

National Corn-to-Ethanol Research Center



Utilizing the National Corn-to-Ethanol Pilot Plant to
Develop a Predictive Model For Distillers' Dried
Grain with Solubles (DDGS) for the Fuel Ethanol
and Animal Feed Industries

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Executive Summary

This research was the first systematic investigation of the effects of ethanol-plant operating conditions on the chemical and physical characteristics of DDGS. The results demonstrate that several operations and processes exerted statistically significant effects on DDGS characteristics and composition, but the effects appear to be highly nonlinear and interactive. Simple multiple-regression statistical models provided poor descriptions of the relationship between DDGS composition and operating conditions. Neural-network models, which are much more complex, provided better descriptions of these relationships, but the best-fit models identified in this work should not be considered to be predictive. Statistically significant treatment effects were also identified for several measures of fermentation performance (*e.g.*, mash DE, sugar consumption rate, ethanol yield), but these relationships are not yet understood well enough for use in process control. Only weak correlations were observed between easily measurable characteristics of the mash and important metrics of fermentation performance, such as ethanol yield.

The results of this research were encouraging with respect to the ability to use sophisticated process-control technologies to improve the consistency and nutritional value of DDGS, which is an important coproduct of the dry-grind ethanol industry. Because nutritional quality depends on several characteristics that are affected differently by processing conditions, use of neural-network models for control of DDGS composition and nutritional quality may be difficult, but such control seems feasible in principle.

One important characteristic that can affect the nutritional value of DDGS is the quality of the protein, and in particular, the assimilable concentrations of essential amino acids, such as lysine. The amino acid concentrations were measured in a preliminary study of the effects of drying on DDGS characteristics. The concentrations were not correlated with any of the dryer parameters that were investigated, but DDGS color, which has been shown to be correlated with protein digestibility, were affected by operating conditions. DDGS color was also measured in pilot-scale experiments that systematically varied front- (*i.e.*, prefermentation) and back-end (*i.e.*, postdistillation) operating conditions. In both studies, dryer temperature and the addition rates of syrup and wet cake were significantly correlated with DDGS color. Moisture content also was affected by dryer temperature and syrup addition rate, but the effects were in the opposite direction (with respect to desired DDGS characteristics) than were the effects on color. Higher dryer temperature reduced DDGS moisture content (increased dry solids concentration) and made the DDGS darker. Dark color is a characteristic of nutritionally poor-quality DDGS. The opposite effects of these operating conditions on desirable characteristics of DDGS suggests that an optimal range of operating conditions may exist, but further investigation will be necessary to identify these conditions.

1. Introduction and Objectives

The objective of this research was to develop and validate a predictive neural network model that relates the composition of distiller's dried Grain with solubles (DDGS), a major coproduct of the dry-grind process for production of fuel ethanol, with plant operating conditions. The relationship was investigated by varying pilot-plant operating conditions, collecting the DDGS that was produced, and determining its chemical composition and physical characteristics. The data were used to develop and test the neural network model. This process can serve as an example that can be emulated at full-scale dry-grind ethanol plants and used for control of DDGS quality.

In the United States, DDGS is used primarily as a protein supplement for formulating feed for ruminants (e.g., cattle). In 2006, dry-grind ethanol plants in the U.S. produced about 8.5 million metric tons (mmt) of DDGS, and production is predicted to increase to 36 mmt by the year 2010 (US Grains Council, 2006). Use of DDGS in swine and poultry diets is limited by uncertainty regarding the nutrient content (US Grains Council, 2006). The expected increase in DDGS supply due to growth of the U.S. fuel-ethanol industry provides greater incentive to develop methods for managing DDGS quality in order to maintain the economic value of DDGS and, therefore, the profitability of dry-grind ethanol plants.

This research was a collaboration between the National Corn-to-Ethanol Research Center (NCERC) at Southern Illinois University-Edwardsville (SIUE), Washington University (WU), Emerson Process Management (EPM), and the Illinois Department of Commerce and Economic Opportunity-Division of Renewable Fuels (DCEO). EPM, DCEO, and SIUE provided matching funds to purchase the hardware and software needed for development of a neural network model. Additional cost-sharing for personnel was provided by SIUE and WU.

The specific objectives of this research were to:

- investigate the operational factors that affect the nutritional composition and overall quality of distillers' dried grains with solubles (DDGS), and
- develop and validate a predictive model for DDGS quality based on plant operating conditions.

2. Background

In the production of fuel-grade ethanol from corn, three products are generated in roughly equal quantities: ethanol, carbon dioxide, and distiller's dried grains with solubles (DDGS). DDGS is an animal feed product composed of the nonfermentable components of corn (e.g., protein, fiber, and fat) and yeast cells that grow during fermentation of starch to ethanol. Every bushel (56 lbs) of corn used for ethanol production also produces about 18 lbs of DDGS. Estimates of the future growth of the ethanol industry suggest that the annual production of DDGS will increase from about 8.5 mmt to as much as 36 mmt per year due to the passage of the renewable fuels standard (US Grains Council, 2006) (1). The majority (80%) of the DDGS used in the U.S. is fed to ruminants (beef and dairy cattle). The remainder is fed to poultry (5%) and swine (15%). DDGS can represent a significant fraction of the revenues of dry-grind ethanol plants, and therefore, it is important to the economic viability of the industry.

The most expensive components of animal feed are energy, amino acids in the form of protein, and phosphorous. The primary sources of these livestock feed ingredients are ground

corn and soybean meal. DDGS is an ideal alternative source of protein, energy, fiber, and phosphorous. Use of this alternative feed material is limited by the variability of its composition and its nutritional quality (Thaler, 2002). Factors that can affect the composition and quality of DDGS include the composition of the grain used in the fermentation process, the operating conditions (e.g., liquefaction conditions, dryer temperature). Table 1 illustrates how the composition of DDGS varies among different sources.

Table 1: Variation in the composition of DDGS among different sources

Component	Shurson <i>et al.</i> cited			
	by U.S. Grains Council (2006)	Cromwell <i>et al.</i> (1993)	Belyea <i>et al.</i> (2004)	
			DDGS	Corn
dry matter (%)		90.5 ± 1.26		
crude protein (%) ^a	30.9 ± 0.5	29.7 ± 1.2	31.3 ± 0.6	9.1 ± 0.2
crude fat (%) ^a	10.7 ± 0.6		11.9 ± 0.3	4.2 ± 0.2
crude fiber (%) ^a	7.2 ± 0.5		10.2 ± 3.3	
NDF (%) ^a		34.9 ± 9.4		
ADF (%) ^a		14.8 ± 4.4	17.2 ± 3.3	
phosphorus (%) ^a	0.75 ± 0.05			
ash (%) ^a	6.0 ± 0.6	5.3 ± 1.1	4.6 ± 0.6	
starch (%) ^a			5.1 ± 0.7	71.4 ± 0.9

^a component concentrations given on a dry mass basis

Because starch represents more than two-thirds of the dry mass of corn kernels and it is almost completely consumed in ethanol fermentation, the protein, fat, fiber, and mineral components that are not metabolized by yeast are concentrated by a factor of three (56 lbs/18 lbs) in DDGS. Thus, variability in the non-starch components of the corn is magnified leading to larger percent variations in DDGS. Despite this concentration effect, the variability in DDGS is not strongly correlated with the variability of the incoming corn (Belyea *et al.*, 2004). This suggests that the majority of variation in DDGS composition comes from the ethanol production process itself. Fermentation, distillation, thin-stillage evaporation, and drying have been identified as having potentially large effects on DDGS nutritional value (Thaler, 2002; U.S. Grains Council, 2006).

The chemical transformations of the components of corn into the forms in which they occur in DDGS are, in general, driven by heat, yeast metabolic processes, or a combination of the two. For example, protein enters the process with the corn, but it is also produced as a byproduct of yeast metabolism, which have a limited ability to hydrolyze protein or to assimilate polypeptides larger than tripeptides (Ingledew, 2003). Because most of the amino acids in corn are not available to support yeast growth, urea and other nutrients are added to allow the yeast to produce the proteins they require. Therefore, fermentation probably increases the total crude protein of the mash relative to what would be observed if protein behaved as a conservative

material. The effects of fermentation on the relative concentrations of the essential amino acids (*i.e.*, those amino acids that cannot be synthesized by the animal and which must be present in the diet) and, therefore, the nutritional quality of the protein in DDGS, however, are unknown.

The unit operations that occur after fermentation do not create or destroy the total amount of crude protein. Since crude protein is a measure of the total reduced nitrogen concentration, this statement means that the post-fermentation unit operations do not remove protein nitrogen by volatilization or oxidation. The heating that occurs during distillation, evaporation, and drying, however, can reduce the amount of protein that an animal can assimilate. The Maillard reaction is one example of such a conversion. The first step in this reaction pathway is shown in Figure 1 for lysine in two forms: free (*i.e.*, as an amino acid) and incorporated in protein.

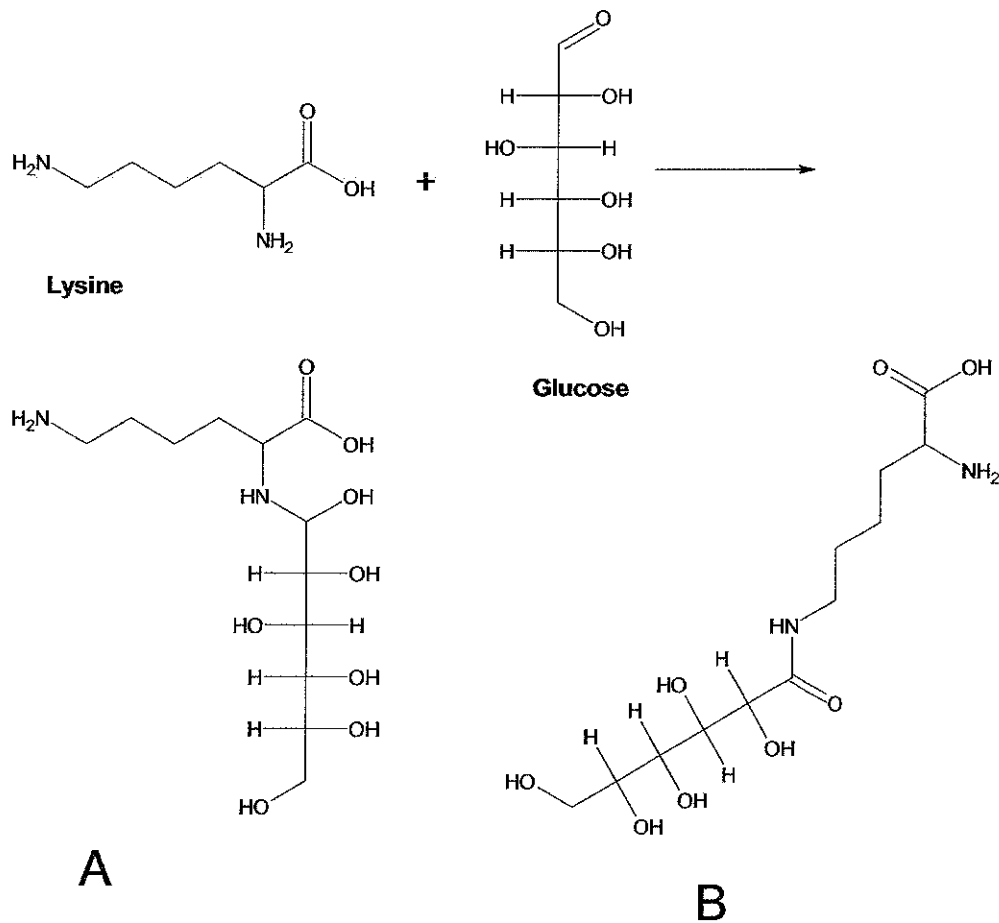


Figure 1: Maillard reaction with lysine. Form A results from free lysine, while form B results from lysine that is bound in proteins. Type B is more common in

The Maillard reaction is initiated when any protein with a free amino group reacts with a sugar to create a glycosylamine. These compounds are not stable and rearrange to form Amadori products, which further react to form polymers called melanoidons. Melanoidons are responsible for the brown color in foods, and the dark color that is often associated with poor quality DDGS is produced through this mechanism.

When protein amino groups bind to sugars, the proteins are no longer susceptible to hydrolysis by proteases, which are enzymes that digest proteins and produce free amino acids. Since protein hydrolysis is an essential prerequisite for metabolism of proteins, this transformation reduces the nutritional availability of these chemically modified proteins to animals. Because lysine has a free amino group even when it is incorporated into protein, it is most susceptible to modification through this reaction mechanism. Lysine is also an essential amino acid. Therefore, Maillard reactions can significantly degrade the nutritional value of DDGS.

Data from swine-feeding studies has been correlated with the presence of amino acids that have undergone the Maillard reaction (Stein *et al.*, 2005). In particular, high performance liquid chromatography (HPLC) has been used to measure furosine, a Maillard reaction product of lysine. An 80% correlation was observed between the furosine concentration of the feed and the ileal digestibility of protein by swine. Stein also found a high correlation between the amounts of reactive lysine (lysine not converted to furosine) in the feed and the amount of protein digested by the swine. Lysine appears to be the best amino acid (2005) for prediction of the nutritional value of the feed.

Other than protein, fat is the primary source of energy available in DDGS. Fat enters the process in corn and is left mostly unchanged by fermentation. Fats are relatively stable except at high temperatures. Therefore, the nutritional value of the fat in DDGS is probably affected primarily by dryer operating conditions. During drying, moisture prevents the temperature of the DDGS from exceeding the point where fat oxidation occurs, but overdried material may experience fat oxidation. The fat content of DDGS may be predictable based on the overall mass balance of fat in the system. Because fat is found primarily in the thin-stillage/syrup fraction after centrifugation, the fat content of the incoming corn and ratio of syrup to wet cake used to produce DDGS may be the most important factors controlling its concentration in the coproduct.

Phosphorous is a key mineral in the nutrition of animals and is one of the most expensive components of animal feed formulations (Neutkens, 2006). It is also a key environmental pollutant that can stimulate algal blooms in surface waters that receive runoff from animal production facilities. Animals can assimilate inorganic phosphorous (Fig. 2B), but not phytate (Fig. 2A). In corn, phosphorous is usually present predominantly as phytate. The availability of

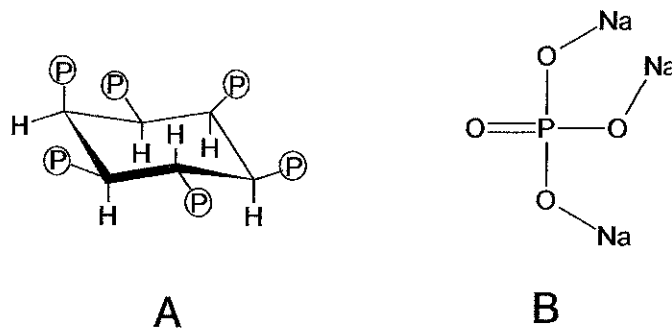


Figure 2: Different types of phosphorous compounds found in the corn-ethanol process. Phytate (A) is an organic form of phosphorous found in corn that is indigestible by animals. B is a phosphate salt that animals can metabolize.

phosphorus in DDGS produced in modern ethanol plants has been reported to be more available for assimilation by swine (92% vs. 87%) than was reported in a highly cited NRC study of animal nutrition (Belyea *et al.*, 2004), but direct evidence of this conversion has not been obtained. Because phosphorous is one of the elements that is concentrated in DDGS relative to corn, cattle fed DDGS ingest more phosphorous than those fed diets in which the energy and protein are provided primarily by corn and soybean meal. This results in a higher concentration of phosphorous in the manure and may increase the phosphorus load to surface and groundwater. Therefore, it is important to be able to predict and (ultimately) control the amount of phosphorous in DDGS based on the plant operating conditions to optimize the nutritional benefits and minimize the negative environmental impacts.

Feeding studies have been conducted to identify the factors that limit the use of DDGS in poultry and swine. In the case of poultry, growth and reproductive response issues have been identified when DDGS is added to the chick diet. It is believed that contributing factors are vitamin B content, available phosphorous content, variability in amino acid composition and availability of amino acids (Noll *et al.*, 2001). These elements vary depending on the source of the DDGS and the conditions under which it was produced. For example, it is believed that lysine degradation or availability is significantly influenced by drying temperatures and drying in the presence of sugars. In the case of swine, a recent study concluded that ethanol plants constructed since 2000 produce DDGS with higher levels of gross energy, phosphorous, lysine, methionine, and threonine relative to the values that were reported in a highly cited NRC study of the use of alternative protein sources in swine diets (Spiehs *et al.*, 2002).

Increasing the use of DDGS in animal feeds, especially in swine and poultry diets, depends on improved nutritional consistency of the product. This research investigated the relationships between ethanol-plant operating conditions and the composition and quality of DDGS by conducting pilot-scale experiments at the National Corn-to-Ethanol Research Center. Statistical and neural-network models were generated to identify the factors that contributed significantly to the observed variability and to describe the relationships. The research provides the basis for the ethanol industry to predict and control the properties of DDGS, possibly tailoring the coproduct for specific animal species.

There are several different types of inputs that could affect the composition and nutritional quality of DDGS. Values such as reaction temperatures, flow rates, and pH are controllable variables. Controllable variables have a defined range of operation. The characteristics of DDGS can also be affected by process parameters that cannot be controlled but can be measured. The relationship between inputs and outputs also depends on time because there may be a lag between the time at which input values change and the DDGS is produced. In a designed experiment, only controllable inputs can be used as factors in the design, but controllable variables may not be the only valuable inputs in a predictive model. Understanding how uncontrollable-but-measurable parameters can affect the process can also provide valuable information and explain some of the variability in DDGS quality. Neural network regression models have the capability to look at both controlled and disturbance variables and determine their effect on an output variable.

A neural network is a statistical regression technique that can account for nonlinearities in processes and can provide predictive capability on processes with enormous complexity (Blevins *et al.*, 2002). However, neural nets do not provide insight into the chemical or physical

processes that cause the observed effects to occur, and they require a large amount of data to be effective. Combining neural network models with standard experimental design principles may facilitate the development of a predictive model for the relationship between DDGS nutritional quality and process parameters while also providing greater understanding of the physical-chemical basis for the observed relationships.

