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# Protein extraction from distiller's grain

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# **Protein extraction from distiller's grain**

By

**Drew John Cookman**

A thesis submitted to the graduate faculty  
in partial fulfillment of the requirements for the degree of  
**MASTER OF SCIENCE**

Major: Chemical Engineering

Program of Study Committee:  
Charles E. Glatz, Major Professor  
Brent H. Shanks  
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Iowa State University

Ames, Iowa

2008

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## **DEDICATION**

This thesis is dedicated to my family, who offered me unconditional love and support throughout the course of this research adventure. In particular, to my wife and parents who taught me with hard work and determination anything is possible.

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## ABSTRACT

The drastic increase in ethanol production over the last 10 years has led to overproduction of distiller's grain (DG), an ethanol byproduct. In this study, we investigated the feasibility of extracting the protein from both unmilled and milled dried, defatted DG (DDDG) leaving a carbohydrate-rich solid phase that could be used for the production of more ethanol. The oil was removed from the DG using a hexane wash and the solids were dried prior to protein extraction. Protein extractions based on aqueous ethanol (both with and without a pretreatment of a reducing agent), alkaline-ethanol, and aqueous enzyme treatments were compared.

Three methods led to a significant amount of protein being extracted from DG. An enzymatic extraction decreased the crude protein content in the solid phase for both the unmilled and the milled DG from 41% (dry weight) to approximately 10% (dry weight) protein, which represented 90% protein yield in the extract. The alkaline-ethanol extraction was similarly effective for the milled DG but not for the unmilled material. Simple extraction with alcohol, both with and without a reducing agent, was not as effective.

## CHAPTER 1 GENERAL INTRODUCTION

### *Introduction*

Distiller's grain (DG) is a byproduct of the dry-grind ethanol process. During ethanol production, corn starch is converted to alcohol and carbon dioxide. As a result of the starch removal, the remaining insoluble nutrients are concentrated nearly threefold (Belyea *et al.*, 2004). One of the byproducts containing these nutrients is DG. Figure 1.1 illustrates a simplified ethanol production process for both the dry grind and the wet mill processes.

