrin (a mixture of polymers of D-glucose units resulting from starch hydrolysis) content was not measured separately, the measured starch content also included dextrin content. Changes in starch/dextrin and total CHO during the dry grind process of corn in Plant 1 (**Figure 2b**) were opposite to those of protein, oil, and ash (**Figure 2a**). At the beginning of the process, starch and dextrin were relatively unchanged, although a decrease from corn to cooked slurry was noticeable. This decrease was apparently due to an increase in the protein, oil, and ash contents discussed earlier. Starch/dextrin decreased substantially upon saccarification and decreased further to about 6% after fermentation. It remained unchanged in the rest of the processing streams. Enzymatic action and fermentation converted most of the starch to ethanol but apparently could not reach complete conversion. Residual starch in coproducts was also reported elsewhere (*8*, *9*). Concomitantly with the starch/dextrin change, total CHO was relatively stable at about 83% until fermentation, where it decreased substantially to about 51%. This value fluctuated slightly in the rest of the processing streams.

Total nonstarch carbohydrate refers to all carbohydrates excluding starch and dextrin. It includes soluble sugars, cellulose, hemicellulose, and lignin. The last three are also known as fiber. Since soluble sugars are mostly absent in whole stillage and the remaining processing streams, the total nonstarch CHO is almost equivalent to total fiber, which is considered nonfermentable in starch-based bioethanol production. In ground corn, starch was a major portion of the total CHO. Therefore, the total nonstarch CHO was around 17% of dry matter (**Figure 2b**). This value remained relatively unchanged until the step of saccharification, where it increased significantly due to the conversion of starch and dextrose to simple sugars. Upon fermentation, the depletion of soluble sugars caused some decrease in total nonstarch CHO, but the value was still about 43%, more than double the value in ground corn. This value fluctuated slightly in the remaining streams.

Changes in the AA Profiles of Processing Streams from Plant 1. Amino acid composition is a major nutritional index of a protein ingredient. It is typically expressed as concentrations (% of sample weight, dry or as it is basis) or relative % (based on the weight of total amino acids or protein in a given sample). DDGS proteins, like other proteins, contain essential and nonessential amino acids (*7*, *9*). In general, changes in AA concentrations, either essential or nonessential, followed the pattern of protein changes during the dry grind process (**Table 1**). Before fermentation, there was a slight change. Upon fermentation, concentrations of all AA increased, resulting from starch depletion. When whole stillage was separated into thin stillage and DG, AA concentrations, just like protein content, were higher in DG than that in thin stillage. When the two were mixed into WDGS, the concentration of each AA became close to that in whole stillage. There was some minor change upon drying into DDGS. Data in **Table 1** also includes yeast AA

Table 2. Changes in Ratios of Amino Acid Concentrations of Downstream Products vs Ground Corn during the Dry Grind Process at Plant 1

amino acid (AA)	ground corn	cooked slurry	liquefied mass	saccharified mass	fermented mass	whole stillage	thin stillage	distiller solubles	distiller grains	WDGS	DDGS
					Essential						
Arg	1.00	1.03	0.95	1.10	3.55	3.39	2.88	2.74	3.74	3.46	3.67
His	1.00	1.13	0.98	1.24	2.55	2.75	2.05	1.90	3.14	2.34	3.19
Ile	1.00	0.79	0.96	0.81	2.87	2.11	1.32	1.88	2.70	2.63	2.50
Leu	1.00	0.83	0.92	0.90	2.59	2.22	1.09	1.31	3.20	2.28	2.76
Lys	1.00	0.99	0.99	0.95	3.49	3.13	2.49	3.01	3.61	3.25	3.37
Met	1.00	1.35	0.99	1.11	2.00	2.06	1.79	1.66	2.04	1.43	2.25
Phe	1.00	0.75	1.04	0.88	2.32	1.85	1.15	1.48	2.41	1.91	2.10
Thr	1.00	1.00	0.95	1.04	2.87	2.61	1.92	2.07	3.22	2.62	2.97
Val	1.00	0.91	0.99	0.92	2.07	1.72	1.36	1.62	2.04	1.85	1.93
					Nonessential						
Ala	1.00	0.99	1.00	0.99	3.09	2.73	1.94	2.18	3.32	2.82	3.14
Asp	1.00	1.05	1.07	1.08	3.29	2.94	2.11	2.37	3.53	2.97	3.32
Cys	1.00	1.13	1.00	1.17	1.98	1.84	1.65	1.54	2.14	1.60	1.93
Glu	1.00	0.93	0.99	0.96	2.95	2.64	1.79	1.95	3.30	2.61	3.06
Gly	1.00	0.98	1.04	1.07	3.44	3.11	2.67	2.97	3.30	3.25	3.34
Pro	1.00	0.67	1.08	0.79	3.27	2.31	1.24	1.93	3.21	2.82	2.87
Ser	1.00	0.97	0.93	1.03	2.78	2.48	1.70	1.82	3.14	2.49	2.83
Tyr	1.00	0.78	1.01	0.71	2.37	1.59	1.15	1.54	2.03	1.51	1.85
minimum	**1.00**	**0.67**	**0.92**	**0.71**	**1.98**	**1.59**	**1.09**	**1.31**	**2.03**	**1.43**	**1.85**
maximum	**1.00**	**1.35**	**1.08**	**1.24**	**3.55**	**3.39**	**2.88**	**3.01**	**3.74**	**3.46**	**3.67**
average	**1.00**	**0.96**	**0.99**	**0.99**	**2.79**	**2.44**	**1.78**	**2.00**	**2.95**	**2.46**	**2.77**
total AA	1.00	0.92	0.99	0.96	2.79	2.41	1.67	1.91	3.00	2.45	2.78