The input variables that were expected to most significantly affect DDGS composition were identified through communications with plant operators, members of the DDGS advisory group, DDGS survey results, and the scientific literature. These include (in order of expected importance): (1) dryer outlet temperature, (2) dryer inlet temperature, (3) dryer moisture content, (4) ratio of backset (*i.e.*, thin stillage) used in preparation of the corn slurry, (5) product DDGS recycle ratio, (6) solids concentration of wet cake, (7) solids concentration of syrup, (8) the ratio of syrup to wet cake in the feed to the dryer, (9) moisture content in dryer feed, (10) moisture content of product DDGS, (11) distillation reboiler temperature, (12) residual starch from fermentation, (13) residual sugar from fermentation, (14) fermentation time, (15) yeast and yeast nutrient concentrations, (16) jet cooker temperature, (17) liquefaction residence time, (18) liquefaction temperature, (19) enzyme (alpha amylase, glucoamylase, phytase) enzyme concentrations, (20) size distribution of particles produced by the hammer mill, and (21) the initial composition of the corn. These factors were used as the basis for the design of the experiments conducted in this research for development of a predictive model of DDGS composition. Many of these factors are potentially controllable, but only a few could be systematically controlled during this research. The experiments that were conducted to evaluate the effects of these factors are described in detail in section 5.

3. Patents

No patents have been applied for or are anticipated in the course of this work. The results of this publicly funded work will be freely communicated to the industry.

4. Publications/Presentations

No papers have yet been submitted for publication but at least two peer-reviewed publications and two trade publications are anticipated. Several presentations including descriptions or results of this research have been made. These presentations are summarized in Table 2.

Table 2: List of presentations on the results of this research

Forum	Title
Wisconsin Showcase of Biorefining, Bioenergy, and Energy Conversion Initiatives (October 2005)	Building Biorefinery Commercial Testing Capabilities
University of Missouri, Rolla (October 2006)	Reducing Coproduct Variability in the Production of Ethanol from Corn
National Ethanol Conference (February 2007)	Quality Control for Fermentation Byproducts: Operational Factors that Affect the Physical and Chemical Properties of DDGS

5. Results

5.1. Phosphorous Conversion in Pilot-Scale Fermentation

A pilot-plant trial was conducted to determine whether phytate concentrations changed during cooking and fermentation of corn slurry. Samples were taken during the fermentations and analyzed for phosphorous composition. Total phosphorus was measured by ashing the samples in a muffle furnace at 500 °C followed by dissolution of the phosphate in 3 N HCl and analysis using the phosphomolybdate method. Phytate was measured by extraction with 2.4% HCl followed by separation of the phytate from the extract by anion-exchange chromatography, ashing of the 0.7 M NaCl eluate and measurement of phosphate by the phosphomolybdate method. Although the phytate concentrations decreased with time during a lab-scale experiment (J. Maurer, personal communication), similar changes were not observed during the pilot plant. Figure 3 shows the ratio of the concentrations of phytate to total phosphorous over time in several pilot-scale fermentations. Although the slopes of three of the four best-fit lines are negative, none of them are significantly different from zero. Therefore, the fraction of total phosphorous that is nutritionally available did not change significantly during the fermentation

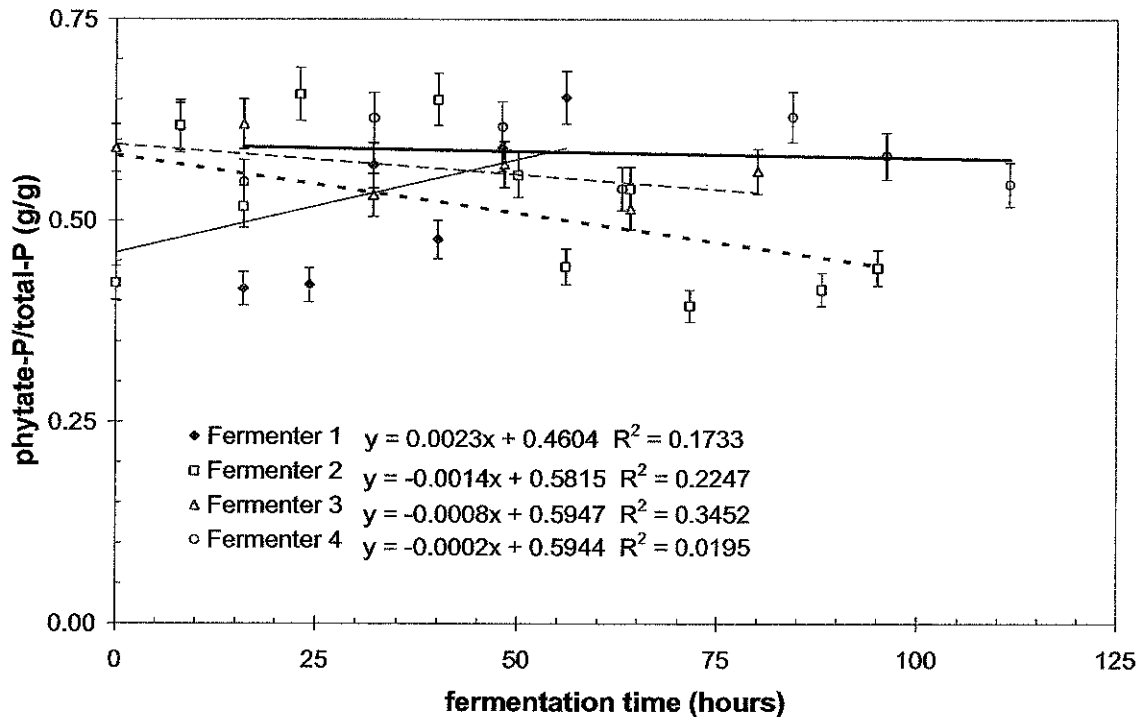


Figure 3: Ratio of the concentrations of phytate phosphorus to total phosphorus in pilot-scale fermentations.

period in any of the four fermenters. Although the temporal trends could be obscured due to continued addition of phytate with corn mash during the approximately 20-hr time period required to fill the fermenters, the slope of the ratio did not change after the fill period was complete, suggesting that transformation did not occur.

5.2. Characteristics of DDGS Prepared using a Dupps Pilot-Scale Dryer

The Dupps Company loaned the NCERC a pilot-scale dryer for these experiments. Because the Dupps dryer has a residence time of about 30 minutes, it can be used to test a wide range of experimental conditions in a relatively short period of time. In addition, the Dupps dryer has the ability to run in two modes: a normal mode which dries with heated air and an airless mode which dries with steam. This provides a unique ability to control the amount of oxygen and moisture that DDGS comes in contact with during the drying process.

The Dupps pilot-scale dryer has several controllable operating parameters, including the temperature of the air or steam entering the dryer (controls the rate of heat input, and therefore, the drying rate), the rotation speed of the drum (determines the mixing rate), the blower speed (controls the flow rate of inlet air or steam, and therefore, the temperature gradient across the dryer), the speed of the auger used to feed the mixture of wet cake and syrup (controls the rate of material, including water, input), and the oxygen concentration of the inlet air (determines the amount of moisture in the inlet air and may affect the degree of oxidation of some components of DDGS). One experiment compared the characteristics of DDGS produced using air as the heat transfer medium to DDGS that was produced using steam to determine the effect of oxygen on the chemical characteristics. The inlet temperature of the air or steam and the blower speed were fixed at 700° F and 40 Hz (fan speed), respectively. The DDGS produced under the two drying conditions was characterized using standard tests for crude protein, fiber, and fat. In addition, the digestibility of the protein in the DDGS was determined using the IDEA™ assay (Novus International, St. Louis, MO). The digestible-protein assay attempts to measure the fraction of protein that is available to the animal.

Table 3 shows the digestibility of several amino acids in the dryer feed (*i.e.*, mixture of wet cake and syrup) and the dry DDGS product. Only about 1% of the digestible protein was lost in a single pass of the dryer. No statistically significant difference was observed between the digestibility of protein in DDGS produced with steam and air. The validity of the IDEA test procedure was evaluate using DDGS dried at a higher temperature. This product showed a significant reduction in the digestibility of the protein.

Table 3: Digestibility of essential amino acids using the IDEA assay

inlet gas composition	Sample	DDGS IDEA-Assay Predicted Amino Acid Digestibility (%)									protein (%)	IDEA Value
		Lys	Met	Cys	Thr	Arg	Val	Ile	Leu	Trp		
air	corn	68.6	87.0	76.1	74.5	85.5	81.3	82.8	88.9	83.0	31.8	0.959
	DDGS	67.3	86.6	75.0	73.7	84.8	80.8	82.3	88.5	82.0	27.9	0.919
steam	corn	70.7	87.5	77.7	75.6	86.4	82.0	83.4	89.4	84.4	28.3	1.019
	DDGS	69.3	87.1	76.6	74.8	85.8	81.5	83.0	89.0	83.4	35.5	0.978
high temp	DDGS	55.2	83.6	65.5	67.0	79.4	76.7	78.3	85.1	73.2	35.0	0.565

A second experiment was conducted to determine the effects of post-distillation processing on the characteristics of DDGS. Whole stillage was separated into a solid (wet cake) and liquid (thin stillage) fraction in a centrifuge, and the thin stillage was concentrated to syrup (30% solids) in an evaporator. The wet cake, syrup, and dried DDGS were combined to create a dryer feed with a fixed moisture content of 30%, but the ratio of syrup to wet cake was varied. The

syrup-to-wet cake ratios that were used (Table 4) represent the upper and lower limits that are used in the ethanol industry. The amount of syrup added to the dryer feed represents variation in the amount of thin stillage that is recycled into the process water. A syrup addition of 50% represents the composition of dryer feed that would exist in a plant that recycles 50% of its thin stillage, while a syrup addition of 100% represents no recycling of the thin stillage. The dryer was seeded with DDGS from a previous plant trial, but because 65% of the dry product was recycled into the next batch of feed during every dryer cycle, the material used to prime the system was eventually displaced by DDGS produced from this study.

Table 4: Composition of mixture fed to the Dupps dryer

Thin Stillage Converted to Syrup	Amount of Syrup Added*		
	50%	75%	100%
Syrup	8 lbs.	11 lbs.	13 lbs.
Wet Cake	26 lbs.	23 lbs.	22 lbs.
DDGS	66 lbs.	66 lbs.	65 lbs.
<i>Total</i> [†]	<i>100 lbs.</i>	<i>100 lbs.</i>	<i>100 lbs.</i>

*the proportion of thin stillage used to make syrup

[†]the moisture content of all input formulations was 30%

In addition to the syrup-to-wet cake ratio, three other dryer operating conditions were varied: inlet temperature, air flow rate, and oxygen concentration. The different feed mixtures were dried at temperatures from 600° F to 700° F and air flows corresponding to 60% and 85% the capacity of the blower (Table 5). The oxygen concentration in the dryer was controlled by using either air (>20%) or steam (< 3%) as the drying medium. The plant distributed control system (DCS) recorded trends on the dryer inlet and outlet temperatures as well as the percentage of oxygen in the dryer. Temperature was measured using J-type thermocouples, while oxygen percentage was measured using a Yokogawa Zirconia Oxygen Analyzer (ZR202G). Other relevant data, such as feed moisture, product moisture, and dryer pressure were measured and logged by operators. The sample points and locations of the dryer measurements are shown in Figure 4.

Table 5: Operating conditions for second Dupps dryer trial

Run #	Inlet Temperature (°F)	Blower VFD Frequency (Hz)	Syrup Addition Rate (%)	Oxygen Concentration (%) [±0.2]	Moisture Content (%) [±0.3]	Outlet Temperature (°F) [±1]
1	650	40	75	0.64	9.25	251.4
2	700	50	50	2.84	6.64	267
3	700	35	100	0.34	14.91	228.8
4	600	50	100	1.57	9.08	249.8
5	600	35	50	1.53	11.82	229.8
6	600	35	100	21.0	14.76	138.9
7	600	50	50	21.0	10.1	159.5
8	700	35	50	20.7	11.43	150.8
9	700	50	100	21.0	9.05	174.1

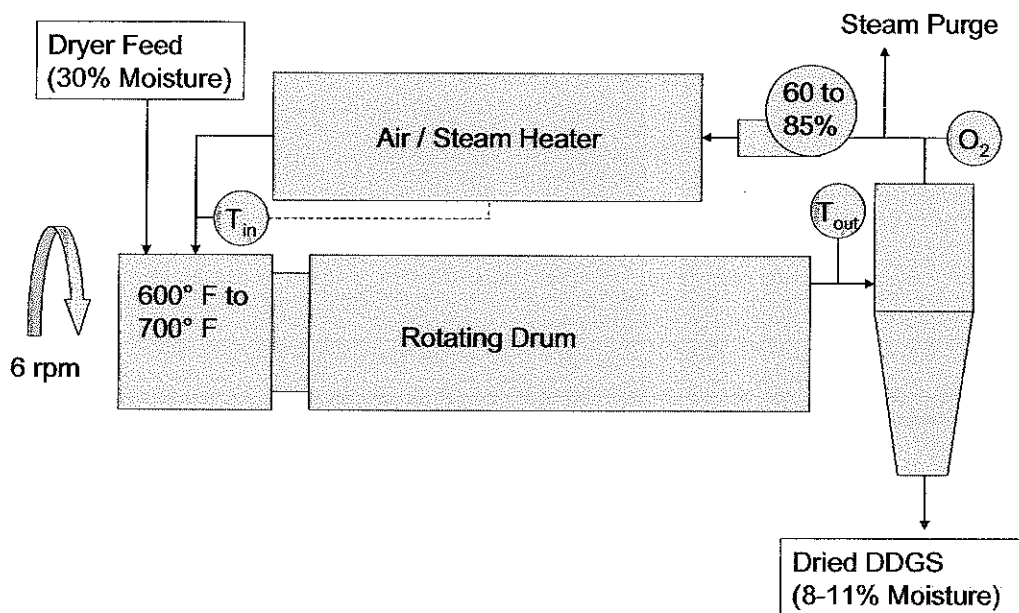


Figure 4: Schematic representation of the Dupps dryer showing the locations at which temperature, oxygen, and moisture measurements were made

Samples were collected after 3 hours of continuous operation at each condition. Since the dryer residence time was approximately 30 minutes, samples were collected after 6 residence times when the system had reached steady state. Five gallon buckets of product were collected and stored in the cold room. Chemical characteristics that were measured included protein, fat, fiber, fatty acid, and amino acid concentrations. In addition, the bulk density and color of the samples were determined.

The color of the DDGS produced in this trial was measured using a donated colorimeter (Hunter Associates Laboratory, Inc., Reston, VA). Color is measured using three parameters: L (lightness-darkness), a (redness-greenness), and b (yellowness-blueness). Lightness is measured on a scale of 0 (black) to 100 (white). The L value has been shown to be most highly correlated with DDGS nutritional value (Cromwell *et al.*, 1993). The color of the DDGS samples for each operating condition is shown in Table 6.

Table 6: DDGS color from second Dupps dryer trial

Run #	Inlet Temperature (°F)	Blower VFD Frequency (Hz)	Syrup Addition Rate (%)	L [±0.2]	a [±0.2]	b [±0.2]
1	650	40	75	55.73	15.93	44.01
2	700	50	50	56.01	15.92	44.31
3	700	35	100	57.85	15.37	46.87
4	600	50	100	60.46	14.88	48.59
5	600	35	50	55.41	15.14	43.27
6	600	35	100	62.42	13.44	49.56
7	600	50	50	61.75	12.95	45.98
8	700	35	50	61.78	12.87	46.16
9	700	50	100	63.48	12.15	48.3

Multiple linear regression was used to determine which parameters affected the color of DDGS to a statistically significant extent. For the light-dark parameter, L, the inlet temperature, feed moisture, product moisture, and oxygen concentration were not significant at the 95% confidence level ($P > 0.05$), but outlet temperature, blower speed, and the syrup addition rate were all significant parameters in the regression model (Table 7). This model was able to predict 94% of the variance in the data ($r^2 = 0.94$). A parity plot of the predicted and actual L values is shown in Figure 5A. Higher outlet temperature tended to darken the DDGS, whereas higher blower speed and syrup addition rate tended to lighten the color. The red-green parameter, a, could also be successfully modeled using multiple linear regression (Fig. 5B; $r^2 = 0.91$). Blower speed and outlet temperature were significant factors for this model, with increased blower speed tending to decrease the redness of the sample and higher outlet temperature increasing the redness. The yellow-green parameter, b, was modeled only moderately well (Fig 5C; $r^2 = 0.83$), but syrup addition and outlet temperature both significantly affected this parameter. Higher syrup addition rates tended to make the DDGS more yellow and higher outlet temperatures tended to make it less yellow.