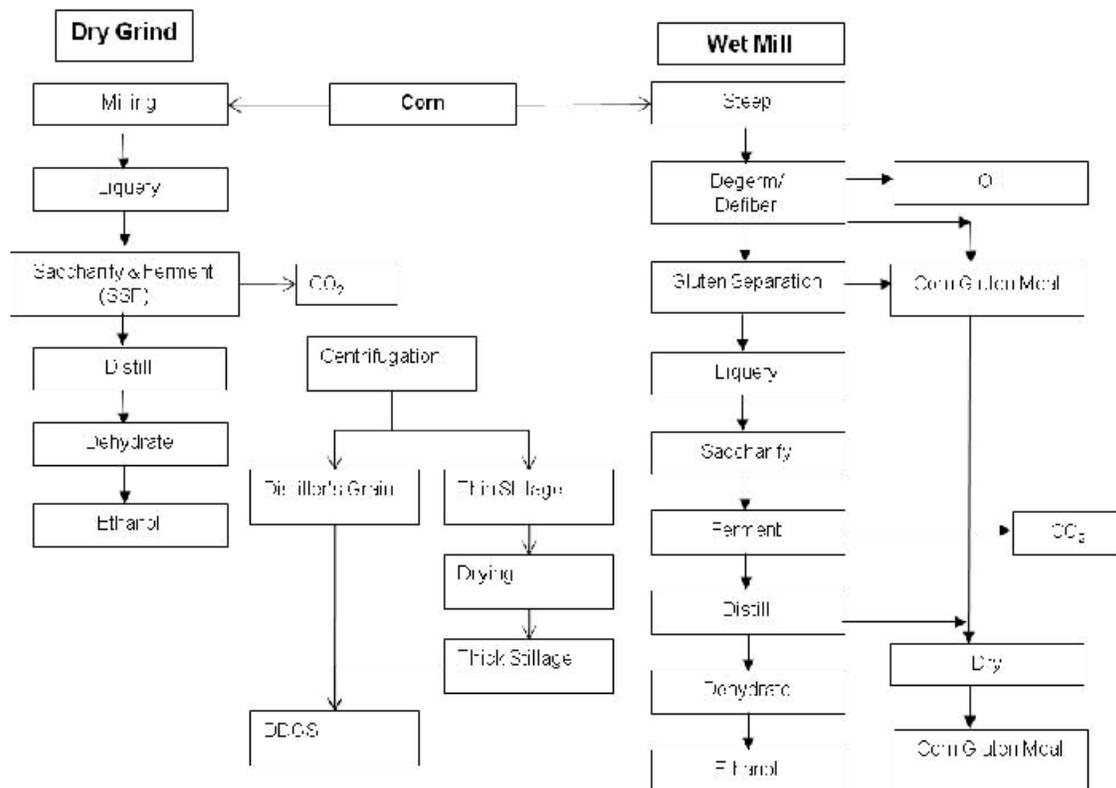


Figure 1.1 Simplified process diagram for the dry grind and wet mill ethanol production process. DG is only produced by the dry grind ethanol process.

The dry grind process produces more ethanol per bushel of corn (approximately 2.8 gallons) than the wet mill process (approximately 2.5 gallons) (Bothast, 2005). As a result, there has been an increase in dry-grind ethanol plants. Currently, most of the DG being produced is being fed to cattle as a high protein feed supplement. However, there is a developing oversupply and recent research has shown addition of DG to cattle feed may result in *E. coli* O157 outbreaks (Hegeman, 2008).

The latter discovery puts more emphasis on finding an alternative use. One option is using the residual carbohydrates in DG as a sugar source for more ethanol production, the oil for biodiesel, and the concentrated proteins as a feed supplement. Shanks' group at Iowa State University has developed a catalytic process to convert residual oligosaccharides to fermentable sugars; however, the feed must be protein-free to avoid poisoning the catalyst (B. Shanks, personal communication). The objective of this research is to extract the oil and proteins from DG leaving a carbohydrate rich starting material for Shanks' process.

### ***Thesis Organization***

This thesis includes this background chapter and a manuscript (Chapter 2) prepared for submission to the journal *Bioresource Technology*. The manuscript focuses on oil and protein extraction from distiller's grain. A final Chapter addresses future work based on the overall conclusions of this work.

## ***Literature Review***

Most of the current research on protein extraction from ethanol byproducts is done using distiller's grain plus solubles (DDGS). DDGS is DG combined with the thick stillage stream and then dried in a drum dryer. The majority of the proteins in both DG and DDGS are zeins (Wolf and Lawton, 1997; Shukla and Cheryan, 2001). Zeins are alcohol soluble storage prolamins comprising 45-50% of the protein in corn endosperm and they remain concentrated in DG (Wang *et al.*, 2005). Zeins have been characterized by size and the variants are denoted  $\alpha$ ,  $\beta$ , and  $\gamma$  (Esen, 1986). The  $\beta$ - and  $\gamma$ -zein differ from the  $\alpha$ -zein only in the number of disulfide linkages (Esen, 1986; Parris and Dickey, 2001). They are disulfide-linked aggregates of components found in native zeins (Esen, 1986). The number of disulfide linkages changes the solubility characteristics of the  $\beta$ - and  $\gamma$ -zein. The  $\alpha$ -zein is soluble in 95% aqueous ethanol, whereas the  $\beta$ - and  $\gamma$ -zein are soluble in 60% aqueous ethanol but not in 95% aqueous ethanol (Esen, 1986; Parris and Dickey, 2001). Extraction of the zeins in DG could be accomplished by simply placing the DG in an environment where the proteins are soluble. However, previous research has shown extraction of zeins from ethanol byproducts to be very difficult. Only about 1.5-3.9% of the crude zein has been extracted from ethanol byproducts using 60% ethanol at 60°C (Wolf and Lawton, 1997).