concentration. From this table, the influence of yeast AA concentration on downstream products appeared to be minimal or unrelated. Results also show that the content of total amino acids was close to the protein content in each sample, but the difference between the two fluctuated between positive and negative, depending on sample type and ethanol plant (**Table 1**). The difference might be due to the difference in nonprotein nitrogen content among samples and the variation resulting from two separate analytical methods.

Although the changing pattern in AA concentrations from ground corn to DDGS generally followed that of protein content, the extent of change for each AA of a given downstream product as compared with that of ground corn varied among AA (**Table 1**). To make this variation more clear, the ratio in concentration of each AA of each downstream product vs ground corn from Plant 1 was calculated and presented in **Table 2**. The ratio in total AA was also calculated. Basically, it was close to the average ratio for all AA or to the ratio in protein content of a downstream product vs ground corn. Results show that the ratio changed with individual AA as well as the type of downstream products. For downstream products before fermentation, the ratio range among AA was small, and the ratio in total AA approached 1 (similar to ground corn value), indicating that there was little change in the total AA concentration of these products from ground corn. Upon fermentation, the total AA ratio increased to 2.79, indicating that total AA concentration increased 2.79-fold from that of ground corn. More importantly, after this step, the extent of change among AA increased significantly, with the ratio ranging from 1.98 to 2.55. This indicates that during fermentation, some amino acids increased in concentration significantly faster than others. For examples, Arg, Lys, Ala, Asp, Gly, and Pro increased faster than the average ratio (or total AA ratio), while Met, Val, Cys, and Try increased less than the average fold of increase from that of ground corn. The concentration increase for the rest of AA was approximately equal to the average fold of increase. This feature of differential increases among AA was also seen in further downstream products, including DDGS, the final product. In addition, for DDGS, the total AA ratio was 2.78, and the ratio range among individual AA was between 1.85 and 3.67. These values were very close to those found in the fermented mass. Although no reports have covered changes in AA concentrations during the dry grind process, information on AA contents of corn (*6*, *7*) and DDGS (*5*−*7*, *9*) is available in the literature. Our data on AA concentrations in corn and DDGS (**Table 1**) generally agreed with previous reports.

When the AA profile is expressed as relative % (based on total AA), it describes the protein quality more than the quantity. Unlike AA concentrations, the change in AA composition in terms of the relative % of an individual AA vs total AA of each sample from Plant 1 (**Table 3**, also converted from **Table 1**) did not follow the trend of protein change (**Figure 2a**). Upon fermentation, some AA (in terms of relative %) increased, others decreased, and still others remained unchanged.

More importantly, the influence of yeast AA composition on downstream products, particularly on those following the fermentation step, became clearer. For example, Arg in corn was 3.62% (of total AA) and 4.89% in yeast; therefore, the trend was increasing from corn to DDGS; Met in corn was 3.35% and 1.79% in yeast; therefore, the trend was decreasing; Ser in corn was 5.02% and 5.52% in yeast; therefore, the trend was relatively flat.