Table 7: Parameters of multiple regression model for color of DDGS produced in second Dupps dryer experiment

color parameter	model parameter	estimate	standard error	t	probability
L (white-black)	intercept	60.88	2.54	23.9	<0.0001
	blower speed	0.173	0.0484	3.57	0.0160
	syrup addition	0.0418	0.0143	2.93	0.0325
	outlet temperature	-0.0577	0.00739	-7.81	0.0006
a (red-green)	intercept	12.38	1.52	8.18	0.000
	blower speed	-0.064	0.003	-2.66	0.037
	outlet temperature	0.028	0.004	7.65	0.000
b (yellow-blue)	intercept	45.78	1.98	23.1	0.000
	syrup addition	0.601	0.015	4.45	0.004
	outlet temperature	-0.021	0.008	-2.84	0.030

The significant effects of outlet temperature on all three color parameters and the effect of dryer residence time (*i.e.*, blower speed) on lightness and redness is consistent with the hypothesis that DDGS color change in a dryer is due to the Maillard reaction. Increasing outlet temperature should accelerate browning associated with proteins reacting with sugars such as hexoses and pentoses, which would make the DDGS darker, more red, and less yellow. Likewise, reducing the dryer residence time at any temperature would tend to reduce the extent of conversion, making the product lighter and less red. Syrup addition, however, was expected to result in darker DDGS due to the associated increase in the protein and sugar concentrations of the product. The tendency of the syrup to lighten the product may have been due to the

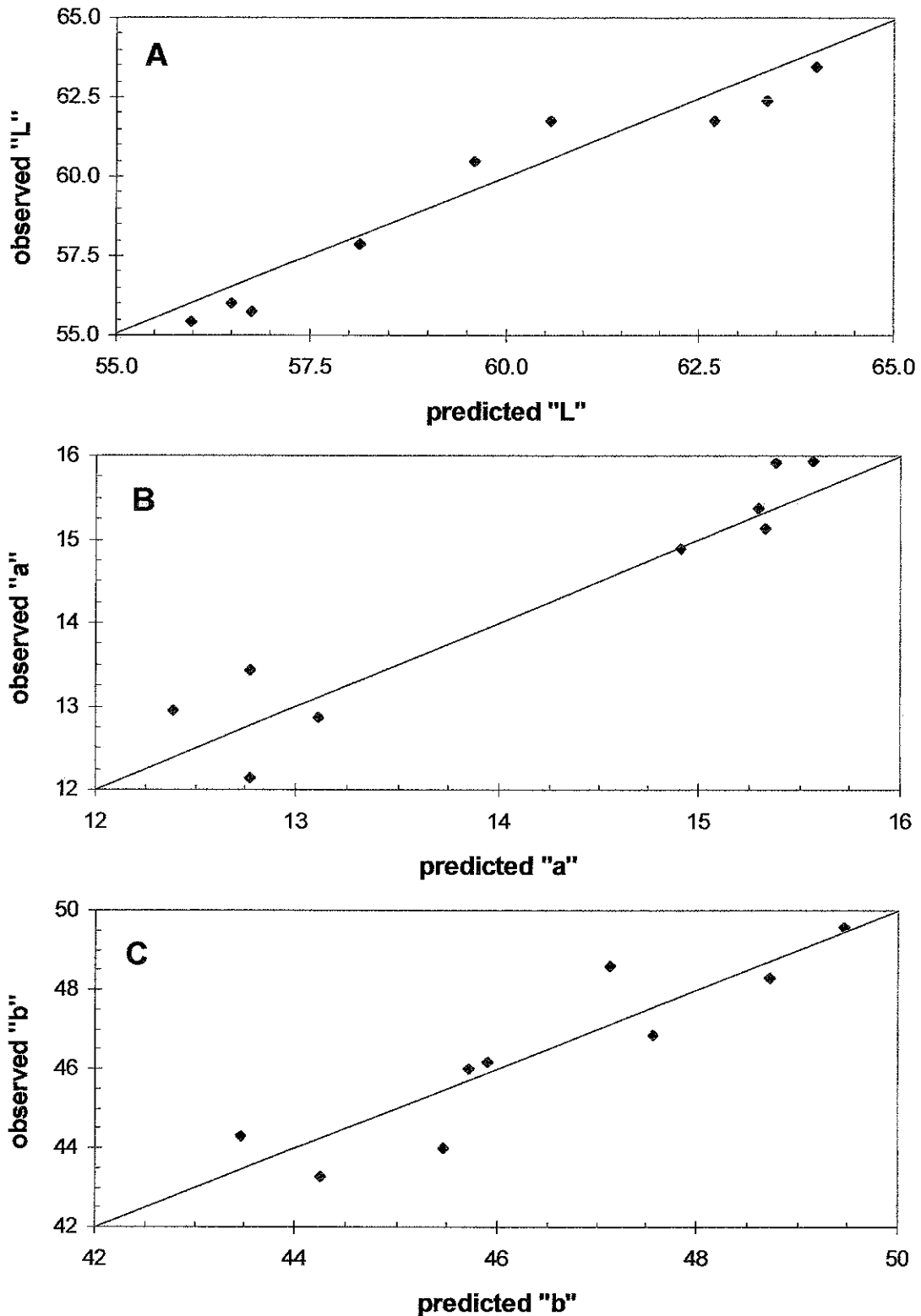


Figure 5: Correlation between the observed and predicted color of DDGS produced in second Dupps dryer experiment using multiple regression modeling: A, light-dark ($r^2 = 0.94$); B, red-green ($r^2 = 0.91$); C, yellow-blue ($r^2 = 0.83$). Model parameters are shown in Table 7.

relatively light color of this syrup compared to the wet cake. The effect of syrup addition rate on the yellowness of the DDGS was probably due to its color, which was somewhat yellow-brown.

The chemical composition of the DDGS samples produced in this study was measured by three feed analysis laboratories: New Jersey Feed Laboratories (NJFL), Minnesota Valley Testing Laboratory (MVTL), and the University of Missouri-Columbia (UMC). All three labs measured moisture content and the concentrations of 18 amino acids. NJFL and MVTL also measured the concentrations of crude protein, crude fiber, fat, free fatty acids, and ash. In addition, at least two of the three labs measured several unusual amino acids, such as ornithine, hydroxylysine, and hydroxyproline. The nutritional quality of the protein was estimated using the pepsin digestion procedure (NJFL and MVTL) or by measuring available lysine (UMC and MVTL). Systematic differences were observed between the results provided by these three laboratories, but the overall trends were consistent. Therefore, the data provided by all three labs was averaged, and the effect of operating conditions on DDGS composition was determined using the average values.

Based on these results, dryer operating conditions did not significantly affect the concentrations of crude protein, crude fiber, fat, or ash. Some of the conditions that were tested did exert significant effects on the moisture content and free fatty acid (FFA) concentrations, however. The dryer inlet temperature and blower speed affected the moisture content of the DDGS, and the blower speed and outlet temperature affected the FFA concentration (Table 8). The effects of dryer temperature and residence time (*i.e.*, blower speed) on moisture content are consistent with the physical characteristics of the drying process: higher temperature results in a dryer product (*i.e.*, lower moisture content) and shorter residence time (*i.e.*, higher blower speed) results in a product with higher moisture content. The effect of dryer operating conditions on the FFA concentration is important because this component is a predictor of rancidity of the feed and affects its shelf-life. DDGS with high FFA concentrations are less palatable than feeds with low FFA concentration. Higher outlet temperatures and shorter residence times both tend to decrease the FFA concentration, which suggests that it may be possible to identify an optimal operating condition because these factors have opposing effects on reaction extent. The relationships between the predicted and observed moisture content and FFA concentrations are shown in Figure 6A and 6B, respectively.

Table 8: Regression model of FFA concentrations in DDGS produced by the Dupps dryer
 DDGS

component	model parameter	estimate	standard error	t	probability
moisture content	intercept	22.91	8.62	2.66	0.038
	blower speed	0.029	0.008	3.62	0.011
	inlet temperature	-0.039	0.011	3.62	0.011
free fatty acid concentration	intercept	13.82	10.87	1.27	0.251
	blower speed	-0.043	0.019	2.30	0.061
	outlet temperature	-0.088	0.027	-3.30	0.016

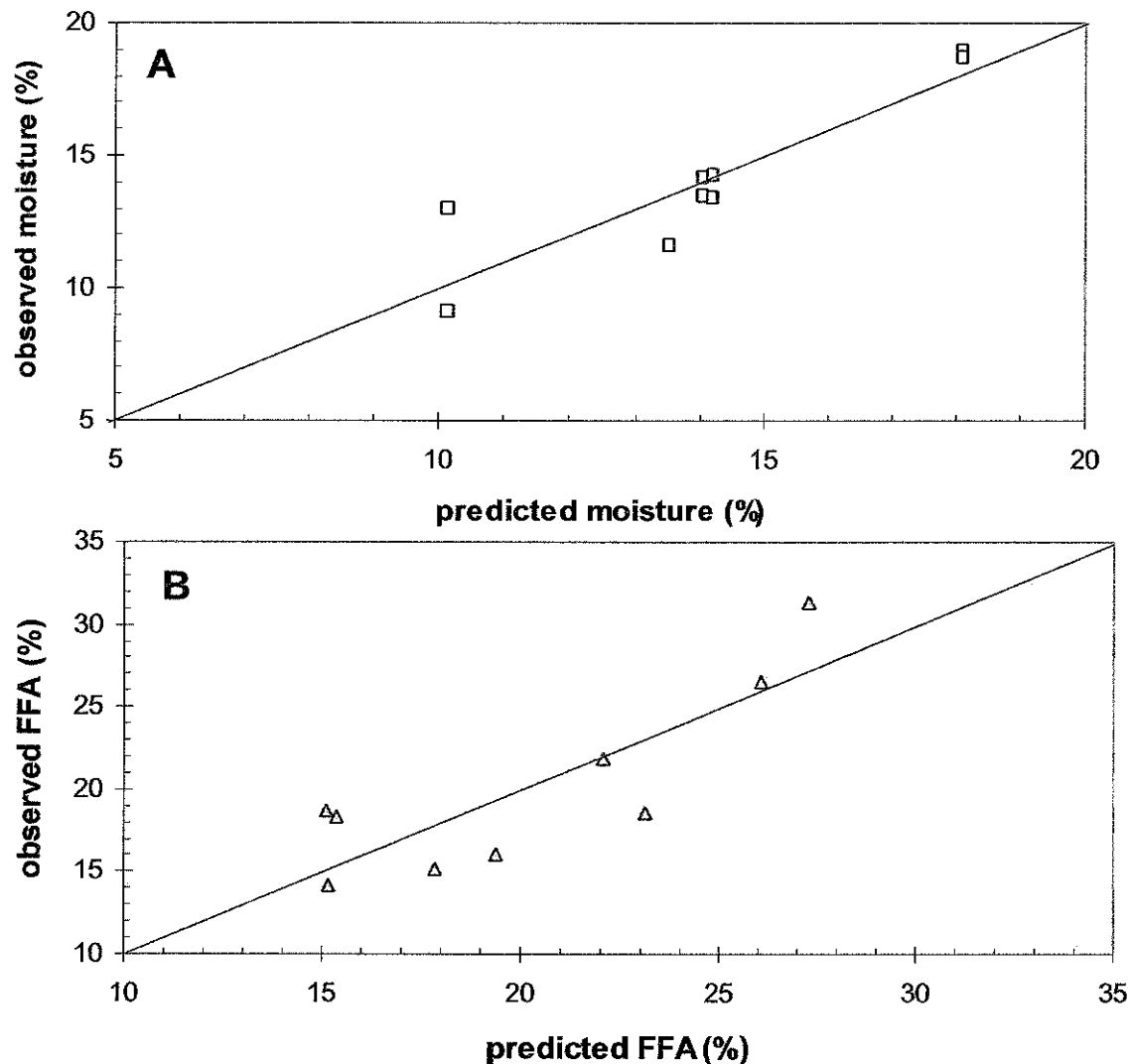


Figure 6: Correlation of the observed (A) moisture content ($r^2 = 0.81$) and (B) free fatty acid concentrations ($r^2 = 0.69$) in DDGS produced in the Dupps dryer with concentrations predicted using multiple linear regression models. The best-fit parameter estimates are given in Table 8.

The effects of dryer operating conditions on protein composition and digestibility was evaluated by measuring the concentrations of amino acids following complete hydrolysis and determining the extent of enzymatic hydrolysis that could be achieved by treatment with pepsin. Protein digestibility was determined using two concentrations of pepsin: low (0.002%) and high (0.2%). The average extent of hydrolysis increased from 57% to 86% at the high pepsin concentration, but neither measure of digestibility was significantly correlated with dryer operating conditions. This is not particularly surprising for the higher pepsin concentration because the coefficient of variance over all dryer conditions was less than 1%; so, little variation existed to be explained. The coefficient of variance was larger for DDGS samples digested with

the lower pepsin concentration (CV = 7.7%), but the variation was not correlated with dryer operating conditions, and the within-treatment variation was not measured for either pepsin concentration. Protein digestibility was also evaluated by measuring available lysine, but no correlation with dryer operating conditions was observed for this parameter either. Available lysine also did not vary greatly among the samples that were collected in this study (CV = 6.1%), and available lysine was only slightly more variable than total lysine (CV = 4.6%). Although lysine concentration is often cited for its importance in determining the nutritional value of DDGS and its susceptibility to reaction with reducing sugars via the Maillard reaction, it was not the most variable of amino acids. That distinction belonged to glutamic acid (CV = 7.6%), but since this is not one of the essential amino acids, this variability is unlikely to affect the nutritional quality of DDGS. Glutamate was also present at the highest concentration (5.2%), neither glutamate nor lysine were significantly correlated with the dryer operating conditions. It seems likely, therefore, that variability in amino acid concentration and protein digestibility is controlled by some factor other than the dryer operating conditions that were evaluated in this study.

5.3. Effects of Front-End Operational Parameters on Mash DE and Ethanol Concentration

A pilot-plant experiment was performed to evaluate the effects of liquefaction and saccharification conditions on fermentation. The purpose of this experiment was to determine how changes in front-end processes affect the starting conditions of fermentation. The experimental design is shown in Table 9. Eleven operational factors were tested at two levels; eight of those factors were also tested at a third (midpoint) level. Plant operating conditions were changed every six hours. Samples of the mash were collected at two-hour intervals; so, three mash samples were collected for each experimental condition (a.k.a., "treatment" or "run"). The solids concentration and dextrose equivalent (DE) concentration were measured in every sample. (DE is a measure of the extent of starch hydrolysis. DE is inversely proportional to the average length of oligosaccharide chains in the sample.) One sample from every treatment was fermented in the lab to determine ethanol yield. The ethanol yield was estimated from the ethanol concentration after fermentation of the mash sample in the lab was complete. The final ethanol concentration was determined gravimetrically (*i.e.*, by loss in the mass of the fermentation flask due to volatilization of carbon dioxide).

The main effects of each experimental factor shown in Table 9 were determined using standard statistical methods. Basically, the main effect of each factor is given by the difference between the average of the response variable (e.g., DE, ethanol concentration) in all treatments in which the factor is present at its high level and all treatments in which the factor is present at its low level. In some experimental designs, interactions between factors can also be evaluated, but the design used in this study involved too few treatments for the number of independent factors that were included. So, interactions could not be tested. Since treatments were not independently replicated, the statistical significance of the effects that were observed was evaluated by plotting the data in a way that allows detection of deviations from a normal distribution. The results of this analysis are shown in Figure 7 (DE) and Figure 8 (ethanol yield).

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Table 9: Experimental conditions for evaluating the effects of upstream processing on fermentation*

Run	mill screen size (in)	process water flow (lbs/min)	target solids conc. (%)	slurry tank temp (°F)	slurry tank level (%)	slurry tank pH	slurry tank enzyme flow (g/hr)	jet cooker temp (°F)	mash tank temp (°F)	mash tank level (%)	mash tank enzyme flow (g/hr)
1	1/16	17.5	36	195	90	5.8	75	225	195	65	150
2	1/16	18.6	32	195	90	5.4	95	235	195	65	150
3	1/16	18.6	32	195	60	5.8	95	225	195	85	190
4	1/16	17.5	36	195	90	5.4	95	225	183	85	150
5	1/16	17.5	36	182	60	5.4	75	235	183	65	150
6	1/16	17.5	36	195	60	5.4	75	235	195	85	190
7	1/16	17.5	36	182	90	5.8	75	225	183	85	190
8	1/16	17.5	36	195	60	5.8	95	235	183	65	190
9	1/16	18.6	32	195	60	5.4	75	225	183	65	190
10	1/16	18.6	32	188.5	75	5.6	85	230	189	75	170
11	1/16	17.5	36	182	90	5.4	95	225	195	65	190
12	1/16	18.6	32	182	90	5.4	95	235	183	85	190
13	1/16	18.6	32	182	90	5.8	75	235	195	65	190
14	1/16	18.6	32	182	60	5.4	75	225	195	85	150
15	1/16	18.6	32	195	90	5.8	75	235	183	85	150
16	1/16	18.6	32	182	60	5.8	95	225	183	65	150
17	1/16	17.5	36	182	60	5.8	95	235	195	85	150
18	7/64	17.5	36	195	90	5.8	95	235	195	85	190
19	7/64	18.6	32	188.5	75	5.6	85	230	189	75	170
20	7/64	18.6	32	195	90	5.8	95	225	183	65	190
21	7/64	18.6	32	182	90	5.8	95	225	195	85	150
22	7/64	17.5	36	195	60	5.4	95	225	195	65	150
23	7/64	17.5	36	195	60	5.8	75	225	183	85	150
24	7/64	17.5	36	182	90	5.8	95	235	183	65	150
25	7/64	18.6	32	195	60	5.8	75	235	195	65	150
26	7/64	17.5	36	195	90	5.4	75	235	183	65	190
27	7/64	18.6	32	182	60	5.8	75	235	183	85	190
28	7/64	18.6	32	195	60	5.4	95	235	183	85	150
29	7/64	18.6	32	195	90	5.4	75	225	195	85	190
30	7/64	18.6	32	182	90	5.4	75	225	183	65	150
31	7/64	18.6	32	182	60	5.4	95	235	195	65	190
32	7/64	17.5	36	182	60	5.8	75	225	195	65	190
33	7/64	17.5	36	182	90	5.4	75	235	195	85	150
34	7/64	17.5	36	182	60	5.4	95	225	183	85	190

*the process water temperature was 200 °F and the jet cooker flow rate was 3 gal/min in all experiments

Normal-order plots are equivalent to plotting data on probability paper: data that are normally distributed plot approximately along a straight line with a slope that is proportional to the standard deviation of the distribution. Data that do not fall on the same straight line are likely to be drawn from different distributions. In Figures 7 and 8, statistically significant treatment effects can be identified by their location away from the straight line that is formed by the other data points. Figure 7 shows that the target solids concentration and the temperature of the mash tank both exerted statistically significant negative effects on the mash DE. That is, the DE was significantly lower when mash was prepared with the higher target solids concentration (36%) and at the higher mash tank temperature (195 °F).

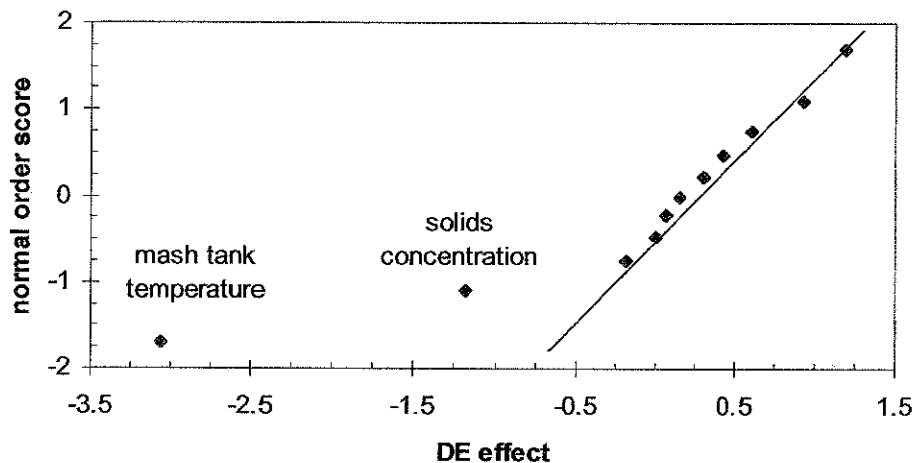


Figure 7: Normal-order plot of treatment main effects on mash DE. Effects that are statistically significant lie off of the straight line drawn through the remaining data points.