The use of reducing agents could increase the protein yields from the aqueous ethanol extractions. Reducing agents have been used to break disulfide linkages in many cereal grains including corn (Jay, 2005). Since the disulfide linkages in zeins changes their solubility characteristics (Esen, 1986), it is possible

that breaking these linkages would make all three variants of zeins soluble at the same alcohol concentration. Sodium bisulfite at a concentration of 100mM improves the digestibility of kafirins (Hamaker *et al.*, 1986). Kafirins are similar to zeins in that they are both alcohol-soluble storage prolamins (Salinas *et al.*, 2006).

An alternative to the aqueous ethanol extraction would consist of using a protease to increase the solubility of zeins. Proteases have been used to make insoluble proteins more soluble (Rothberg and Axilrod, 1967). Methods consisting of an aqueous enzyme treatment have been developed to extract proteins from plant products such as soy beans (Jung *et al.*, 2006) and corn germ (Moreau, 2004). Hydrolyzing the proteins not only makes the proteins easier to extract, it also reduces antigenicity (Ludlow, 2005). The attractive characteristics of hydrolyzed proteins would increase the value of the proteins in DG. Several studies have shown that turkeys fed up to 10% hydrolyzed DG in place of soybean concentrate have similar weight gain as turkeys fed their normal diet of corn and soybean concentrate (Noll, 2001; Tucker *et al.*, 2004) Hence, hydrolyzed DG could partially replace the higher-priced soybean concentrate.

Intact zeins have many industrial uses as coatings, fibers, adhesives, and textiles (Core, 2002). One problem with using zeins for these purposes is the difficulty associated with extracting them. The extraction of zeins has been limited to approximately 500 tons a year for use in specialty products (Shukla and Cheryan, 2001). If a process could be developed to extract zeins from DG it could drastically reduce the cost of these proteins (Core, 2002). Extraction of zeins would also

accomplish the goal of refining the residual carbohydrates so they could be used to produce more ethanol.

A protein-rich extract of either intact zeins or hydrolysates for soy concentrate replacement offers potential economic benefit over selling it as part of DG. While higher in price than could be expected for the film market, zeins have a value of \$19-\$22/kg for use as specialty products (Lawton, 2003). Soy concentrate, for example ARCON<sup>®</sup>SM, is 70% protein and sells for \$3.25/kg (per Chicago Sweeteners, Chicago, IL) or \$4.64/kg of protein. The market value of DDG is \$170 a ton (May 2008, Commodities Specialty Co., Minneapolis, MN). For 32% protein content DDG, this corresponds to \$0.58/kg of protein.

Extraction of oil from DG would also add value to DG. A commercial extraction process has been developed by Veridium Corporation that extracts the oil for use in biodiesel (Silverstein, 2006). Most of the oil in corn is found in the germ. Some ethanol production facilities are removing the corn germ prior to fermentation which causes a drastic reduction in the fat content in DG (Van Hulzen and Forster, 2007). It is reasonable to think that most of the oil in DG is from the corn germ which means methods used for obtaining oil from corn germ could apply. Current approaches to the extraction of oil from corn germ include techniques such as screw processing, hexane extraction, and enzymatic extraction (Shukla and Cheryan, 2001). Due to safety considerations, hexane extraction is less favorable, but it has shown the best yields and is the technique currently used in industry. Liquid hexane can accumulate static charge from flow or agitation and the vapor can be readily

ignited by static discharge. The cost of operation associated with using hexane is greatly increased because of these safety concerns.

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## CHAPTER 2 Extraction of Oil and Protein from Distiller's Grain

A manuscript submitted to Bioresource Technology

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### ***Abstract***

We have investigated the feasibility of extracting the oil and protein from distiller's grain (DG) to obtain a higher-valued protein-rich product and a carbohydrate-rich residue better suited for conversion to fermentable sugars. Protein extractions based on aqueous ethanol, alkaline-ethanol, and aqueous enzyme treatments were compared.

Three of the methods extracted a significant amount of the protein from dried, defatted DG (DDD<sub>2</sub>G). The enzymatic extraction decreased the crude protein content in the solid phase for both milled and unmilled DDD<sub>2</sub>G from 41% (dry weight) to approximately 10% (dry weight) protein, which represented 90% protein yield in the extract. The alkaline-ethanol extraction was similarly effective for milled but not unmilled DG. Simple extraction with alcohol was not as effective. Amino acid analysis of each protein extract was consistent with it consisting mainly of zeins. For

the protease-assisted extractions, 95% the proteins were in the form of peptides smaller than 10 kDa.