Although changing trends in relative composition for most AA appeared to depend on the difference in AA composition between yeast and corn, there were some exceptions. For example, Pro showed little change, but its content in yeast was much lower than that in ground corn (2.18% vs 6.67%). One possible explanation for this discrepancy is that during alcohol fermentation, the main source of nitrogen for yeast growth is free amino acids in the fermentation mass. However, yeasts take up the free amino acids

Table 3. Changes in Amino Acid Composition (Relative % of an Individual AA vs Total AA in Each Sample) during the Dry Grind Ethanol Process from Corn at Plant 1[a]

amino acid (AA)	ground corn	cooked slurry	liquefie dmass	saccharified mass	fermented mass	whole stillage	thin stillage	distiller olubles	distiller grains	WDGS	DDGS	yeast
						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 ± 0.08
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] Means ± standard deviation. The rest are values of single measurement.

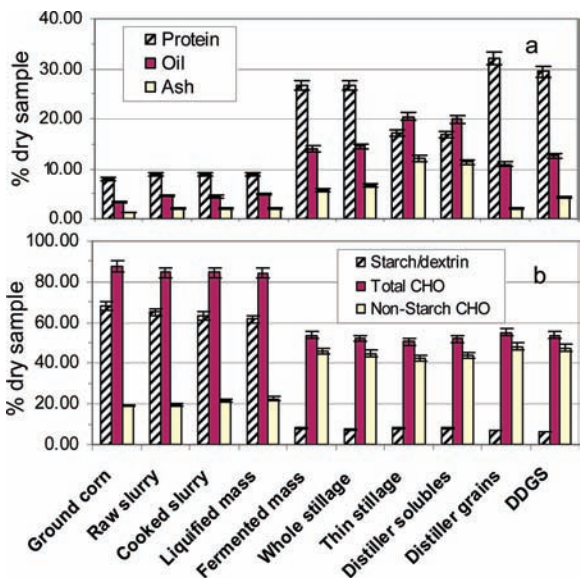

Figure 3. Changes in chemical composition during dry grind ethanol processing from corn at Plant 2. (**a**) Contents of protein, oil and ash and (**b**) contents of starch/dextrin, total CHO (carbohydrates), and total nonstarch CHO. Error bars represent starndard deviations.

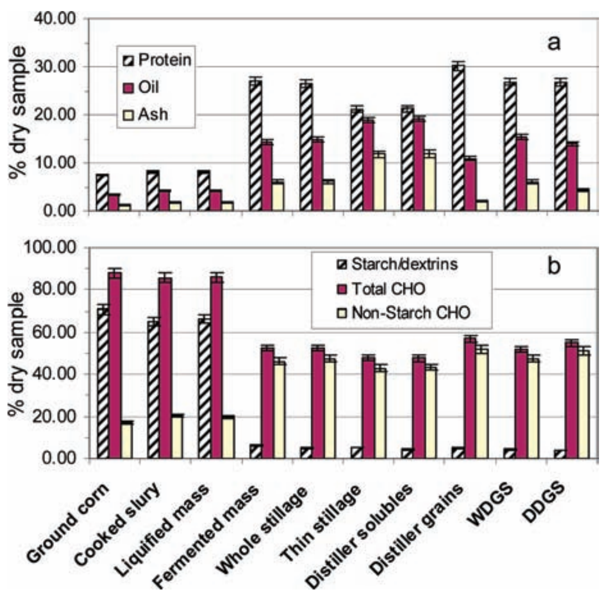

Figure 4. Changes in chemical composition during dry grind ethanol processing from corn at Plant 3. (**a**) Contents of protein, oil and ash and (**b**) contents of starch/dextrin, total CHO (carbohydrates), and total nonstarch CHO. Error bars represented standard deviations.

in an orderly manner with different amino acids removed at various points in the fermentation cycle (*13*). According to Piece (*14*), Pro in the wort/mash is absorbed and assimilated most slowly by yeast during fermentation. Therefore, for this AA, contribution from yeast toward the final product is expected to be minimal.

Confirmation from the Data Sets from Plants 2 and 3. The sample set from Plant 2 did not include the saccharified mass, while the Plant 3 sample set did not include both saccharified mass and WDGS. Although the exact steps of processing from each plant are commercial trade secrets, apparently, Plant 1 followed the traditional process of dry grind, where saccharification and fermentation were separate steps, whereas Plants 2 and 3 used simultaneous saccharification and fermentation (*1*,*2*). Yet, in spite of this difference, the changing patterns of chemical attributes in streams of the dry grind process in Plants 2 and 3 (**Figures 3** and **4**, respectively) followed those found with the Plant 1 sample set (**Figure 2**). The results for Plants 2 and 3 essentially confirmed all of the observations on chemical changes during the dry grind process of corn at Plant 1.