Figure 8 shows that three factors exerted significant effects on ethanol concentration: higher target solids concentrations and higher mash tank temperatures increased the final ethanol concentration relative to the lower values for both factors (32% and 183 °F, respectively), and higher process water feed rate (18.6 lbs/min) reduced the ethanol concentration relative to the lower feed rate (17.5 lbs/min). Note that process water feed rate and target solids concentrations are inversely proportional, such that the higher water feed rate always corresponds to the lower target solids concentration. So, it is not surprising that these two variables have different effects on the final ethanol concentration. The fact that process water feed rate did not exert a significant effect on DE, whereas target solids concentration did, appears to reflect the nature of the distribution of treatment effects. As expected, the main effects of these two factors on DE were identical and of opposite sign (solids effect = -1.183 vs. process water feed rate effect = 1.183), but the process water feed rate effect was consistent with the distribution of the other treatment effects, whereas the solids concentration effect was not.

Because some treatment factors (e.g., slurry tank pH, mash solids concentration) could not be controlled well enough to insure that the actual level was equal to the target level, multiple

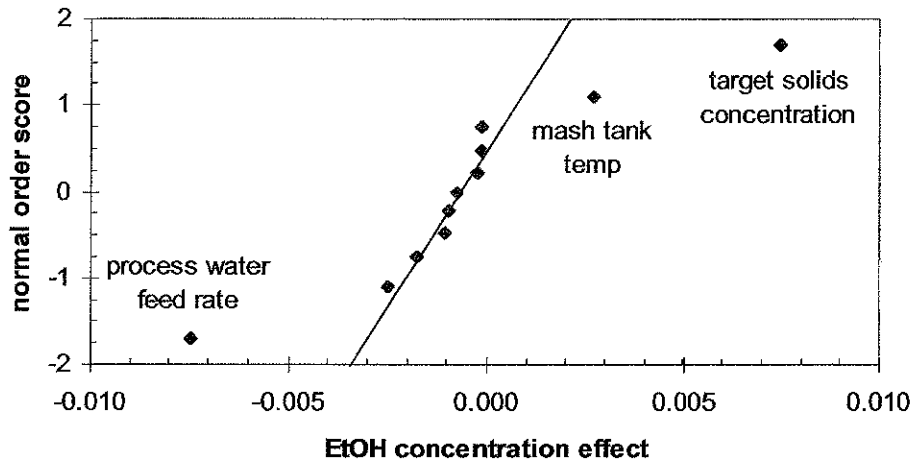


Figure 8: Normal-order plot of treatment main effects on the ethanol yield, measured as the ethanol concentration after lab-scale fermentation was complete. Note that treatment run 2 was excluded from this analysis because it was determined to be an outlier.

linear regression was used to further evaluate the relationships between operational parameters and the performance variables of interest (*i.e.*, mash DE and ethanol concentration). This approach also allowed us to evaluate the effects of parameters that could be measured but which were not systematically varied and parameters that varied in response to the treatment conditions. For example, the effect of mash DE on ethanol yield can be included in a multiple linear regression model, but it cannot be considered in the factor analysis shown in Figs. 7 and 8. Multiple linear regression models have the following form:

$$\hat{y} = a_0 + a_1x_1 + a_2x_2 + \dots + a_ix_i + \dots + a_nx_n \quad (1)$$

where \hat{y} is the predicted value of the response variable of interest, x_i is the value of the “i”th independent variable, and a_i is the coefficient of the “i”th independent variable. The model coefficients, a_i , were estimated using the standard least squares approach.

The results of the multiple linear regression analysis are shown in Figs. 9 (mash DE) and 10 (final ethanol concentration). In these figures, the observed response variables are plotted versus the predicted response given by the best-fit model with the fewest independent variables. Only independent variables with coefficients that were significantly different from zero at the 95% confidence level were retained in the final model. The independent variables that were included in the final models (x_i), the best-fit model parameters (a_i), and the standard errors for each model parameter are shown in Table 10.

For both response variables, independent variables with model parameters that were significantly different from zero (Table 10) were also identified as exerting significant effects in the factor analysis (Figs. 7 and 8). The only difference is the mash tank temperature, which was important in the factor analysis for final ethanol concentration but not in the multiple linear regression. Since the mash tank temperature affected mash DE, and mash DE was included in

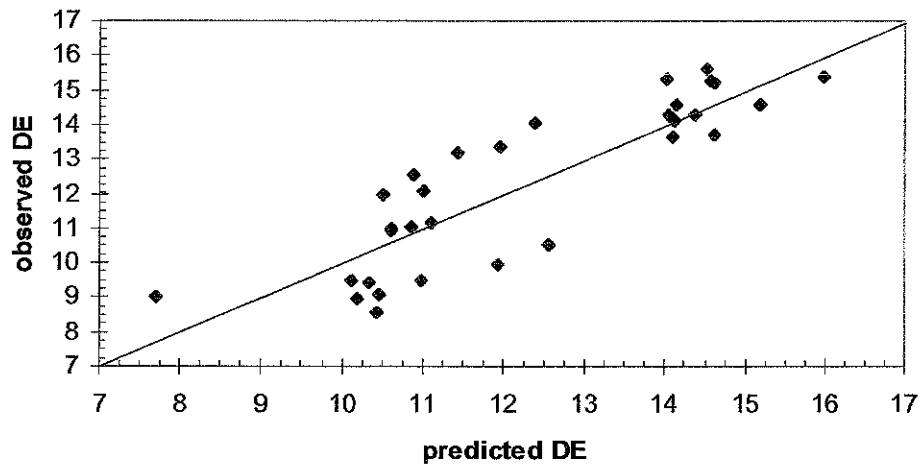


Figure 9: Best-fit multiple linear regression model for mash DE based on the operational parameters that were varied or measured in the mash-preparation experiment described in this report. The R^2 for this fit is 0.757, indicating that about three-quarters of the observed variation in mash DE is explained by the model. The measured mash solids concentration and the mash tank temperature were the only independent variables with model coefficients that were significantly different from zero. The diagonal line represents the expected relationship between observed and predicted mash DE if the model described the data perfectly.

the multiple-regression model, the positive effect of mash tank temperature on final ethanol concentration in the multiple-regression model might have been confounded by an opposing negative effect of mash DE.

Table 10: Best-fit parameters for multiple linear regression models for the effects of mash preparation conditions on mash DE and final ethanol concentration

response variable (y)	independent variable (x _i)	coefficient (a _i)	standard error
mash DE	intercept	82.04	7.41
	measured solids concentration (%)	-0.379	0.171
	mash tank temp (°F)	-0.303	0.04
final ethanol concentration (%)	intercept	11.8	2.91
	process water feed rate (lbs/min)	-0.383	0.103
	measured solids concentration (%)	0.194	0.047
	mash DE	-0.070	0.026

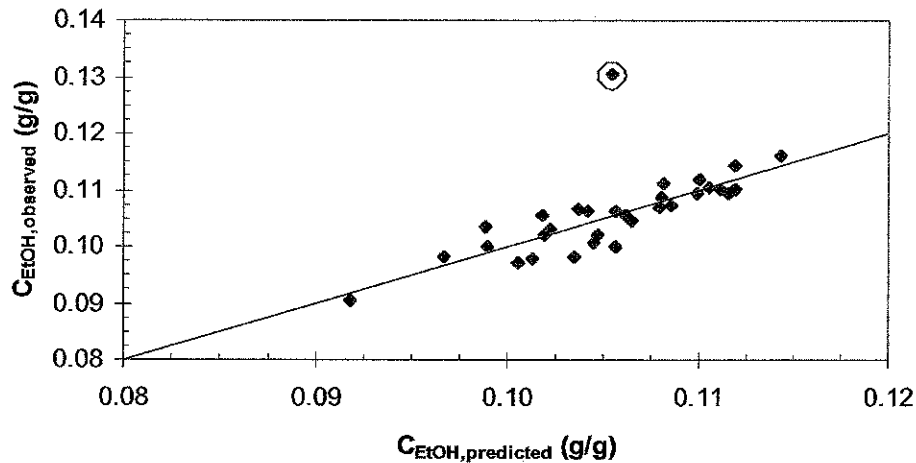


Figure 10: Best-fit multiple linear regression model for final ethanol concentration based on the operational parameters that were varied or measured in the STAC II pilot-plant study described in this section. The R^2 for this fit is 0.787, indicating that about three-quarters of the observed variation in final ethanol concentration is explained by the model. Process water feed rate, measured mash solids concentration, and mash DE were the only independent variables with coefficients that were significantly different from zero. The diagonal line represents the expected relationship between observed and predicted mash DE if the model described the data perfectly. The circled data point represents the observed final ethanol concentration for treatment run 2 (see Table 9 for experimental conditions), which was determined to be an outlier and, therefore, was not included in the regression analysis or the factor analysis.

Mash tank temperature and mash solids concentration exerted negative effects on DE, which is a measure of the extent of starch hydrolysis. Higher DE indicates more hydrolysis and, consequently, smaller (on average) polysaccharide polymers. Therefore, the negative effect of these operational parameters on DE suggests that these factors inhibited starch hydrolysis. The effect of temperature may be explainable by the sensitivity of the amylase enzyme that was added to the mash tank along with the corn-flour slurry. The rate of enzyme-catalyzed reactions typically increases with increasing temperature until the maximum temperature is reached, after which the rate decreases rapidly with further increase in temperature. The maximum temperature of the enzyme appears to be closer to the low end of the temperature range used in this experiment (183 °F) than the high end (195 °F). The best-fit coefficient for mash tank temperature in the multiple regression model suggests that the DE should decrease by about 4 units in going from the lowest to the highest temperature. The actual range of observed DE values was about 7 units, suggesting that the temperature effect could explain about half of the observed variation.

The effect of mash solids concentration may have a more mundane explanation: since DE is a ratio, if the change in solids concentration increases the total starch concentration more than it increases the reaction rate, DE will decrease. The dextrose equivalent (DE) value is basically a

measure of the number of reducing sugar units relative to the total number of sugar monomers in the starch polymer. The change in the starch concentration—and therefore, the total number of sugar monomers in the polymer—is linearly proportional to the change in the solids concentration, but the rate of enzyme catalyzed reactions is not a linear function of substrate concentration. Instead, the reaction rate, r , can be described by a function similar to the Michaelis-Menten equation:

$$r = \frac{V_{\max} S}{K_m + S} \quad (2)$$

where V_{\max} is the maximum rate of the reaction, which occurs when the enzyme is saturated with substrate, S is the substrate (starch in this case) concentration, and K_m is the half-saturation concentration (i.e., the substrate concentration at which the reaction rate is half maximal). Equation (2) shows that the reaction rate is linearly proportional to the substrate concentration only at substrate concentrations that are very low relative to K_m . Assuming that corn flour is about 70% starch by mass, the starch concentration at the lowest target solids concentration was about 22% (220 g/L), which is probably high relative to the K_m . So, any increase in the starch concentration that occurred in response to increasing solids concentration is unlikely to be matched by a proportional increase in the reaction rate; in fact, it is more likely that the reaction rate changed very little. Therefore, the DE would decrease because the denominator of the ratio increased faster than the numerator. The best-fit coefficient for the effect of solids concentration on mash DE (Table 10) suggests that the DE should have decreased by about 1.5 units over the range of values that were tested in this experiment. Therefore, the effect of solids concentration on DE was smaller than the effect of temperature.

The two factors with the biggest effect on the final ethanol concentration were highly correlated: mash solids concentration and process water feed rate. Increasing the process water feed rate at a constant rate of corn feed to the hammer mill resulted in a proportional decrease in the solids concentration of the slurry, which was reflected in the solids concentration of the mash. Therefore, it is not surprising that these two operational parameters had opposite effects on the final ethanol concentration. Dextrose equivalents also affected the final ethanol concentration, but the magnitude of the effect was relatively small (i.e., the observed range in DE accounted for less than one-fifth of the observed range of final ethanol concentrations) and opposite to the expected direction (i.e., higher mash DE produced lower final ethanol concentrations). The effects of solids concentration and process water feed rate, on the other hand, accounted for about two-thirds of the observed variation, and the effect was in the expected direction. The importance of mash solids concentration on ethanol yield was not surprising because the fermentation was allowed to go to completion, and a higher initial substrate (starch) concentration was expected to produce a higher final product (ethanol) concentration. The relatively small effect of DE on final ethanol concentration probably reflected the fact that this was an endpoint measurement. The effect of DE might have been greater (and positive) if ethanol production kinetics had been measured instead of the final ethanol concentration.

The results of a neural network model of the DE value with respect to plant process parameters are shown in Figures 11 and 12, which show plots of the predicted mash DE versus measured DE during the STAC II trial. The fit shown in Figure 12 used a different and more

rigorous method of cross-validation, called random hold-back. The R^2 value for neural nets was much greater than for traditional regression, and both analysis methods exhibited good predictive power. This shows that neural network models with high correlation between the DE number and the plant process parameters can be produced. Unfortunately, these models do not provide an estimate of the effect of a single process variable. Instead, they provide predictions based on the overall state of the process.

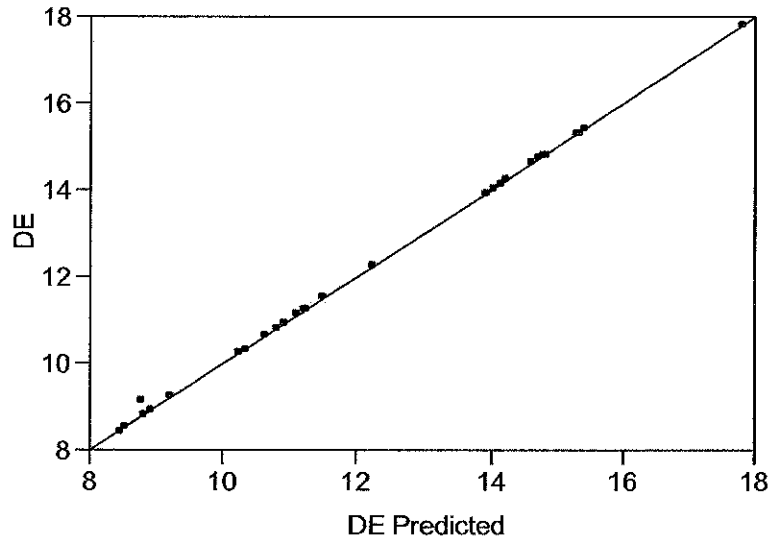


Figure 11: Neural-network-model predicted mash DE versus observed DE using K-fold cross-validation

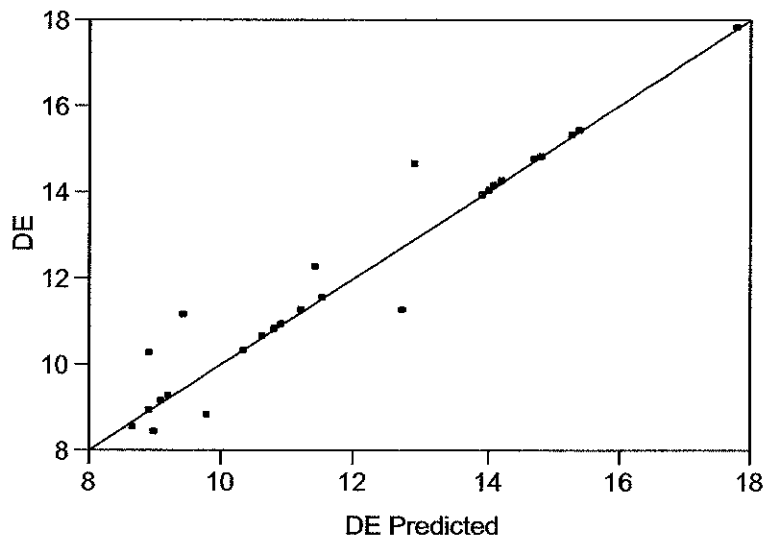


Figure 12: Neural-network-model predicted mash DE versus observed DE using random holdback (66% training, 33% testing) fitting procedure

5.4. Effects of Pilot-Plant Operating Conditions on DDGS Composition

5.4.1. Experimental Design

The NCERC performed two pilot-plant experiments investigate the effects of liquefaction, saccharification, and solids-processing conditions on fermentation and DDGS quality. The purpose of these experiments was to determine how changes in plant operating conditions affect the rate and yield of fermentation and the quality of the DDGS coproduct. The experimental design is shown in Table 11. Ten operational factors were tested at two levels in these experiments; nine of those factors were also tested at a third (midpoint) level. Other plant conditions were consistent in all treatment conditions (a.k.a., "runs"). The consistent conditions are shown in Table 12. Each treatment condition was maintained long enough to completely fill a single fermenter (about 20 hrs). This experimental design was conducted in two pilot-plant trials, one of which was conducted in October 2006 and the other of which was conducted in January 2007. The back-end conditions were arbitrarily varied during the October 2006 trial. In the January 2007 trial, the back-end conditions were varied systematically as shown in Table 13. The dryer temperature was held at a constant temperature for 12 hours, then it was switched to the other temperature for 12 hours. The centrifuge flow rate was switched between the high and low values every six hours. Whole stillage produced by every fermenter was processed through the entire range of back-end conditions.

Slurry, mash, and beer samples were collected at approximately eight-hour intervals. The solids concentration and dextrose equivalents (DE) value were measured in slurry and wort samples, soluble carbohydrates (*e.g.*, glucose and oligoaccharides) and ethanol were measured in beer samples. In addition to the variation in front-end conditions, several post-distillation (a.k.a., back-end) conditions were varied. Several characteristics related to the nutritional value, including the concentrations of crude protein, crude fat, neutral detergent fiber (NDF; a measure of the concentration of cellulose, plus hemicellulose, plus lignin), and acid detergent fiber (ADF; a measure of the concentration of cellulose plus lignin), were measured in DDGS samples. The soluble carbohydrate and ethanol concentrations were used to estimate fermentation kinetic parameters.