Keywords: protein, DG, distiller's grain, ethanol, extraction, composition

## ***Introduction***

Production of byproduct distiller's grain (DG) has increased along with the number of dry grind ethanol production facilities. In the dry-grind ethanol process, DG is the solid residue recovered from the still bottoms following fermentation and distillation. It is dried to be sold as distiller's dried grain (DDG) or blended with the dried solubles in the bottoms and sold as distiller's dried grain plus solubles (DDGS). Currently most of the DG is being used as cattle feed in one of these forms, but oversupply and an increase in *E. coli* O157 (Hegeman, 2008) outbreaks fuel the search for alternative uses. DG contains protein, some oil, and non-starch carbohydrates from the corn along with some portion of yeast material (Kim *et al.*, 2008). An analysis of the composition of DG from the same source as used in this work showed 34.4% crude protein, 10.9% ether extractable fat, 52.7% carbohydrates, and 2.0% ash (Kim *et al.*, 2008).

A significant effort is being made to obtain additional fermentable sugars from the carbohydrate (mainly cellulose) portion of the DG (Ladisich, *et al.*, 2008). Because of the complex nature of these carbohydrates, acid hydrolysis has advantages over enzymes in being able to hydrolyze multiple types of linkages, with solid acid catalysts favored for reduced degradation of the desired monosaccharide products (Bootsma, *et al.*, 2008). That route for obtaining the sugars is to extract them from the DG (or DDGS), leaving behind a protein-enriched DG. However, some proteins are also extracted and/or degraded leading to poisoning of the hydrolysis catalyst (Bootsma, *et al.*, 2008) and a less valuable feed (Perkis *et al.*,

2008). The current work explores direct extraction of proteins with potentially greater value than DG, while leaving behind a carbohydrate residue with reduced fouling potential.

The proteins in DG are believed to be mainly zeins (Wolf and Lawton, 1997; Shukla and Cheryan, 2001). Zeins are alcohol soluble storage prolamins comprising 45-50% of the proteins found in the corn endosperm (Wang *et al.*, 2005). Shukla and Cheryan (2001) have reviewed zein extraction from corn and corn gluten meal, the zein-containing fraction from corn wet-milling. Zeins have been characterized by size and the variants are denoted  $\alpha$ ,  $\beta$ , and  $\gamma$  (Esen, 1986). The  $\beta$ - and  $\gamma$ -zein differ from the  $\alpha$ -zein in the number of disulfide linkages (Esen, 1986; Parris and Dickey, 2001) and have been characterized as disulfide-linked aggregates of components found in native zeins (Esen, 1986). The disulfide linkages change the solubility characteristics. The  $\alpha$ -zein is soluble in 95% aqueous ethanol, whereas the  $\beta$ - and  $\gamma$ -zein are soluble in 60% aqueous ethanol but not in 95% aqueous ethanol (Esen, 1986; Parris and Dickey, 2001).

DG is in contact with alcohol at much lower concentrations during the ethanol process and thus zeins are unlikely to have been extracted. The extraction of zeins from DG has been shown to be quite difficult. Extraction at 60% ethanol and 60°C was only capable of extracting 1.5-3.9% of the crude zein in ethanol byproducts (Wolf and Lawton, 1997). Alkaline-ethanol extractants have shown better extraction of proteins from DG (Rosentrater *et al.*, 2006). Here we have considered use of a protease to produce more soluble peptides or pretreatment with a reducing agent to

break disulfide linkages present in the native zein or formed during process to eliminate insoluble aggregates.

Reducing agents have been used to break disulfide linkages in many cereal grains including corn (Jay, 2005). Sodium bisulfite at a concentration of 100mM improved the digestibility of kafirins from sorghum (Hamaker, 1986). Kafirins are similar to zeins in that they are both alcohol-soluble storage prolamins (Salinas *et al.*, 2006).