On the basis of **Figures 2−4**, on average, contents of protein, oil, ash, starch, total CHO, and total nonstarch CHO in ground corn were 7.65, 3.26, 1.29, 70.23, 87.79, and 17.57, % dry matter,

Table 4. Changes in Amino Acid Concentration (% Dry Weight) during Dry Grind Ethanol Processing from Corn at Plant 2[a]

amino acid (AA)	ground corn	raw slurry	cooked slurry	liquefied mass	fermented mass	whole stillage	thin stillage	distiller solubles	distiller grains	DDGS	yeast
					Essential						
Arg	0.32 ± 0.00	0.34	0.34	0.33	1.26	1.27	0.83	0.79	1.37	1.40 ± 0.06	1.56 ± 0.05
His	0.35 ± 0.04	0.30	0.33	0.30	0.79	0.89	0.49	0.51	0.96	0.91 ± 0.01	0.82 ± 0.01
Ile	0.31 ± 0.08	0.22	0.34	0.35	1.00	0.76	0.51	0.41	0.95	1.25 ± 0.01	1.31 ± 0.04
Leu	1.21 ± 0.09	0.92	1.17	1.12	3.14	3.05	1.32	1.17	3.89	3.91 ± 0.01	2.24 ± 0.02
Lys	0.29 ± 0.03	0.27	0.33	0.32	1.04	1.03	0.70	0.64	1.15	1.15 ± 0.02	2.45 ± 0.04
Met	0.41 ± 0.08	0.40	0.35	0.31	0.62	0.77	0.50	0.51	0.81	0.75 ± 0.00	0.66 ± 0.01
Phe	0.60 ± 0.10	0.49	0.67	0.66	1.49	1.40	0.84	0.64	1.56	1.76 ± 0.04	1.38 ± 0.01
Thr	0.40 ± 0.01	0.36	0.38	0.36	1.09	1.15	0.68	0.66	1.23	1.26 ± 0.00	1.79 ± 0.02
Val	0.80 ± 0.06	0.69	0.76	0.73	1.54	1.52	1.08	0.93	1.50	1.80 ± 0.01	1.64 ± 0.01
					Nonessential						
Ala	0.67 ± 0.02	0.58	0.65	0.63	1.89	1.91	1.10	1.01	2.19	2.27 ± 0.02	1.73 ± 0.05
Asp	0.63 ± 0.02	0.55	0.62	0.61	1.84	2.00	1.12	1.05	2.05	2.16 ± 0.02	3.28 ± 0.01
Cys	0.36 ± 0.04	0.35	0.32	0.29	0.58	0.64	0.47	0.48	0.61	0.60 ± 0.02	0.41 ± 0.01
Glu	1.80 ± 0.09	1.56	1.78	1.70	4.93	4.98	2.90	2.68	5.74	6.01 ± 0.05	6.32 ± 0.06
Gly	0.33 ± 0.02	0.30	0.36	0.35	1.15	1.18	0.86	0.78	1.15	1.31 ± 0.02	1.40 ± 0.00
Pro	0.65 ± 0.19	0.39	0.76	0.69	2.18	1.79	1.10	0.74	2.21	2.63 ± 0.01	0.67 ± 0.02
Ser	0.50 ± 0.01	0.47	0.48	0.46	1.36	1.40	0.79	0.77	1.57	1.58 ± 0.01	1.63 ± 0.01
Tyr	0.48 ± 0.15	0.30	0.54	0.40	1.16	1.04	0.58	0.48	0.98	1.29 ± 0.05	0.89 ± 0.06
total AA	10.11 ± 0.67	8.44	10.17	9.59	27.03	26.80	15.88	14.21	29.89	32.05 ± 0.17	30.19 ± 0.41
protein	7.82 ± 0.04	8.93 ± 0.01	8.96 ± 0.04	8.95 ± 0.05	26.55 ± 0.040	26.77 ± 0.36	17.19 ± 0.28	16.96 ± 0.30	32.02 ± 0.61	29.42 ± 0.06	36.19 ± 0.11