5.4.2. Effects of Front-End Treatment Factors on Fermentation

Evaluation of the effects of front-end conditions in this study differs from the similar study that was described in the previous section (5.3) in that each treatment combination was used to completely fill a pilot-scale fermenter (about 3600 gallons). The fermentations were allowed to proceed to completion before the beer was distilled and the solids were processed to produce DDGS. The progress of each fermentation was monitored by measuring the concentrations of soluble carbohydrates, ethanol, and several potential alternative fermentation products (*e.g.*, lactic acid). Several important characteristics of the mash and beer, and the rates of sugar consumption and ethanol production, are reported in Table 14. The mash characteristics include the initial concentration of total soluble sugars (measured by HPLC), the initial concentration of glucose plus the low-molecular-weight glucose oligomers (*i.e.*, maltose and maltotriose, a.k.a., DP2 and DP3), the slurry DE, and the mash DE. Examples of several fermentations are shown in Figures 13 (hammer mill screen size 4/16"; conducted in October 2006) and 14 (hammer mill screen size 7/16"; conducted in January 2007). Both figures show the concentrations of total dissolved sugars (expressed as glucose equivalents) and ethanol. Two different treatment

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Table 11: Front-end treatment conditions for pilot-plant experiments*

Run	hammer mill screen size (in)	process water flow (lbs/min)	target solids conc. (%)	slurry tank temp (°F)	slurry tank level (%)	slurry tank enzyme flow (g/hr)	jet cooker temp (°F)	mash tank temp (°F)	mash tank level (%)	mash tank enzyme flow (g/hr)
1	4/16	15.1	32	182	90	95	225	181	85	190
2	4/16	18.1	28	195	60	95	235	192	85	150
3	4/16	16.6	30	188.5	75	85	230	186.5	75	170
4	4/16	15.1	32	195	90	95	225	192	65	190
5	4/16	18.1	28	182	60	95	235	181	65	150
6	4/16	15.1	32	195	60	75	235	181	85	190
7	4/16	18.1	28	182	90	75	225	192	85	150
8	4/16	15.1	32	182	60	75	235	192	65	190
9	4/16	18.1	28	195	90	75	225	181	65	150
10	7/16	15.1	32	182	90	75	235	192	65	150
11	7/16	18.1	28	195	60	75	225	181	65	190
12	7/16	15.1	32	195	90	75	235	181	85	150
13	7/16	18.1	28	182	60	75	225	192	85	190
14	7/16	16.6	32	188.5	75	85	230	186.5	75	170
15	7/16	15.1	30	195	60	95	225	192	65	150
16	7/16	18.1	28	182	90	95	235	181	65	190
17	7/16	15.1	32	182	60	95	225	181	85	150
18	7/64	18.1	28	195	90	95	235	192	85	190

*the first pilot-plant experiment (Oct. '06) included runs 1-9 and the second pilot-plant experiment (Jan. '07) including runs 10-18

Table 12: Consistent conditions for pilot-plant experiments

Condition	Set point
corn feed rate	510 lbs/hr
process water temperature	200 °F
jet cooker flow rate	3 gal/min
second mash tank (TA-1030) level	75%
third mash tank (TA-1220) level	75%
fermenter volume	3600 gals
fermenter temperature	90 °F

Table 13: Back-end treatment conditions for January 2007 pilot-plant experiment

Treatment Condition	centrifuge flow rate (gal/min)	dryer outlet temperature (°F)
1	2.3	220
2	1.3	220
3	2.3	250
4	1.3	250

combinations are shown in both figures. The top panel in both figures shows the results of two independent replicate fermentations (*i.e.*, different fermenters) for a single treatment condition. Overall, four treatment combinations were independently replicated, and as illustrated by Figs. 13 and 14, the results were adequately repeatable. One treatment combination (run 4) had to be discarded due to poor quality data and/or poor control of the process. Note that the rates of soluble carbohydrate consumption and ethanol production both appear to be approximately zero order with respect to carbohydrate concentration. Ethanol production appeared to stop at approximately the same time that carbohydrate consumption ceased. Alternative fermentation products (*e.g.*, glycerol, lactic acid, acetic acid) were always a small fraction of the ethanol concentration and did not increase steadily over time as did the ethanol concentration.

The effects of front-end process conditions on the chemical characteristics of wort, the kinetics of fermentation, and the final ethanol concentration were evaluated using the general linear model tool in Systat 10 (SPSS, Inc., Chicago, IL). Because this experiment varied ten front-end conditions but include only eighteen independent treatment combinations, only treatment main effects could be evaluated.

The treatment factors that exerted statistically significant effects on the initial concentration of soluble sugars included the hammer mill screen size, the slurry temperature, and the slurry residence time. The rate of enzyme (alpha amylase) addition to the slurry system was nearly significant at the 95% confidence level ($P = 0.054$). The effects and the relevant probabilities are shown in Table 15, and the correlation between the predicted and observed initial soluble-sugars concentrations are shown in Figure 15. The initial concentrations of soluble sugars increased with increasing screen size and slurry-tank residence time and decreased with the increasing slurry tank temperature and enzyme addition rate. It is somewhat surprising that the slurry-tank conditions affected the initial concentrations of soluble sugars but the liquefaction-tank conditions did not. This may indicate that extraction of the starch in the slurry system is more important than the conditions for enzymatic hydrolysis in the liquefaction system.

The treatment factors that exerted significant effects on the initial concentration of glucose plus maltose and maltotriose are shown in Table 16, and the factors that affected the mash DE are shown in Table 17. The correlations between the observed and predicted values for these response variables are shown in Figs. 16 and 17, respectively. None of the treatment factors exerted significant effects on slurry DE. Unlike the initial concentration of soluble sugars, the initial concentrations of glucose and low molecular weight oligomers and the mash DE were primarily affected by conditions in the liquefaction process, which is not surprising because both of these response variables indicate the extent of starch hydrolysis, not simply the extent of extraction.

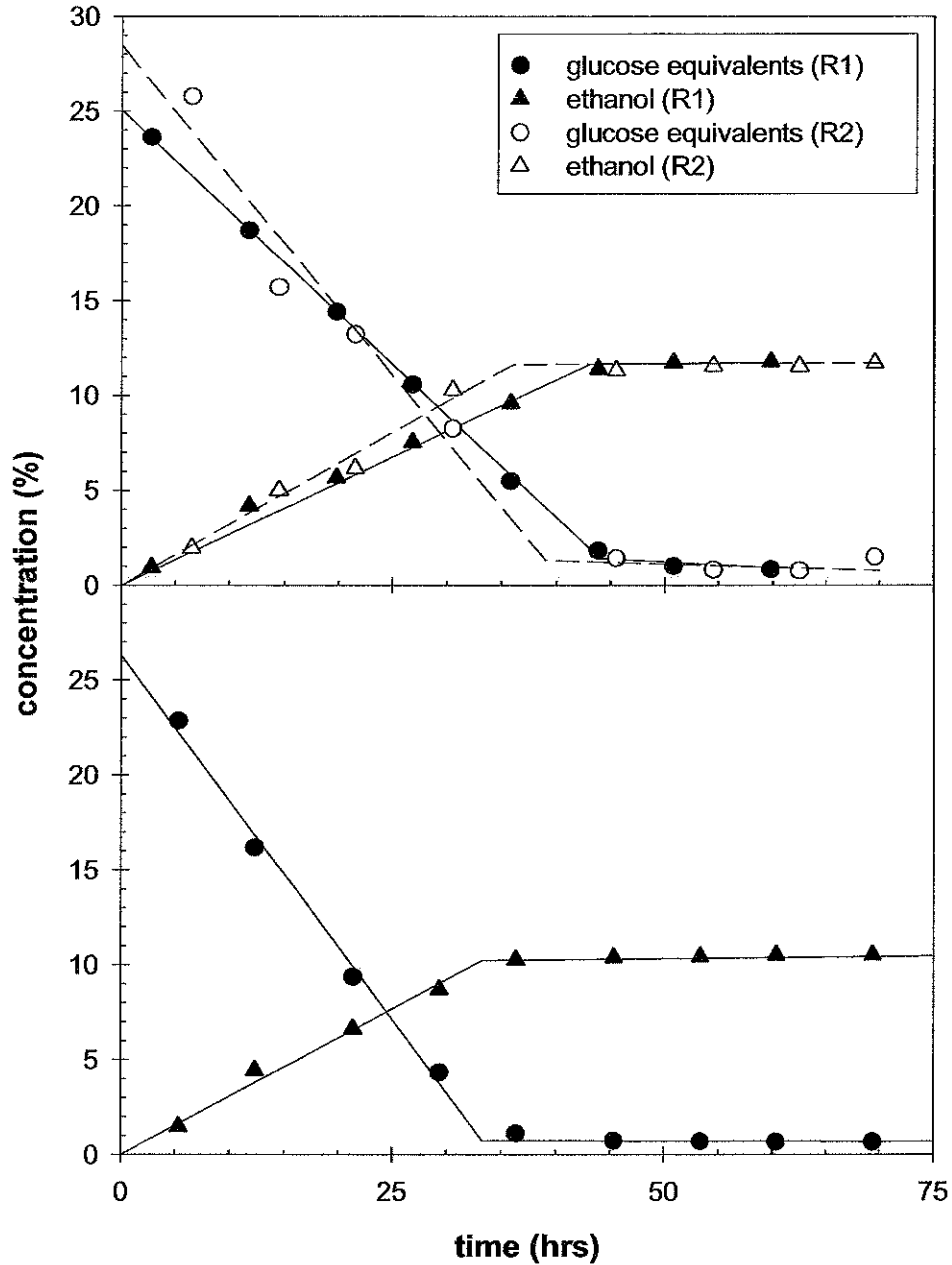


Figure 13: Progress of representative fermentations from the pilot-plant experiment conducted in October 2006 (hammer mill screen size 4/16"). The top panel shows the progress of fermentation for treatment combination (*i.e.*, "run") 1, and the bottom panel shows the progress for treatment combination 7. The data for two independent fermenters (R1 and R2) that were filled with wort prepared using treatment combination 1 are shown in the top panel.

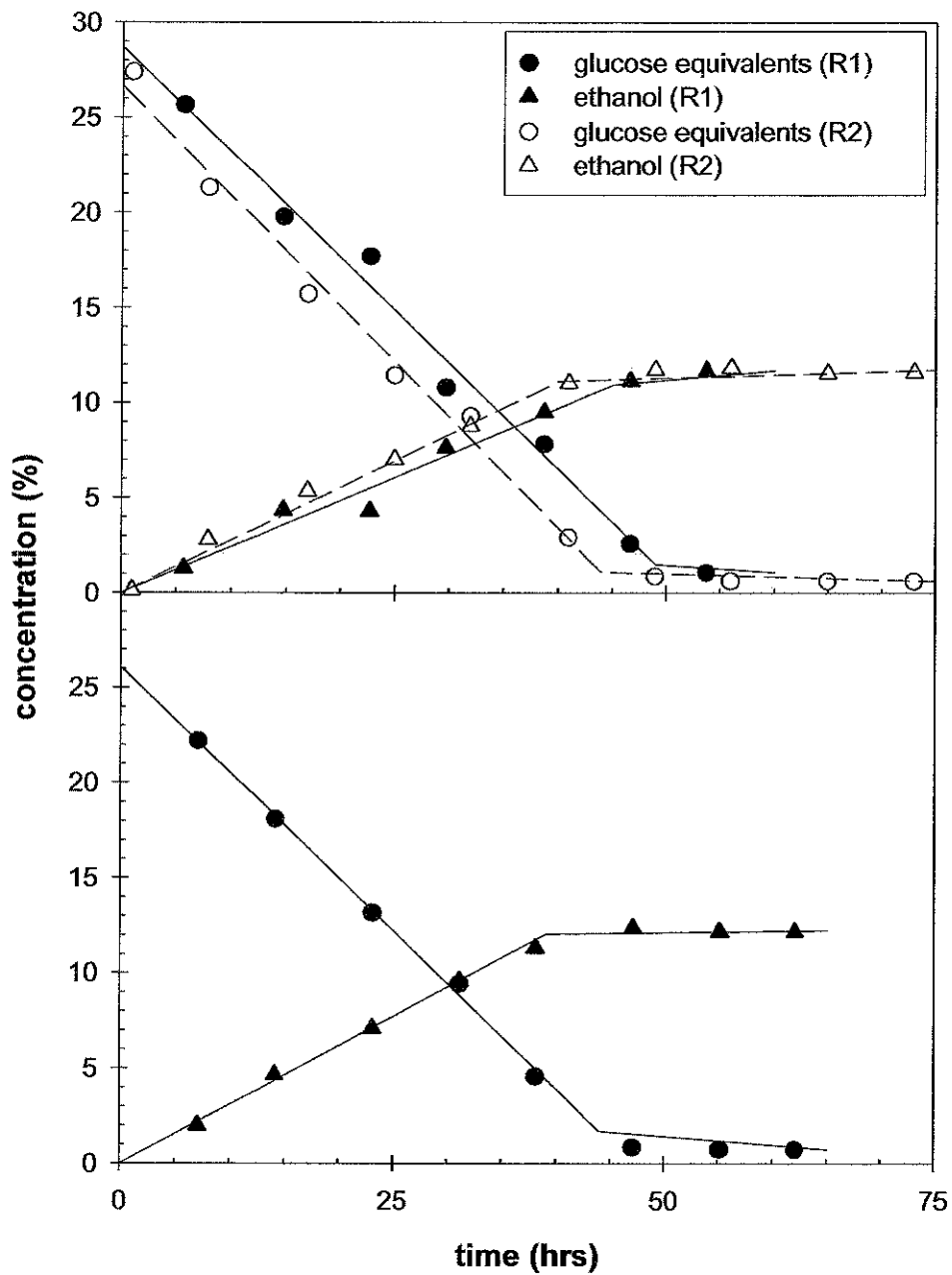


Figure 14: Progress of representative fermentations from the pilot-plant experiment conducted in January 2007 (hammer mill screen size 7/16"). The top panel shows the progress of fermentation for treatment combination (*i.e.*, "run") 12, and the bottom panel shows the progress for treatment combination 15. The data for two independent fermenters (R1 and R2) that were filled with wort prepared using treatment combination 12 are shown in the top panel.

Table 14: Characteristics of mash, beer, and fermentation rates for pilot-scale experiments

Run	initial conc. soluble sugars (%)	initial conc. glucose to maltotriose (%)	final conc. ethanol (%)	slurry DE	mash DE	sugar consump. rate (%/hr)	ethanol production rate (%/hr)
1	23.63	16.37	11.76	18.25	18.30	0.537	0.271
1 (R2)	25.78	14.23	11.72	10.30	16.05	0.696	0.324
2	21.67	11.52	10.23	9.40	12.70	0.615	0.289
3	22.40	14.16	10.84	10.90	14.67	0.538	0.285
5	20.89	7.54	10.52	13.05	14.07	0.712	0.286
6	21.39	14.62	11.82	17.10	18.00	0.526	0.290
7	22.86	12.83	10.49	13.65	11.35	0.769	0.307
8	22.19	12.10	11.61	8.50	11.70	0.551	0.306
9	21.02	7.33	10.51	8.25	12.60	0.576	0.295
10 (R1)	29.92	13.74	11.80	10.17	14.37	0.635	0.287
10 (R2)	29.95	15.03	11.57	11.27	14.13	0.668	0.294
11 (R1)	22.69	13.31	10.28	15.25	17.85	0.605	0.279
11 (R2)	23.77	13.48	10.11	6.50	17.35	0.648	0.286
12 (R1)	25.67	11.22	11.70	7.15	17.20	0.556	0.243
12 (R2)	27.39	13.38	11.71	7.37	14.77	0.582	0.278
13	26.25	13.84	10.81	9.43	13.17	0.625	0.291
14	24.57	13.23	10.95	8.50	14.30	0.626	0.289
15	22.20	9.86	12.24	9.77	12.43	0.555	0.310
16	26.47	10.66	10.22	11.83	15.33	0.672	0.300
17	21.89	11.67	11.52	7.87	15.33	0.545	0.288
18	22.52	11.57	10.41	8.45	15.00	0.656	0.284

Table 15: Front-end treatment factors that exerted significant main effects on the initial concentration of soluble sugars

Factor	Effect	P
hammer mill screen size	$61.6 \pm 15.3 \text{ \%}\cdot\text{in}^{-1}$	0.001
slurry-tank temperature	$-0.17 \pm 0.06 \text{ \%}\cdot\text{°F}^{-1}$	0.011
slurry-tank residence time	$0.24 \pm 0.08 \text{ \%}\cdot\text{min}^{-1}$	0.005
slurry-tank enzyme addition rate	$-0.079 \pm 0.038 \text{ \%}\cdot\text{hr}\cdot\text{g}^{-1}$	0.054

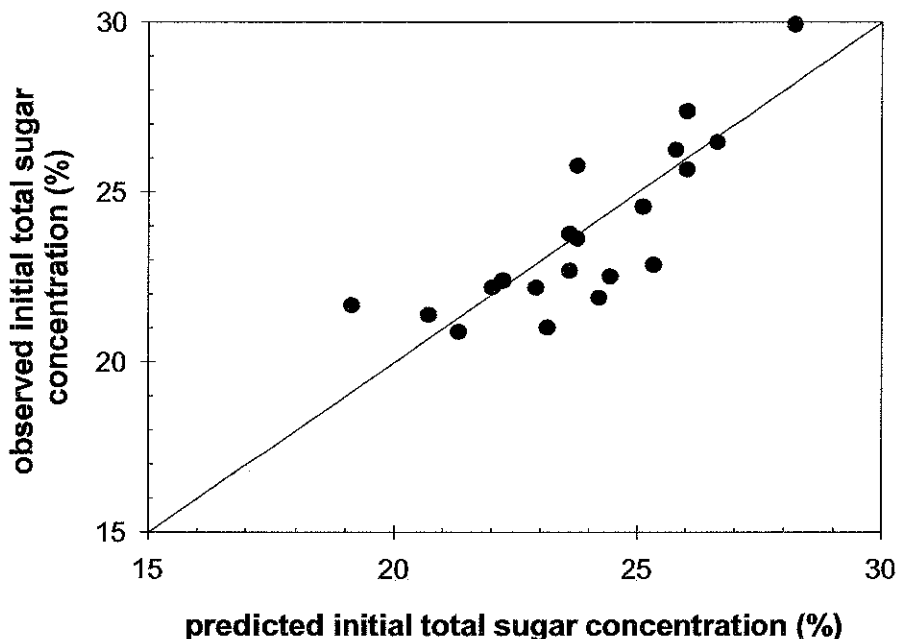


Figure 15: Correlation between observed and predicted initial soluble sugar concentrations using multiple linear regression model including the treatment effects shown in Table 14. The diagonal line represents the expected relationship assuming the model provides perfect prediction of the response variable.