An alternative to breaking the disulfide linkages and aqueous alcohol extractions would be hydrolyzing the proteins. Proteases have been used to increase protein solubility (Rothberg and Axilrod, 1967). Methods have been developed using proteases to successfully extract proteins from soybeans (Jung *et al.*, 2006) and corn germ (Moreau, 2004). Hydrolyzed proteins are attractive feed supplements because they have decreased antigenicity and are easier to digest (Ludlow, 2005). Up to 10% hydrolyzed DG has been used to replace soybean concentrate with no reduction in the weight gain of turkeys normally fed corn and soybean concentrate (Tucker *et al.*, 2004). Hence, hydrolyzed DG could be used to replace the higher priced soybean concentrate.

A protein-rich extract of either intact zeins or hydrolysates for soy concentrate replacement offers potential economic benefit over selling it as part of DG. While higher in price than could be expected for the film market, zeins have a value of \$19-\$22/kg for use as specialty products (Lawton, 2003). Soy concentrate, for example ARCON<sup>®</sup>SM, is 70% protein and sells for \$3.25/kg (per Chicago Sweetners, Chicago, IL) or \$4.64/kg of protein. The market value of DDG is \$170 a ton (May

2008, Commodities Specialty Co., Minneapolis, MN). For 32% protein content DDG, this corresponds to \$0.58/kg of protein.

Extractions were performed here using aqueous ethanol, alkaline-ethanol, and aqueous enzyme solutions. Pretreatments of milling and reduction with sodium bisulfite (for the aqueous ethanol extractions) were also evaluated. The extractions were assessed on the basis of protein extracted, protein in the residual fiber fraction, and extracted protein size and amino acid composition.

## ***Materials and Methods***

### **Materials**

All chemicals were from Fisher Scientific unless otherwise noted. DG was provided by Big River Resources, West Burlington, Iowa. The DG contained 67% moisture and was stored frozen (-20°C) until use. The extractions were performed in 250 mL beakers using 150 mL of the solvents listed in Table 1 and 10 grams of dried, defatted DG (DDDGG).

Type of Extraction	Solvent Used
Aqueous Ethanol	60% Ethanol
Aqueous Ethanol	95% Ethanol
Alkaline-Ethanol	45% Ethanol and 55% 1M NaOH
Aqueous Enzyme	0.67% v/v Protex 6L in deionized water (pH 8.0)

Table 1. Solvents used for protein extraction from DDDGG.

Solutions containing ethanol were prepared using absolute ethanol. The enzyme used was Protex 6L (an alkaline serine-endopeptidase, active range pH 7.0-10.0, 30-70°C) provided by Genencor International Inc, Rochester, NY.

### **Oil Extraction**

A hexane wash was used for both the proximate analysis of the DG and prior to any protein extraction step. The hexane-extractable oil content of the DG was determined by mass difference of the dry weights before and after a hexane wash. For the hexane wash, 50 grams of DG was stirred with 250 mL hexane in an ice bath for 1 hour. The DG and hexane were separated by centrifugation in 250 mL centrifuge bottles at 3,000 *g* for 15 minutes and 25°C (Sovall RC-5B, Newton, CT) using a HS-4 Swinging Bucket rotor. The supernatant was decanted and the extraction was repeated. The remaining solids were dried at room temperature overnight.

### **Extraction with Ethanol**

Extractions were performed using 60% and 95% aqueous ethanol, and 45% ethanol in 1 M NaOH, at a solid:liquid ratio of 1:15. The solution was stirred at 520 rpm for 2 - 10 hours at 50°C in a water bath. The solids and the extract were separated by centrifugation (25°C, 20 minutes at 3000 *g*). The solid fraction was analyzed for residual mass and crude protein content after drying for 6 hours at 130°C. Amount of nonprotein solids extracted was calculated by difference. Proteins were precipitated from the extract by pH adjustment to 7.0 using 5 M HCl.

The precipitate was characterized by amino acid analysis, SDS-PAGE, and total N analysis.