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

Table 5. Changes in amino acid concentration (% dry weight) during dry grind ethanol processing from corn at Plant 3[a]

amino acid (AA)	ground corn	cooked slurry	liquefied mass	fermented mass	whole stillage	thin stillage	distiller solubles	distiller grains	WDGS	DDGS	yeast
						Essential					
Arg	0.35 ± 0.02	0.34	0.30	1.26 ± 0.03	1.20	1.07	1.02	1.28	1.19	1.16 ± 0.09	1.73
His	0.30 ± 0.02	0.30	0.31	0.81 ± 0.00	0.79	0.67	0.63	0.89	0.81	0.82 ± 0.06	0.80
Ile	0.36 ± 0.02	0.35	0.25	1.03 ± 0.17	1.02	0.74	0.49	1.24	1.04	0.92 ± 0.19	1.68
Leu	1.13 ± 0.06	1.12	0.88	3.23 ± 0.21	3.10	1.81	1.45	4.19	3.26	3.18 ± 0.21	2.62
Lys	0.32 ± 0.01	0.32	0.27	1.01 ± 0.03	1.01	0.95	0.78	1.10	1.00	0.88 ± 0.06	2.71
Met	0.33 ± 0.08	0.34	0.37	0.67 ± 0.06	0.66	0.54	0.69	0.69	0.72	0.65 ± 0.16	0.63
Phe	0.64 ± 0.06	0.69	0.50	1.36 ± 0.17	1.45	1.00	0.81	1.74	1.50	1.37 ± 0.02	1.69
Thr	0.36 ± 0.02	0.38	0.36	1.10 ± 0.02	1.07	0.86	0.79	1.24	1.10	1.06 ± 0.02	1.92
Val	0.75 ± 0.08	0.75	0.66	1.56 ± 0.15	1.55	1.31	1.09	1.70	1.59	1.40 ± 0.12	2.03
						Nonessential					
Ala	0.63 ± 0.02	0.61	0.54	1.87 ± 0.09	1.89	1.40	1.23	2.29	1.94	1.86 ± 0.14	2.08
Asp	0.61 ± 0.02	0.59	0.54	1.79 ± 0.07	1.79	1.49	1.31	2.06	1.84	1.77 ± 0.08	3.71
Cys	0.30 ± 0.05	0.32	0.32	0.61 ± 0.02	0.55	0.53	0.54	0.57	0.57	0.53 ± 0.07	0.41
Glu	1.66 ± 0.09	1.66	1.47	5.11 ± 0.21	4.95	3.67	3.20	5.95	5.18	4.94 ± 0.25	7.48
Gly	0.35 ± 0.01	0.35	0.31	1.16 ± 0.05	1.17	1.12	1.00	1.21	1.18	1.11 ± 0.09	1.61
Pro	0.68 ± 0.03	0.71	0.41	2.01 ± 0.37	2.18	1.53	1.04	2.80	2.30	2.01 ± 0.30	1.25
Ser	0.46 ± 0.02	0.47	0.44	1.35 ± 0.05	1.32	1.00	0.93	1.58	1.36	1.32 ± 0.05	1.77
Tyr	0.50 ± 0.17	0.56	0.34	0.98 ± 0.21	1.10	0.84	0.64	1.23	1.13	0.87 ± 0.05	1.28
total AA	9.73 ± 0.76	9.85	8.25	26.37 ± 1.71	26.81	20.52	17.60	31.79	27.75	25.83 ± 1.29	35.42
protein	7.45 ± 0.03	8.09 ± 0.00	8.08 ± 0.02	27.16 ± 0.74	26.55 ± 0.05	21.14 ± 0.03	21.24 ± 0.84	30.21 ± 0.05	26.89 ± 0.30	26.67 ± 0.09	37.4 ± 0.32

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

respectively; in whole stillage, 27.61, 14.82, 6.33, 6.12, 51.25, and 45.13, respectively, and in DDGS, 28.52, 12.79, 4.33, 5.93, 54.36, and 48.43, respectively. Although the literature on changes in chemical composition during the dry grind process is lacking, information on the chemical composition of corn (*3*, *6*, *7*) and DDGS (*3*, *5−8*) is available. Our data on the chemical composition of corn and DDGS were generally agreeable with these previous reports, although some variation existed among reports.

Furthermore, careful comparison of **Figures 2−4** shows that among the sample sets of the three different processing plants variation existed with regard to the relative difference between oil and protein content in thin stillage and DS. The protein content was higher than oil content in these two samples from Plant 1 (**Figure 2a**) and Plant 3 (**Figure 4a**). However, for the Plant 2 samples (**Figure 3a**), the oil content was higher than the protein content.