Table 16: Front-end treatment factors that exerted significant main effects on the initial concentration of low molecular weight glucose oligomers (glucose, maltose, and maltotriose)

Factor	Effect	P
process water flow rate	$-0.74 \pm 0.24 \text{ \%}\cdot\text{min}\cdot\text{lb}^{-1}$	0.020
liquefaction-process enzyme addition rate	$0.058 \pm 0.022 \text{ \%}\cdot\text{hr}\cdot\text{g}^{-1}$	0.016

Table 17: Front-end treatment factors that exerted significant main effects on mash DE

Factor	Effect	P
liquefaction-process temperature	$-0.25 \pm 0.06 \text{ \%}\cdot^{\circ}\text{F}^{-1}$	0.001
liquefaction-process enzyme addition rate	$0.038 \pm 0.016 \text{ \%}\cdot\text{hr}\cdot\text{g}^{-1}$	0.032

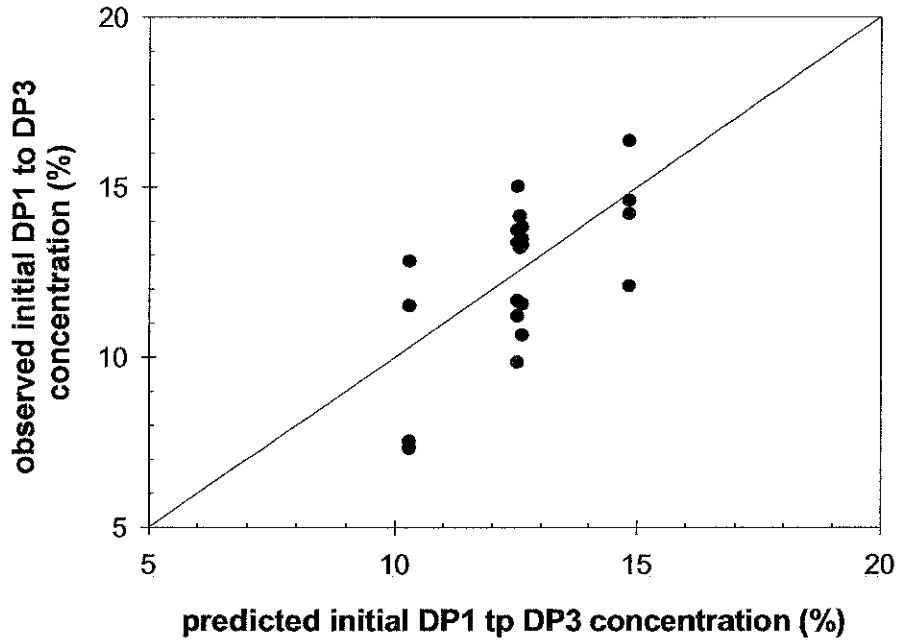


Figure 16: Correlation between observed and predicted initial concentrations of low molecular weight glucose oligomers using multiple linear regression model including the treatment effects shown in Table 15.

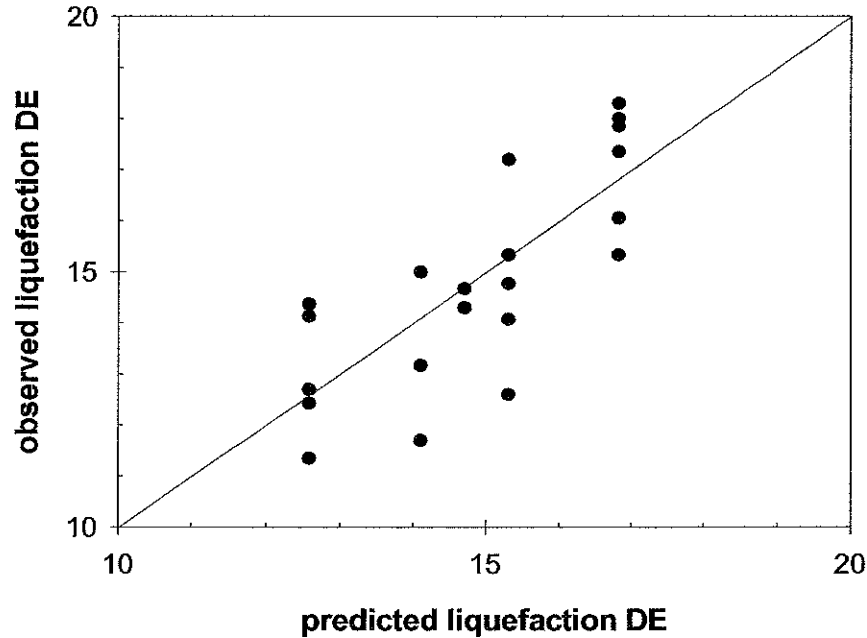


Figure 17: Correlation between observed and predicted liquefaction-process (*i.e.*, mash) DE using multiple linear regression model including the treatment effects shown in Table 16.

The only treatment factor that affected the final ethanol concentration was the process-water flow rate, which determined the slurry solids concentration because the rate of corn input was constant (510 lbs/hr). Decreasing rate of process water input was correlated with an increase in the final ethanol concentration ($-0.45 \pm 0.031 \text{ \%}\cdot\text{hr}\cdot\text{lb}^{-1}$; $P < 0.001$) because the concentration of solids in the slurry increased. The ethanol yield was significantly affected by the process water flow rate, the slurry-tank residence time, and the slurry pH. The main effects of these parameters are shown in Table 18, and the relationship between the predicted and observed ethanol yield is shown in Figure 18. (Note that the reported ethanol yield was calculated based on the ratio of the rates of ethanol production and soluble sugar consumption, not based on the ratio of the final ethanol concentration to the initial concentration of corn or starch. The ethanol yield was calculated both ways, however, and it did not affect the trends that were observed.) Increases in all three factors resulted in decreased ethanol yield. It is interesting that slurry-process conditions affected ethanol yield, but liquefaction process conditions did not. This suggests that starch extraction is more important than starch hydrolysis with respect to determination of ethanol yield. In this respect, however, it is somewhat surprising that an increase in slurry-tank residence time resulted in a decrease in the ethanol yield because one would expect a longer residence time to result in greater extraction. Apparently the process is more complicated than would be predicted by a simple model of starch extraction.

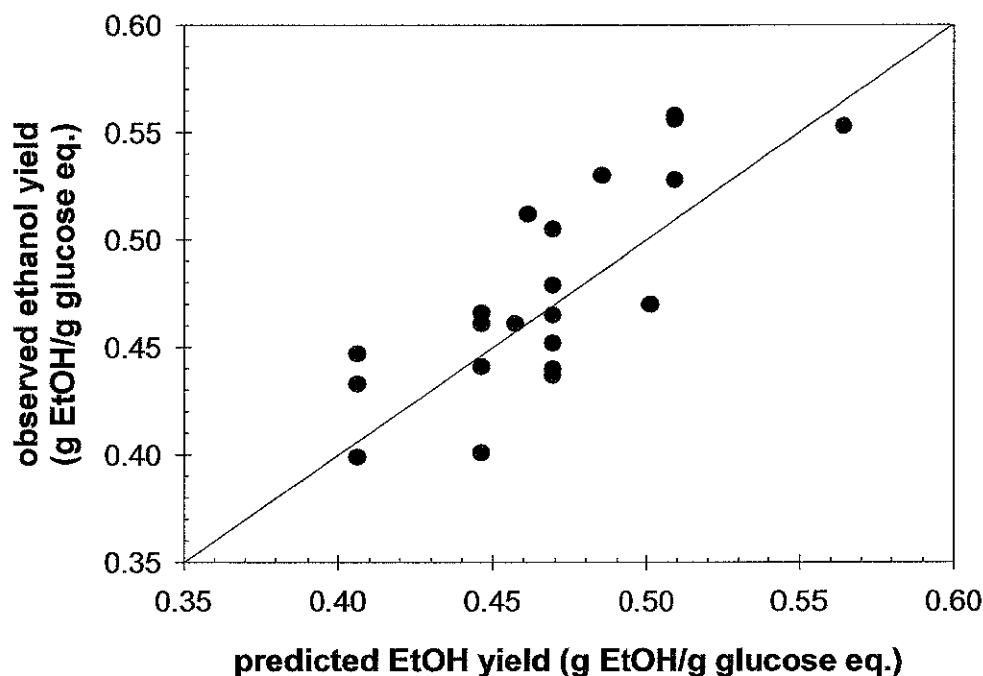


Figure 18: Correlation between observed and predicted ethanol yield using multiple linear regression model including the treatment effects shown in Table 17. The diagonal line represents the expected relationship assuming the model provides perfect prediction of the response variable.

Table 18: Front-end treatment factors that exerted significant main effects on ethanol yield

Factor	Effect	P
process-water flow rate	-0.021 ± 0.005 g EtOH-hr-g glucose ⁻¹ -lb ⁻¹	0.001
slurry-tank residence time	-0.004 ± 0.002 g EtOH-g glucose ⁻¹ -min ⁻¹	0.021
slurry-tank pH	-0.139 ± 0.051 g EtOH-g glucose ⁻¹	0.014

A potential explanation for the negative effect of slurry-tank residence time on ethanol yield is that this treatment factor exerted a positive effect on sugar consumption rate but had no effect on ethanol production rate. (In fact, none of the treatment factors significantly affected the ethanol production rate.) Although the effect of slurry residence time on the sugar consumption rate was only significant at the 90% (not 95%) confidence level, because it is in the denominator of the ethanol yield calculation, any increase that is not accompanied by a corresponding increase in ethanol production rate will result in a decrease in the ethanol yield. A similar argument can be made for the effect of the process water flow rate. Since process-water flow rate exerted a significant positive effect on the sugar consumption rate, its effect on ethanol yield would be negative if no corresponding effect were exerted on the ethanol production rate (which it is not). The effects of front-end treatment factors on the soluble sugar consumption rate are shown in Table 19, and the relationship between the predicted and observed rates are shown in Figure 19. The very close correspondence between the effects of process water flow rate and slurry tank residence time on ethanol yield and soluble-sugar consumption rate (compare Tables 18 and 19) provides additional support for the argument that these processes are correlated. Note that Fig. 19 shows the predicted soluble-sugar consumption rate including (filled circles) and excluding (open squares) the effect of slurry-tank residence time. When the slurry system residence time is included, the multiple regression model over predicts the soluble-sugar consumption rate, but when it is excluded, the model slightly under predicts the rate.

Table 19: Front-end treatment factors that exerted significant main effects on the soluble-sugar consumption rate

Factor	Effect	P
process-water flow rate	0.028 ± 0.008 %-lb ⁻¹	0.001
slurry-tank temperature	-0.004 ± 0.002 %-hr ⁻¹ -°F ⁻¹	0.024
slurry-tank residence time	-0.004 ± 0.002 %-hr ⁻¹ -min ⁻¹	0.091

Although this analysis has demonstrated several clear relationships between operating conditions and the characteristics of the slurry or the mash, its value may be limited. Ultimately, optimization of fermentation must be based on some measure of process efficiency, such as final ethanol concentration, ethanol yield, or ethanol production kinetics. Unfortunately, none of the efficiency measures are strongly correlated with the characteristics of the slurry or mash. For example, the final ethanol concentration is only weakly correlated with the initial concentration

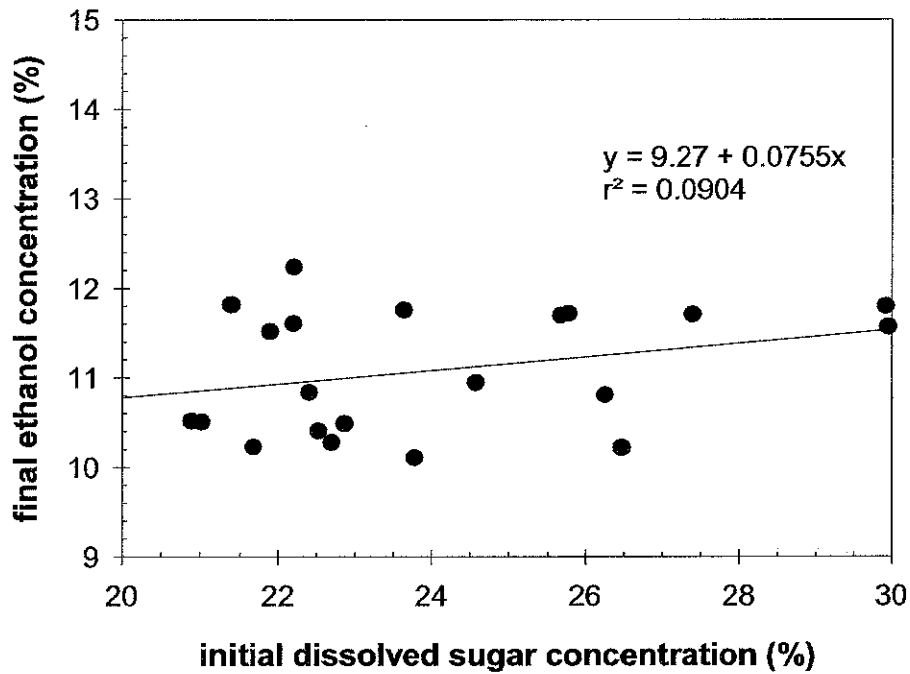


Figure 20: Correlation between the final ethanol concentration and the initial concentration of soluble sugars. The initial soluble sugar concentration describes only about 9% of the variance associated with the final ethanol concentration.

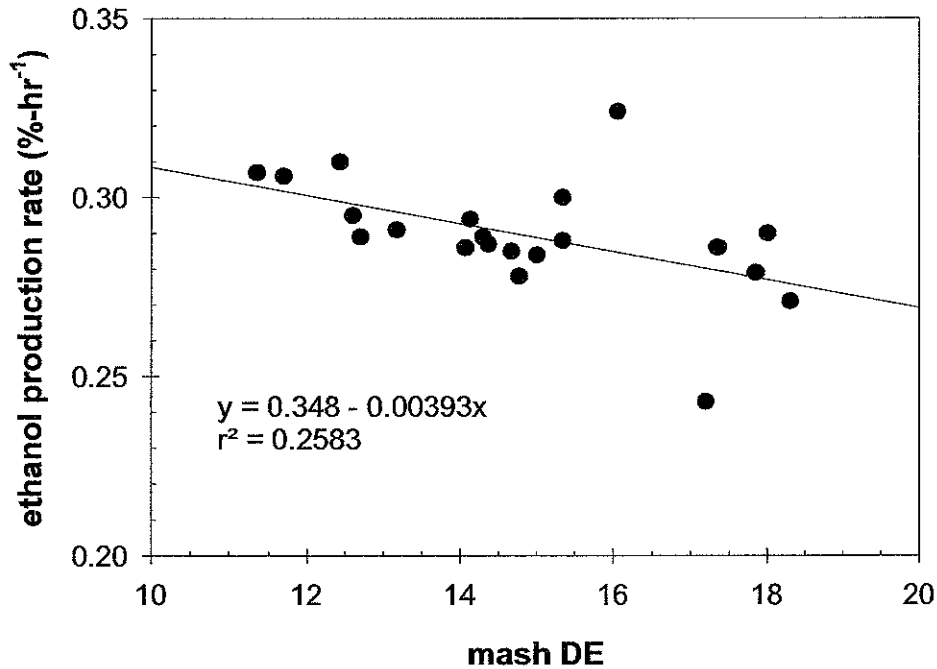


Figure 21: Correlation between ethanol production rate and mash DE. Mash DE explains only about 26% of the variance associated with the ethanol production rate.

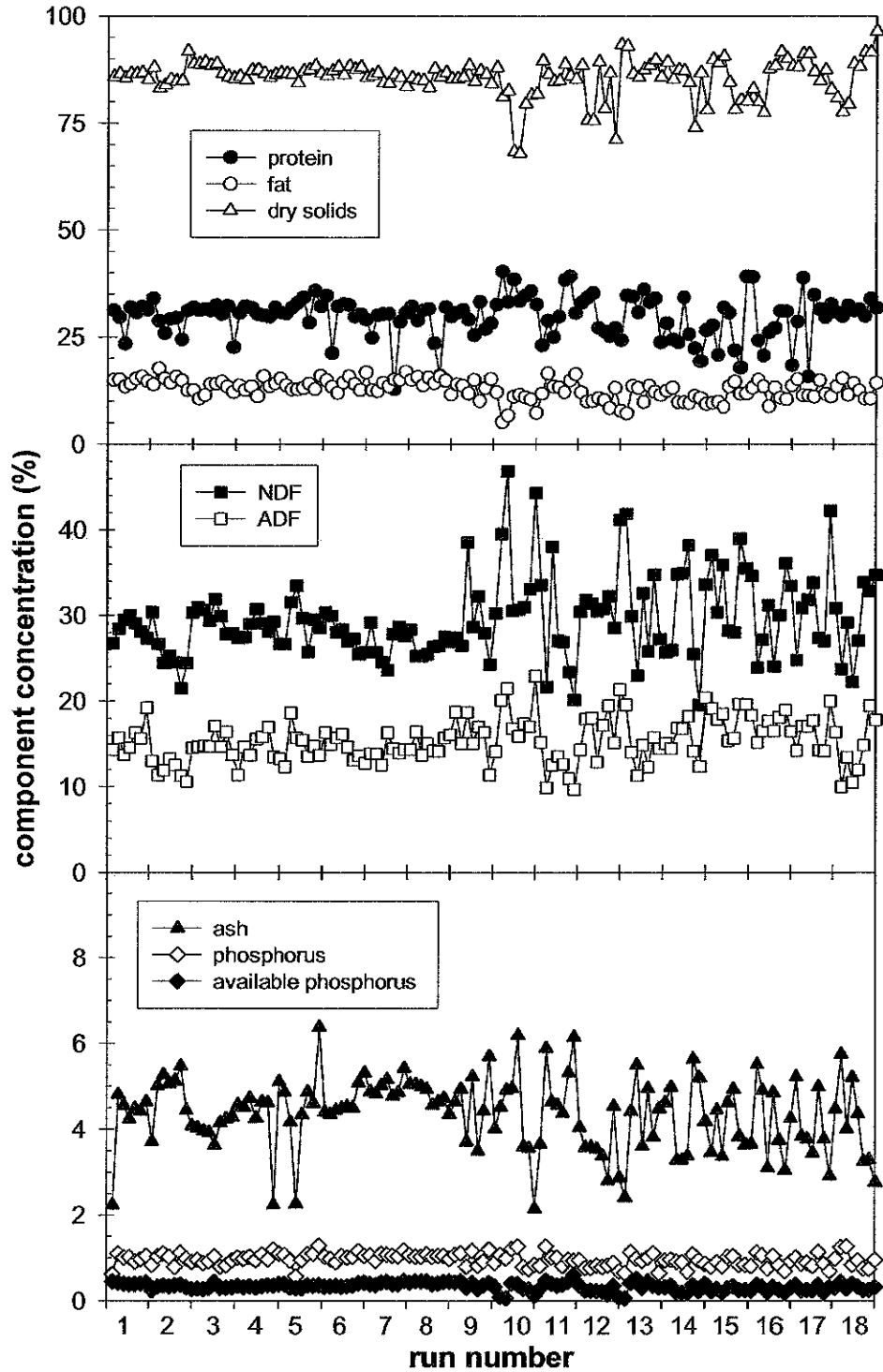


Figure 22: Concentrations of several important components of DDGS produced in the two pilot-plant studies. Run numbers correspond to the front-end treatment combinations that are listed in Table 11.