### **Enzyme-Aided Extraction**

A mixture of 10 g of DDDG, 1 mL Protex 6L and 150 mL deionized water was stirred at 520 rpm for 2 hours at pH 8.0 in a 50°C water bath. The pH was maintained at 8.0 with 2 N NaOH using an automatic titrator (Metrohm titrator, model STAT Titrino 718). The solids and extract were separated by centrifugation in 50 mL centrifuge bottles at 3000 g for 20 minutes and 25°C. The size of the proteins in the extract were analyzed using size exclusion chromatography (SEC) and the solid fraction was analyzed for crude protein content after drying for 6 hours at 130°C.

### **Disulfide Bond Reduction**

A mixture of 10 grams of DDDG and 150 mL of 100 mM NaHSO<sub>3</sub> was stirred at 520 rpm at 50°C for 2 hours. The solid and liquid fractions were separated by centrifugation (25°C, 20 minutes at 3000 g). The supernatant was decanted and the solid phase was extracted with 60% and 95% aqueous ethanol solutions.

### **Particle Size Distribution/Size Reduction**

The volume- and surface-weighted mean diameters ( $D_{4,3}$  and  $D_{3,2}$ ) of the DDDG were measured using a laser light scattering particle size analyzer (Mastersizer 2000 S, Malvern Instruments, Ltd., Chicago, IL) before and after milling (Retsch mill with a 0.5 mm screen).

## **Protein Content Determination**

The crude protein (N x 5.9) (Kim *et al.*, 2008) of the solids was based on the nitrogen content determined by combustion analysis (Berner and Brown, 1994) (Rapid NIII combustion analyzer; Elementar Americas, Inc., Mt. Laurel, NJ).

## **Protein Analysis of Alkaline-Ethanol Extract**

The alkaline-ethanol extract was pH adjusted to 7.0 using 5 M HCl (Ludlow, 2005). The pH adjustment caused precipitation of a fraction of the proteins from the extract. The precipitated proteins were separated by centrifugation (25° C, 20 min at 3015 g) and dried in an oven for 6 hours at 130°C.

Approximately 1 g of dried protein pellet was dispersed in 10 mL of 2% SDS, 8M urea, and 50 mM DTT. The solution was diluted (1:2, 1:5, 1:10, and 1:20 v/v) in sample buffer containing 200 mM Tris-HCl pH 6.8, 2% SDS, 40% glycerol, 0.04% bromophenol blue and 350 mM of DTT, and heated at 100°C for 5 minutes. Samples of 15 µL and 25 µL were loaded on the electrophoresis gel (Tris-HCL 4-15% acrylamide linear gradient gel, Bio-Rad, Cat #161-1104 Hercules, CA) and the gel was run at 200 V for 40 minutes in a Mini-PROTEAN® II Electrophoresis Cell (Bio-Rad, Hercules, CA). The gels were stained with Coomassie Brilliant Blue and the molecular weights were determined by comparison to molecular weight markers (Precision Plus Protein Standard, Bio-Rad, Catalog #161-0363) run in parallel.

## **Hydrolyzed Protein Size Distribution**

The molecular weight distribution of proteins in the protease-assisted extract was determined by size exclusion chromatography (SEC) using a 300 mm x 7.8 mm Biobasic SEC 120 column (Bio-Rad, Hercules, CA). The column was equilibrated for 1 hour using phosphate buffer (pH 7.5) as the mobile phase. Molecular weight markers were: aprotinin from bovine lung (6.5 kDa), insulin chain B (3.6 kDa), angiotensin II human acetate (1.1 kDa), and leucine enkephalin acetate hydrate (0.5 kDa). Samples were filtered through a 0.45  $\mu\text{m}$  regenerated cellulose filter (Millipore Corporation, Billerica, MA) and diluted to 1  $\mu\text{g}$  protein/ $\mu\text{L}$  with the phosphate buffer. The mobile phase flow rate was 1.0 mL/min and the injection volume was 10  $\mu\text{L}$ . The absorbance was measured at 215 nm.

## **Amino Acid Analysis**

Amino acid profiles were determined at the University of Missouri-Columbia Experiment Station Chemical Labs using AOAC Official Method 982.30.

## ***Results and Discussion***

### **Proximate Analysis of DG**

The DG used in this study contained 67% moisture and on a dry basis was 8% hexane-extractable oil and 32% protein.