Similarly, the changing patterns of AA composition (as % dry sample) during the dry grind process from Plants 2 and 3 (**Tables 4** and **5**, respectively) followed the change pattern in the Plant 1 data set (**Table 1**). When AA concentration data in **Tables 4** and **5**

Table 6. Multiple Linear Regression for Amino Acid Composition (Relative % of an Individual Amino Acid vs Total Amino Acids in Each Sample) of Downstream Products with Ground Corn and Yeast Amino Acid Compositions As Variants X1 and X2, Respectively[a]

sample source	parameter	raw slurry	cooked slurry	liquefied mass	saccharified mass	fermented mass	whole stillage	thin stillage	distiller solubles	distiller grains	WDGS	DDGS	average
Plant 1	A		0.783	0.967	0.84	0.84	0.745	0.428	0.535	0.924	0.783	0.843	**0.769**
	B		0.162	0.013	0.121	0.191	0.304	0.482	0.366	0.198	0.258	0.244	**0.234**
	C		0.332	0.116	0.24	−0.188	−0.295	0.531	0.58	−0.719	−0.244	−0.516	**-0.016**
	r^2		0.965	0.993	0.98	0.963	0.965	0.926	0.945	0.963	0.949	0.962	**0.961**
Plant 2	A	0.842	0.969	0.947		0.821	0.794	0.565	0.476	0.892		0.895	**0.800**
	B	0.151	0	0.048		0.177	0.224	0.33	0.433	0.199		0.156	**0.191**
	C	0.048	0.165	0.03		0.019	−0.109	0.617	0.545	−0.53		−0.32	**0.052**
	r^2	0.975	0.985	0.984		0.94	0.965	0.94	0.949	0.942		0.94	**0.958**
Plant 3	A		1.026	0.845		0.846	0.885	0.555	0.444	1.061	0.924	0.939	**0.836**
	B		−0.038	0.218		0.218	0.175	0.354	0.426	0.082	0.157	0.178	**0.197**
	C		0.062	0.136		−0.378	−0.361	0.535	0.767	−0.847	−0.484	−0.728	**-0.144**
	r^2		0.996	0.96		0.959	0.963	0.952	0.929	0.947	0.963	0.956	**0.958**
combined	A		0.918	0.926		0.835	0.802	0.513	0.488	0.95	0.843	0.889	**0.796**
	B		0.046	0.06		0.196	0.238	0.39	0.407	0.165	0.214	0.195	**0.212**
	C		0.208	0.08		−0.18	0.24	0.571	0.622	−0.68	−0.343	−0.511	**0.001**
	r^2		0.977	0.978		0.954	0.963	0.936	0.94	0.949	0.955	0.951	**0.956**

[a] Regression was based on a multiple leanier model $Y = AX1 + BX2 + C$. Whereas Y = relative % of an individual amino acid in an downstream product, X1 = relative % of the amino acid in ground corn. X2 = relative % of the amino acid in yeast.

were also converted into ratios of amino acid concentrations of downstream products vs ground corn (data not shown), or to relative % (based on total AA; data not shown), the variation in the ratios or relative % among AA of a given downstream product and the changing patterns in these values for an individual AA throughout the process in Plants 2 and 3 were also similar to those found in Plant 1 (**Tables 2** and **3**, respectively). This also confirms all of the observations on changes in the AA profile and the influence of yeast AA during the dry grind process of corn at Plant 1.

Determination of Yeast Contribution to the Total DDGS Proteins. Yeast growth requires the uptake of nitrogen for the synthesis of protein and other nitrogenous components of the cell, but yeasts can only utilize low molecular weight nitrogenous materials (*13*), such as inorganic ammonium ions, urea, free amino acids, or small peptides, collectively known as free amino nitrogen (FAN). Although grain-based feedstock, such as corn, contains proteins (about 8%), yeast cannot take up these proteins or break them down to FAN due to the lack of extracellular proteolytic activity. A small amount of FAN is produced during the prefermentation process, but this amount is not sufficient to support fermentation at an acceptable rate. Fermentation is usually accelerated by supplementing the mash with nitrogen forms that can be assimilated (such as urea or ammonia) or by generating FAN from grain proteins though hydrolysis with a protease supplied exogenously (*1*, *15*).