The concentrations of several DDGS components are plotted as a function of run number in Figure 22. The component concentrations that were measured include crude protein, crude fat, neutral-detergent fiber (NDF), acid-detergent fiber (ADF), ash, total phosphorus, and available phosphorus. Several of the components show significant variation over time, either between or within fermentations. In general, the variability of component concentrations was greater during the pilot-plant trial that was conducted in January 2007 (runs 10-18) than in the one conducted in October 2006 (runs 1-9). In the January 2007 experiment, the back-end conditions, including dryer temperature and centrifuge flow rate, were systematically varied to test a wide range of conditions. In order to maintain a constant moisture content in the material that entered the dryer, the syrup addition rate was also varied. So, the ratio of syrup to wet cake varied in the January 2007 experiment during processing of whole stillage from a single fermenter. This seems to have affected the characteristics of the DDGS as much or more than did the fermentation conditions. Therefore, statistical analysis of the effects of treatment factors on DDGS composition also included the mass rates of syrup and wet cake addition (expressed as mass of dry solids per unit time) instead of the centrifuge flow rate.

The statistically significant main effects identified by the GLM analysis for each treatment factor are shown in Table 20 for several nutritionally relevant component concentrations. At least one treatment factor exerted a significant effect on every component listed in Table 20. Although statistical significance is often arbitrarily defined to be less than a 5% chance that the observed difference could have been observed due to chance, factors that affected the concentration with at least 90% confidence were included in this table in order to capture effects that may be important because this analysis was conducted primarily as a screening tool.

Other characteristics that are known to be correlated with DDGS quality include the dry solids concentration (or, alternatively, moisture content) and color. The statistically significant main effects that affected these physical characteristics of the DDGS are listed in Table 21. Like the chemical characteristics that are listed in Table 21, at least one treatment factor significantly affected each of the physical characteristics that are listed. Color was determined using a HunterLab Colorflex colorimeter and were quantified using the “L” (white-black), “a” (red-green), and “b” (yellow-blue) scales. Previous research has shown that acid-indigestible nitrogen (AIDN) was negatively correlated with the “L” and “b” scores (Cromwell *et al.*, 1993). So, protein digestibility decreases as the DDGS color becomes darker (*i.e.*, lower “L”) and less yellow (*i.e.*, lower “b”). Note that, although dryer exit temperature did not significantly affect any of the chemical characteristics of the DDGS, it did affect two of its physical characteristics: the dry solids concentration and the relative darkness. In both cases, the effect of dryer temperature was in the expected direction. That is, higher dryer temperature increased the dry solids concentration (*i.e.*, decreased the moisture content) and decreased the lightness of the DDGS (*i.e.*, made the product darker). Higher wet cake addition rates tended to produce a lighter-colored product (*i.e.*, increased the “L” value), and higher syrup addition rates tended to make the product redder (*i.e.*, increased the “a” score).

Although the factors listed in Tables 20 and 21 exerted significant main effects on DDGS composition, the linear model used to evaluate these effects did not describe the observed characteristics well. The coefficients of determination (r^2) for the linear models involving only main effects (*i.e.*, no interactions) ranged from 0.055 (crude protein) to 0.56 (crude fat). Because the linear models were not adequately descriptive, neural network modeling was used to provide a better description of the effects of operating conditions on DDGS characteristics.

Table 20: Statistically significant main treatment effects for DDGS chemical composition

component	treatment factor	effect	P
crude protein	slurry temperature	$0.197 \pm 0.071 \text{ \%} \cdot \text{°F}^{-1}$	0.006
	screen size	$-21.77 \pm 6.07 \text{ \%} \cdot \text{in}^{-1}$	0.000
crude fat	liquefaction enzyme	$0.012 \pm 0.003 \text{ \%} \cdot \text{hr} \cdot \text{g}^{-1}$	0.000
	syrup addition rate	$0.153 \pm 0.043 \text{ \%} \cdot \text{hr} \cdot \text{kg}^{-1}$	0.001
	wet cake addition rate	$-0.080 \pm 0.019 \text{ \%} \cdot \text{hr} \cdot \text{kg}^{-1}$	0.000
NDF	process water flow rate	$-0.469 \pm 0.236 \text{ \%} \cdot \text{min} \cdot \text{lb}^{-1}$	0.050
	liquefaction enzyme	$-0.018 \pm 0.006 \text{ \%} \cdot \text{hr} \cdot \text{g}^{-1}$	0.005
	syrup addition rate	$-0.195 \pm 0.105 \text{ \%} \cdot \text{hr} \cdot \text{kg}^{-1}$	0.064
	wet cake addition rate	$0.274 \pm 0.043 \text{ \%} \cdot \text{hr} \cdot \text{kg}^{-1}$	0.000
ADF	process water flow rate	$-0.327 \pm 0.138 \text{ \%} \cdot \text{min} \cdot \text{lb}^{-1}$	0.019
	slurry temperature	$-0.105 \pm 0.034 \text{ \%} \cdot \text{°F}^{-1}$	0.002
	liquefaction enzyme	$-0.014 \pm 0.004 \text{ \%} \cdot \text{hr} \cdot \text{g}^{-1}$	0.000
	syrup addition rate	$-0.107 \pm 0.059 \text{ \%} \cdot \text{hr} \cdot \text{kg}^{-1}$	0.070
	wet cake addition rate	$0.115 \pm 0.025 \text{ \%} \cdot \text{hr} \cdot \text{kg}^{-1}$	0.000
total phosphorus	slurry temperature	$0.003 \pm 0.002 \text{ \%} \cdot \text{°F}^{-1}$	0.096
	slurry pH	$-0.112 \pm 0.065 \text{ \%} \cdot \text{unit}^{-1}$	0.089
	wet cake	$-0.008 \pm 0.001 \text{ \%} \cdot \text{hr} \cdot \text{kg}^{-1}$	0.000
available phosphorus	screen size	$-0.451 \pm 0.257 \text{ \%} \cdot \text{in}^{-1}$	0.082
	liquefaction temperature	$-0.005 \pm 0.001 \text{ \%} \cdot \text{°F}^{-1}$	0.001
	liquefaction residence time	$-0.001 \pm 0.000 \text{ \%} \cdot \text{min}^{-1}$	0.029
	syrup addition rate	$0.004 \pm 0.002 \text{ \%} \cdot \text{hr} \cdot \text{kg}^{-1}$	0.040
	wet cake addition rate	$-0.005 \pm 0.001 \text{ \%} \cdot \text{hr} \cdot \text{kg}^{-1}$	0.000

The neural network modeling was conducted using the JMP software package (SAS Institute, Inc., Cary, NC). The number of hidden nodes was varied from 3 to 6, and an over-fitting penalty was used to compensate for the effects of the larger number of fitting parameters used in models with more hidden nodes. The over-fitting penalty increased by a factor of two with each additional node. The best-fit model for each case (*i.e.*, number of hidden nodes) was estimated using 30 randomly selected initial guesses (“tours”) to reduce the likelihood of finding local rather than global minima. The random-hold-back method was used generate training and validation data sets. One-third of the samples were randomly removed from each data set and used for validation. The fitting process was conducted at least three times for each case, and each time, different training and validation data sets were generated.

Table 21: Statistically significant main treatment effects for DDGS physical characteristics

characteristic	treatment factor	effect	P
solids concentration	screen size	$-26.62 \pm 12.55 \text{ \%}\cdot\text{in}^{-1}$	0.036
	process water flow rate	$-0.493 \pm 0.209 \text{ \%}\cdot\text{min}\cdot\text{lb}^{-1}$	0.020
	liquefaction residence time	$0.035 \pm 0.018 \text{ \%}\cdot\text{min}^{-1}$	0.056
	liquefaction enzyme	$0.020 \pm 0.005 \text{ \%}\cdot\text{hr}\cdot\text{g}^{-1}$	0.000
	syrup addition rate	$-0.456 \pm 0.097 \text{ \%}\cdot\text{hr}\cdot\text{kg}^{-1}$	0.000
	dryer temperature	$0.169 \pm 0.026 \text{ \%}\cdot^{\circ}\text{F}^{-1}$	0.000
color "L"	slurry pH	$-3.52 \pm 0.94 \text{ unit}^{-1}$	0.000
	wet cake addition rate	$0.068 \pm 0.019 \text{ hr}\cdot\text{kg}^{-1}$	0.001
	dryer temperature	$-0.047 \pm 0.013 \text{ }^{\circ}\text{F}^{-1}$	0.001
color "a"	screen size	$-13.32 \pm 2.57 \text{ in}^{-1}$	0.000
	liquefaction temperature	$-0.049 \pm 0.015 \text{ }^{\circ}\text{F}^{-1}$	0.001
	liquefaction enzyme	$0.005 \pm 0.001 \text{ hr}\cdot\text{g}^{-1}$	0.000
	syrup addition rate	$0.113 \pm 0.019 \text{ hr}\cdot\text{kg}^{-1}$	0.000
color "b"	screen size	$-44.45 \pm 5.74 \text{ in}^{-1}$	0.000
	slurry pH	$-4.37 \pm 0.82 \text{ unit}^{-1}$	0.000
	liquefaction temperature	$-0.074 \pm 0.035 \text{ }^{\circ}\text{F}^{-1}$	0.034
	liquefaction enzyme	$0.010 \pm 0.003 \text{ hr}\cdot\text{g}^{-1}$	0.001

The best neural network models for crude protein, crude fat, NDF, ADF, and total phosphorus are shown in Figures 23 to 27 for all four cases. The high degree of nonlinearity and interactivity allowed by neural network models provided significantly better descriptions of each DDGS nutritional parameter than did the corresponding multiple linear regression models. In addition, the fits improved significantly with increasing number of hidden nodes, illustrating the importance of interactivity among the treatment factors. Some component concentrations (*e.g.*, fat and phosphorus) were predicted more successfully than others (*e.g.*, protein). For protein, the best model explained only about 73% of the observed variation the data, whereas the best model explained 94% of the observed variation in the observed fat concentration. The average coefficients of determination for the four neural-network-model cases are compared to each other and the coefficient of determination for the multiple linear regression models in Table 22. Although the model fits increased dramatically when the number of hidden nodes was increased from three to four or five, very little additional improvement resulted from increasing the number of hidden nodes beyond five. This suggests that the models were over fit to the training data set when five or six hidden nodes are used, and the lack of fit was due primarily to the validation set. It seems likely that factors that were not considered in this study also contributed a significant amount of variability to the chemical composition of DDGS.

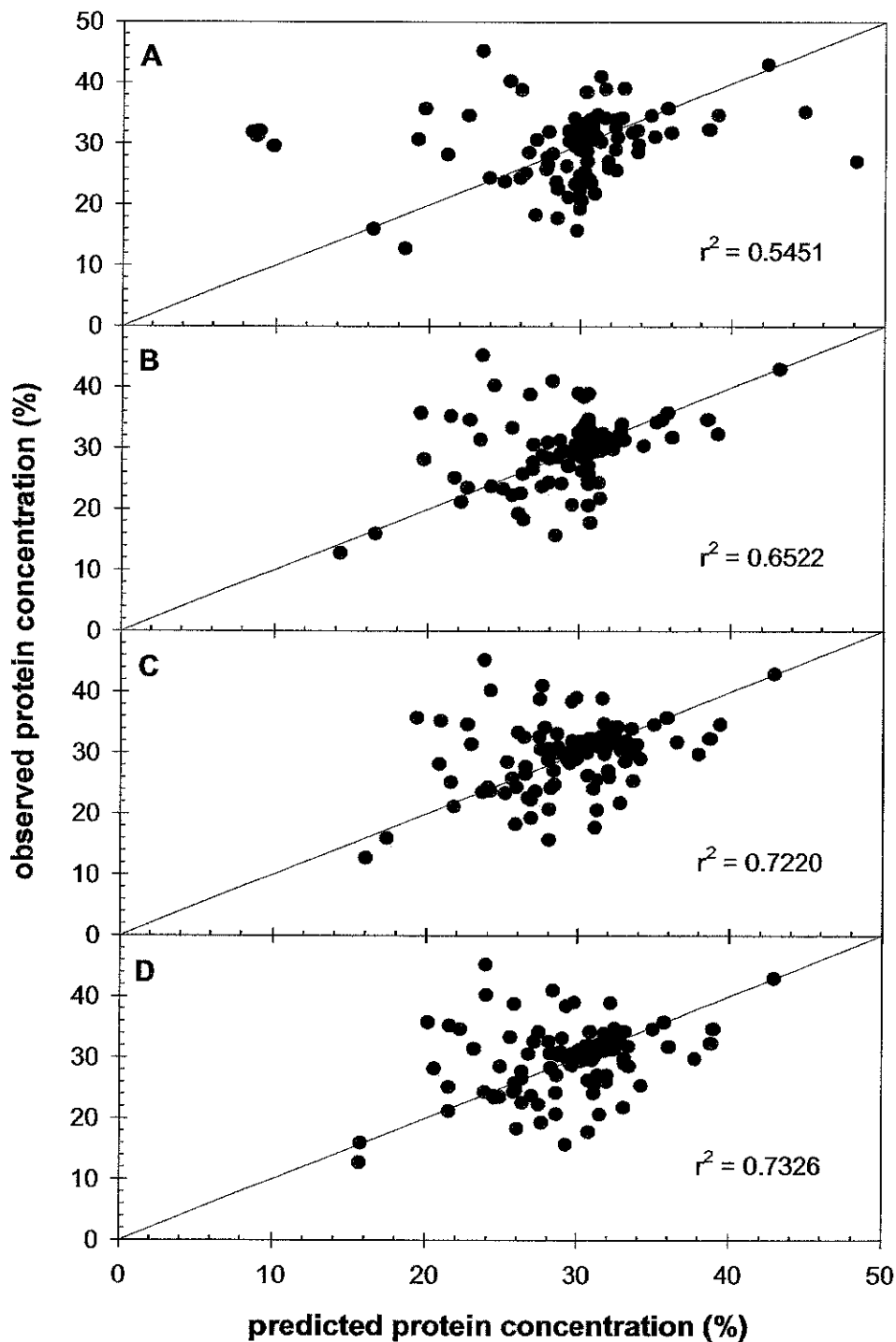


Figure 23: Parity plots comparing the observed protein concentrations to the concentrations predicted by the best-fit neural network models for DDGS samples produced in pilot-plant trials conducted in October 2006 and January 2007. The models were fit using (A) 3, (B) 4, (C) 5, or (D) 6 hidden nodes. The diagonal lines represent perfect agreement between observed and predicted concentrations.

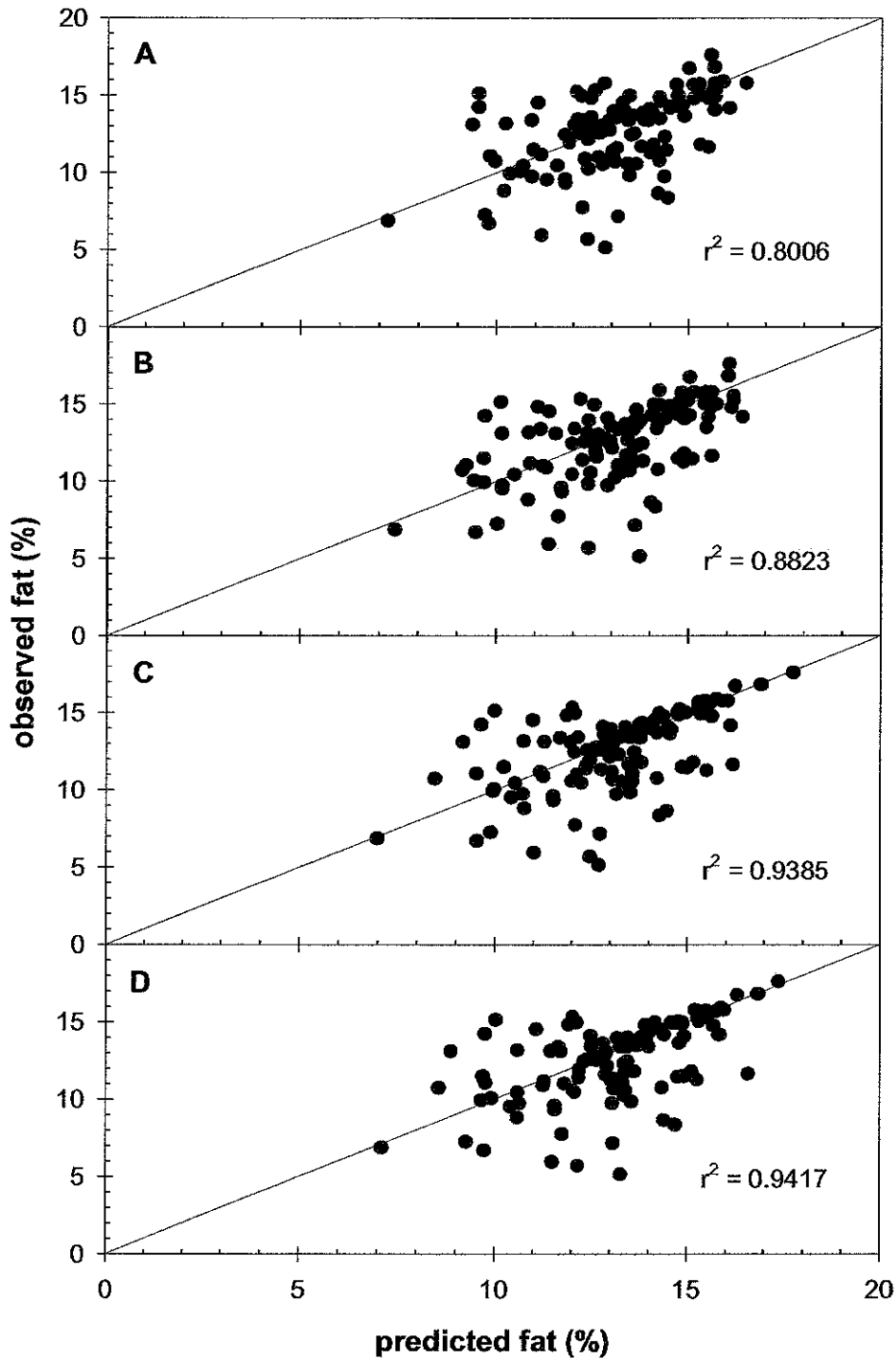


Figure 24: Parity plots comparing the observed fat concentrations to the concentrations predicted by the best-fit neural network models for DDGS samples produced in pilot-plant trials conducted in October 2006 and January 2007. The models were fit using (A) 3, (B) 4, (C) 5, or (D) 6 hidden nodes. The diagonal lines represent perfect agreement between observed and predicted concentrations.