## Extraction

Figure 1 below shows the protein content in the solid phase after a 2 hour extraction time for both unmilled and milled DDDG. Extractions longer than 2 hours did not extract significantly more protein (results not shown).

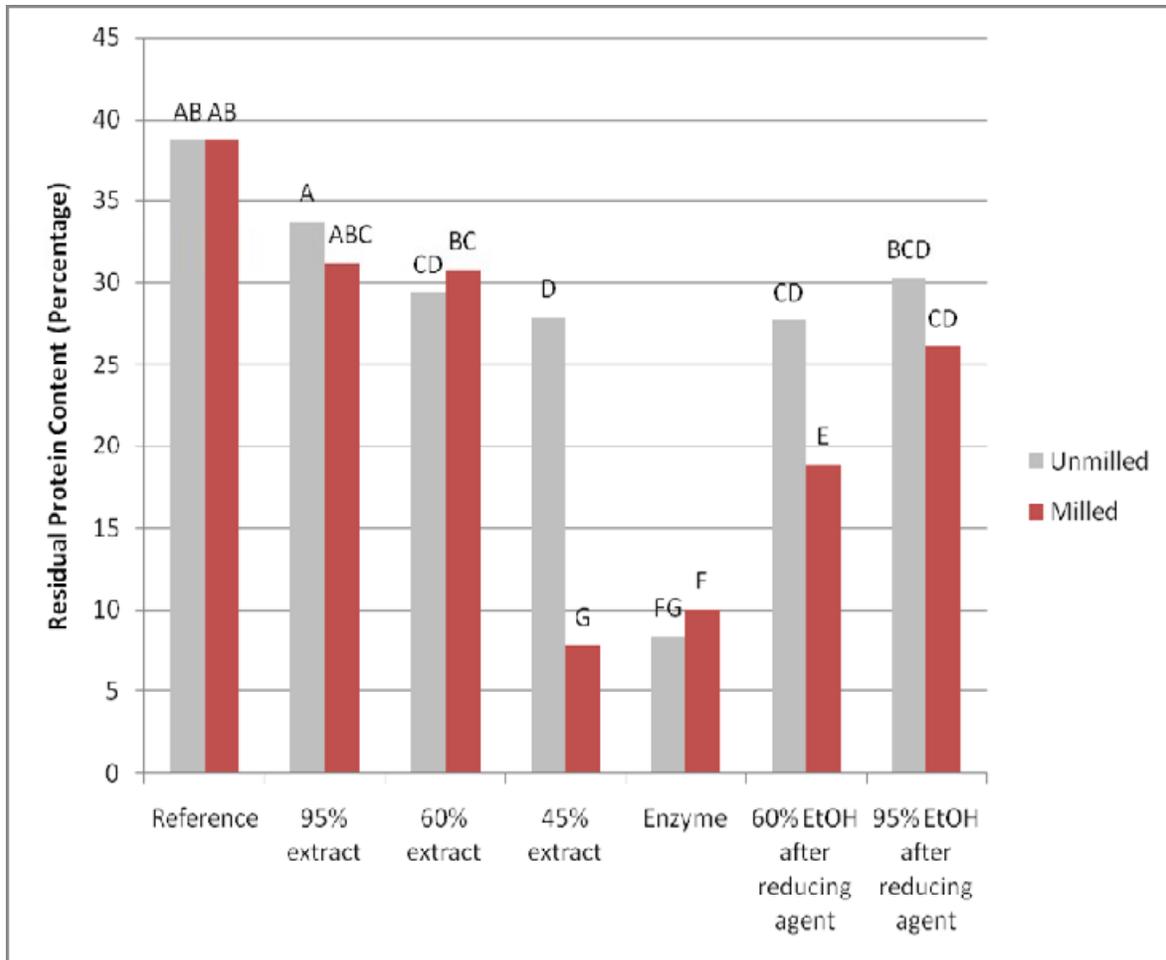


Figure 1. Percent of protein in the solid phase after 2 hour extractions with different solvents. The starting (reference) material was DDDG. A multiple comparison test using Tukey's Procedure (Devore, 2004) was performed on the data. Bars with the same letter above them means there is no significant difference in protein content ( $\alpha=0.05$ ).