Regardless of rates, during fermentation, yeasts grow and produce cell mass that contains a much higher amount of protein (36−60%, 15) than DDGS. When yeast cells undergo autolysis, a process of self-degradation following the death of the cell, intracellular proteolysis, and other enzymatic activities occur. During this process, protein and other compounds are released. Thus, DDGS proteins are widely believed to come from two sources, corn and yeast. However, the proportion of yeast protein to corn protein in DDGS is not well documented in the literature. Belyea et al. (*6*) measured essential amino acid concentrations of yeast, corn, and DDGS and found that the ratio of DDGS amino acid concentrations vs that of yeast varied considerably among amino acids, but most ranged from 0.45 to 0.70. They calculated the average ratio to be 0.55, and on the basis of this, they suggested that yeast protein may make up approximately half of the protein in DDGS. Unfortunately, this approach is questionable since the average ratio was basically the ratio in protein content between DDGS and yeast. It also ignored the influence of corn AA composition.

In this study, we found that when amino acid composition is expressed as % of dry sample weight, there was little information about the influence of yeast AA composition. However, when AA is expressed as relative % of protein (total AA) weight, the influence of yeast AA on DDGS AA becomes clear. Therefore, we proposed that, in terms of relative % (rather than absolute concentration), AA composition of DDGS or an intermediate product (response variable) is determined by AA composition of corn (independent variable 1) and AA composition of yeast (independent variable 2) based on the following multiple linear regression model:

$$Y = AX_1 + BX_2 + C$$

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

On the basis of the above proposed model, regression results (**Table 6**) show that parameters A, B, and C varied greatly with the type of downstream products and slightly with the sample source (Plant no.). When regression is made for the combined data set, results show that before fermentation, the value of parameter A was about 0.92, and B was around 0.05. This implies that an average AA composition (% of total AA) for cooked slurry or liquefied mass from all three plants would increase by an average factor of 0.92 if the AA composition of ground corn increased by 1%, while the AA composition of yeast remained unchanged. Similarly, a 1% increase in yeast AA composition with corn AA held fixed would only increase the mean AA of cooked slurry or liquefied mass by an average factor of 0.05.

After fermentation, value A was reduced to about 0.84, and value B increased to about 0.20. This implies that the average AA composition for fermented mass from all three plants would increase by a factor of 0.84 if AA composition of ground corn increased by 1% and the AA composition of yeast remained fixed. Similarly, a 1% increase in yeast AA composition, with corn AA held fixed, would now increase the mean AA of fermented mass by a factor of 0.20. Furthermore, upon centrifugation, the B value increased in thin stillage and its condensed form (DS), but decreased in distiller grains. The A value changed accordingly, decreasing in thin stillage and DS but increasing in distiller grains. The two parameters in both WDGS and DDGS became similar to those found in whole stillage and fermented mass.

The A and B values in **Table 6** indicate that before fermentation, yeast protein contributed about 5% toward the protein in the intermediate products. However, upon fermentation, contribution of yeast protein increased to about 20%. The contribution of yeast protein was further increased in thin stillage and DS, but reduced in DG. When the two were combined together into WDGS and then dried to DDGS, the contribution of yeast protein returned to about 20%. This estimation is much lower than the value estimated by Belyea et al. (*6*) who suggested that yeast protein may make up approximately half of the protein in DDGS.

Apparently, the increase in yeast protein contribution after fermentation was due to the addition of yeast culture, growth, and autolysis of yeast biomass. It is expected that a considerable portion of yeast protein was in the form of free amino acids and soluble peptides resulting from the autolysis of yeast cells at the end of fermentation. This would explain the observation that there was a higher concentration of yeast protein in thin stillage than in distiller grains after centrifugation. The minor contribution of yeast protein to cooked slurry and liquefied mass is possible because a portion of thin stillage was recycled as backset to make slurry from ground corn. The higher r^2 values (with an average value higher than 0.95, **Table 6**) indicate the higher strength of the linear relationships in AA composition (relative %) among a downstream product, corn, and yeast.

Finally, it is important to note that although the multiple linear regression data in **Table 6** suggests that yeast contributed about 20% of DDGS protein and that the rest, 80% protein, came from ground corn, this estimation does not mean that yeast biomass also contributed about 20% toward the DDGS mass. The reason is that during fermentation, yeasts used corn sugars and other nutrients as substrates and converted them into their biomass, including yeast protein.

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