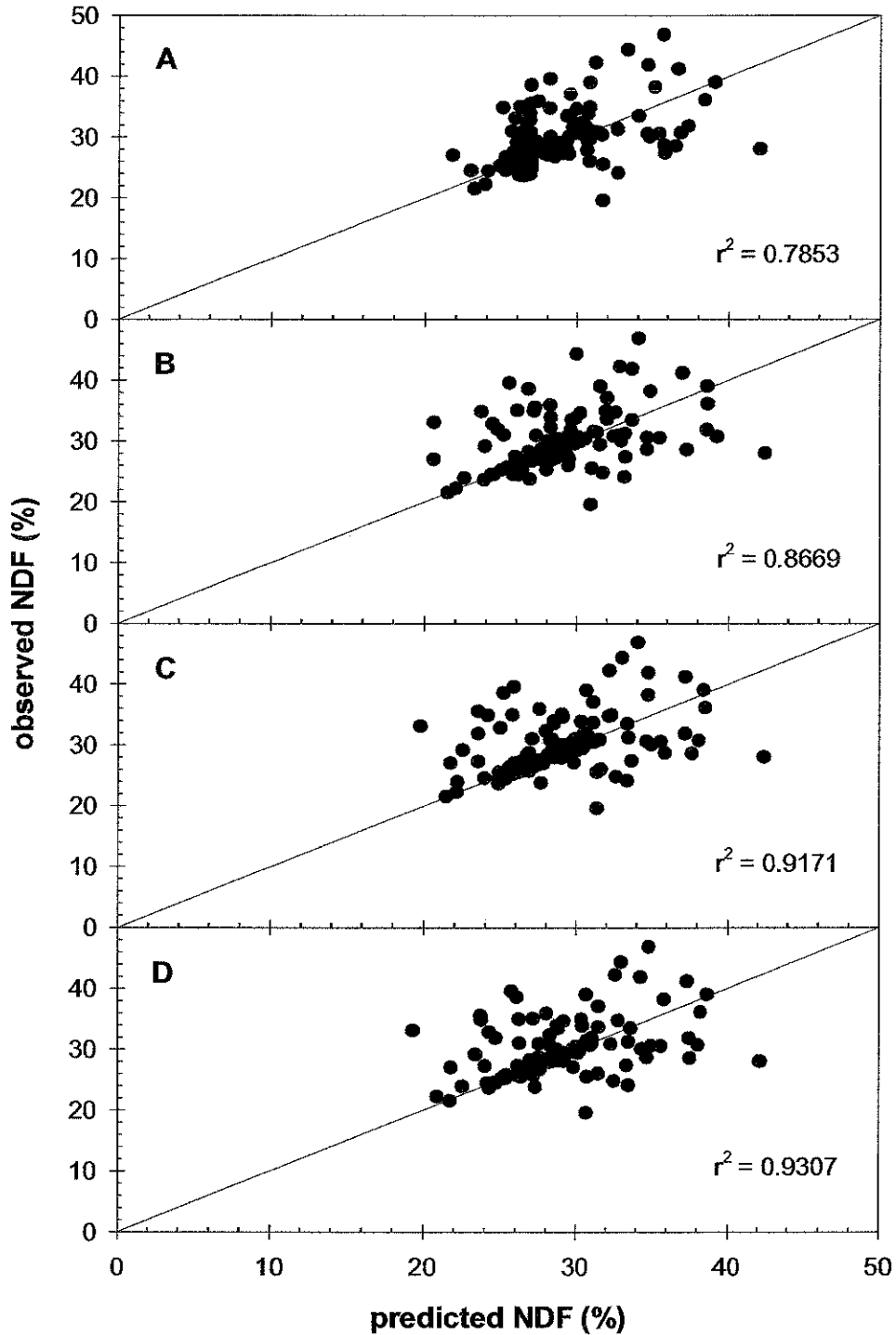


Figure 25: Parity plots comparing the observed NDF concentrations to the concentrations predicted by the best-fit neural network models for DDGS samples produced in pilot-plant trials conducted in October 2006 and January 2007. The models were fit using (A) 3, (B) 4, (C) 5, or (D) 6 hidden nodes. The diagonal lines represent perfect agreement between observed and predicted concentrations.

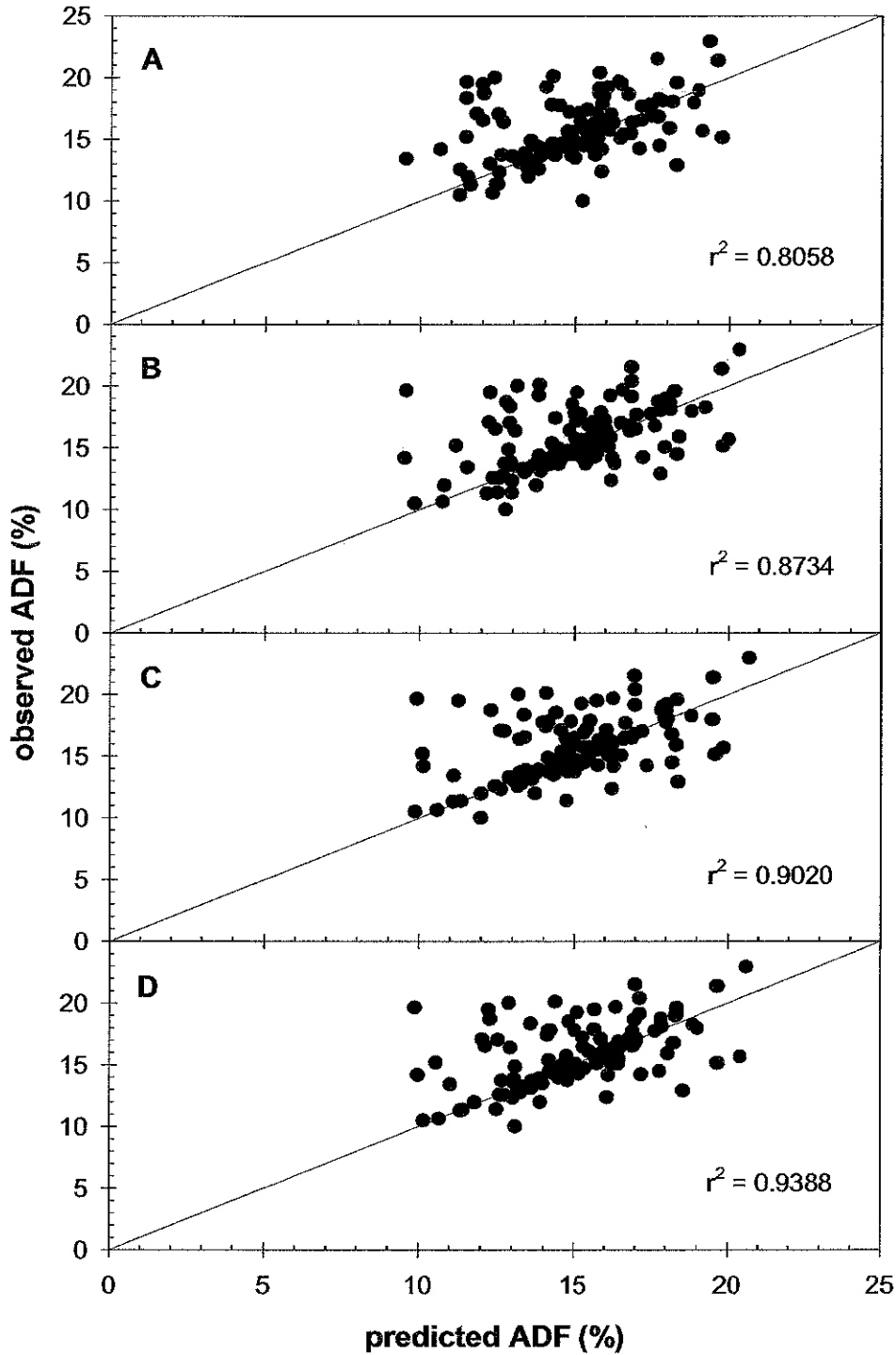


Figure 26: Parity plots comparing the observed ADF concentrations to the concentrations predicted by the best-fit neural network models for DDGS samples produced in pilot-plant trials conducted in October 2006 and January 2007. The models were fit using (A) 3, (B) 4, (C) 5, or (D) 6 hidden nodes. The diagonal lines represent perfect agreement between observed and predicted concentrations.

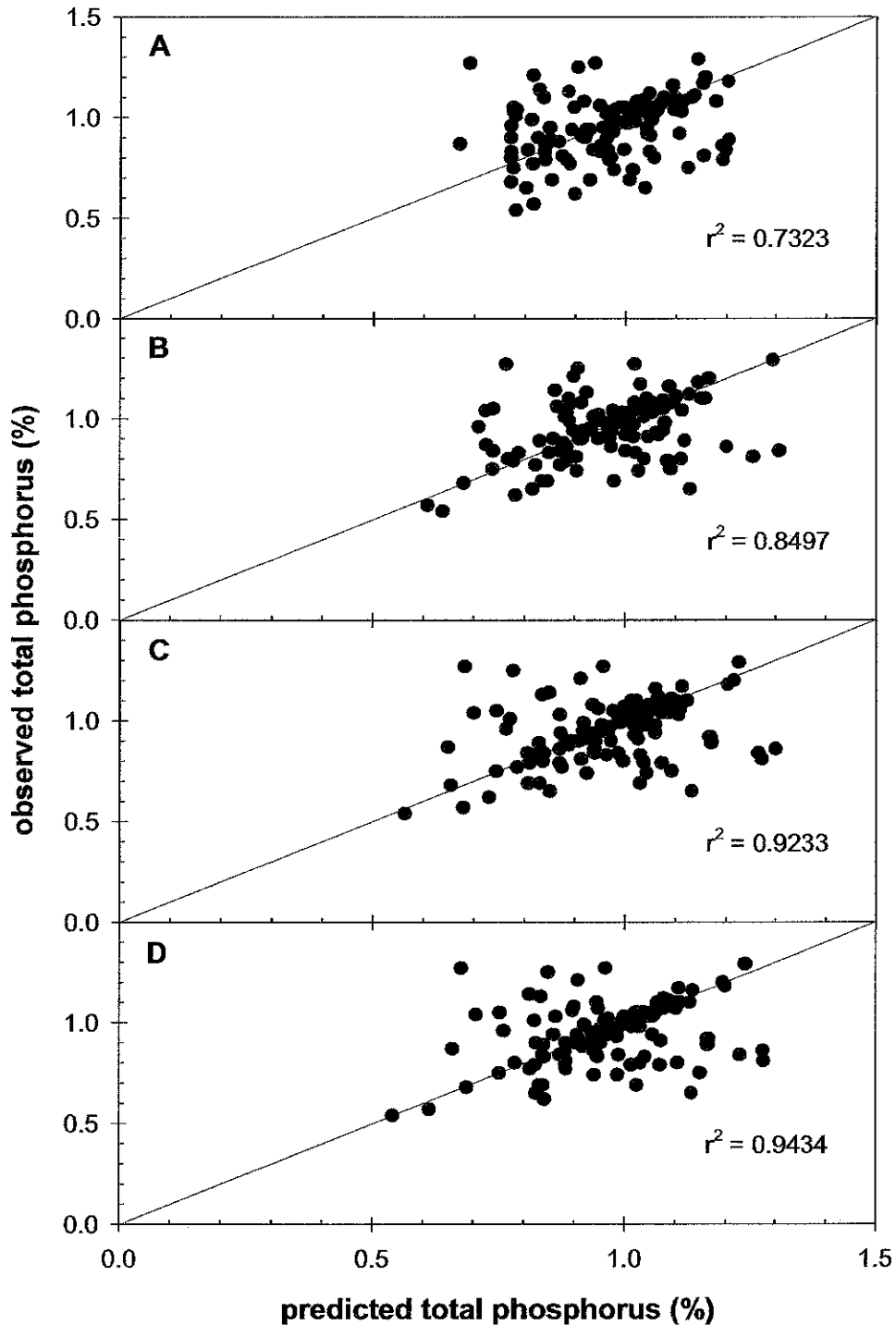


Figure 27: Parity plots comparing the observed total phosphorus concentrations to the concentrations predicted by the best-fit neural network models for DDGS samples produced in pilot-plant trials conducted in October 2006 and January 2007. The models were fit using (A) 3, (B) 4, (C) 5, or (D) 6 hidden nodes. The diagonal lines represent perfect agreement between observed and predicted

Table 22: Comparison of the coefficients of determination for multiple linear regression and several neural network models of the chemical composition of DDGS

component	coefficients of determination (r^2)*				
	linear regression model	3 hidden nodes	4 hidden nodes	5 hidden nodes	6 hidden nodes
crude protein	0.055	0.526 ± 0.030	0.638 ± 0.013	0.701 ± 0.025	0.729 ± 0.003
crude fat	0.556	0.762 ± 0.048	0.864 ± 0.017	0.908 ± 0.027	0.933 ± 0.013
NDF	0.463	0.689 ± 0.089	0.863 ± 0.006	0.905 ± 0.014	0.927 ± 0.016
ADF	0.390	0.773 ± 0.033	0.852 ± 0.029	0.890 ± 0.017	0.933 ± 0.006
total phosphorus	0.275	0.719 ± 0.019	0.817 ± 0.029	0.903 ± 0.020	0.933 ± 0.015

*The coefficients of determination for the neural-network models are based on the training and validation data sets, whereas the entire data set was used for parameter estimation (training) in the linear regression models

6. Conclusions and Recommendations

This research was the first systematic investigation of the effects of ethanol-plant operating conditions on the chemical and physical characteristics of DDGS. The results demonstrate that several operations and processes exerted statistically significant effects on DDGS characteristics and composition, but the effects appeared to be highly nonlinear and interactive. Simple multiple-regression statistical models provided poor descriptions of the relationship between DDGS composition and operating conditions. The more complex neural-network models provided better descriptions of these relationships, but the best-fit models identified in this work should not be considered to be predictive. Similar statistically significant treatment effects were also identified for several measures of fermentation performance (*e.g.*, mash DE, sugar consumption rate, ethanol yield), but these relationships are not yet understood well enough for use in process control. With respect to fermentation, in particular, only weak correlations were observed between easily measurable characteristics of the mash and important performance metrics, such as ethanol yield.

Use of neural network models for control of DDGS composition and nutritional quality may be difficult for a variety of reasons, but such control seems feasible in principle. Even if feasible, however, additional research will be required to determine the best approach to DDGS quality control. Because nutritional quality is complex and not dependent on any one characteristic of DDGS, the interactions between nutritionally important characteristics and their control parameters must be studied in more detail. Nutritional quality depends on several characteristics that are affected differently by processing conditions. In some cases, the same process-control strategy may have opposite effects on different characteristics that are of interest. For example, the nutritional value of DDGS depends on the quality of the protein, and in particular, the concentrations of essential amino acids, such as lysine. Although the amino acid concentrations were not correlated with any of the dryer parameters that were investigated in a preliminary study using the Dupps dryer and were not measured in DDGS produced in the complete-system

pilot-plant experiments that were conducted in October 2006 and January 2007, DDGS color has been shown to be correlated with protein digestibility. The color of the DDGS produced using these two dryers depended, among other factors, on dryer temperature and the addition rates of syrup and wet cake. Moisture content also was affected by dryer temperature and syrup addition rate, but these affected the desired DDGS characteristics (*i.e.*, lower moisture) than they affected color. Higher dryer temperature reduced DDGS moisture content (increased dry solids concentration) and made the DDGS darker. Conversely, higher syrup addition rates tended to improve the color of the DDGS but also made it more difficult to dry. The caloric value of DDGS, on the other hand, depends mainly on the protein and fat content, and the protein and fat concentrations appeared to vary independently with operating conditions (slurry-tank temperature was the only important factor affecting protein concentration, whereas the syrup and wet-cake addition rates, the application rate of alpha amylase during liquefaction, and the particle-size distribution all affected the fat content). Therefore, although complex, it may be possible to control the plant operating conditions to allow optimization of caloric content and protein digestibility, but simultaneous control of moisture content may be more difficult.

The results of this research are encouraging with respect to the ability to use sophisticated process-control technologies to improve the consistency and nutritional value of DDGS, which is an important coproduct of the dry-grind ethanol industry. More research is required, however, to understand the ways in which plant operating conditions affect specific aspects of DDGS quality and composition. Many of the factors that were shown to affect DDGS quality were consistent throughout at least two completely independent dryer studies, and these significant factors were consistent with their expected effects based on fundamental principles. The relatively poor predictive power of the neural-network models that were developed, however, suggest that other factors may also be important, and the effects appeared to be highly nonlinear and interactive. Additional research should focus specifically on the factors that were identified as exerting significant treatment effects to more clearly determine the nature of these nonlinearities and interactions.

7. References

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