Without milling, the aqueous enzyme treatment was the only method that extracted a significant amount of protein. After size reduction, the aqueous enzyme treatment and the alkaline-ethanol treatment were the most effective at reducing the

protein content in the solid phase. For the milled alkaline-ethanol extraction and the enzyme extractions, these residual protein contents correspond to approximately 90% protein extraction. The alkaline-ethanol extraction extracted more of the nonprotein material (35%) from the distiller's grain than the enzyme extraction (20%). During the alkaline-ethanol extraction about a third of the solvent evaporated; however, an alternative procedure using a condenser to reflux the solvent gave the same residual protein content as the standard procedure. To a lesser extent the 60% ethanol extraction of bisulfite-pretreated, milled DDDG was also effective. The aqueous ethanol treatments without a sodium bisulfite pretreatment did not extract a significant amount of protein from either the milled or the unmilled material. Increased surface area, shorter diffusion lengths and additional cellular disruption accompanying milling would explain why milling improved the extraction with ethanol. Further size analysis was performed to determine why milling did not also improve the enzyme-aided extraction.

### **Milling Results**

The milling process reduced the  $D_{4,3}$  from 170  $\mu\text{m}$  to 75  $\mu\text{m}$  and  $D_{3,2}$  as shown in Figure 2, resulting in an increased surface area for greater contact with the solvent.

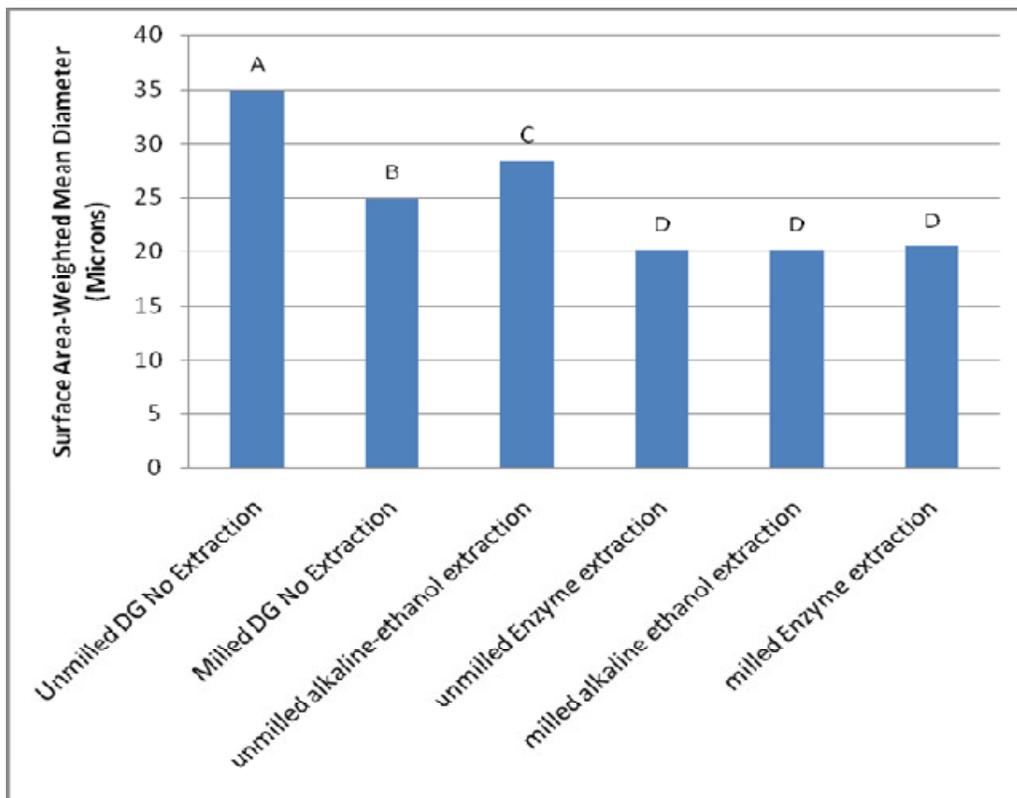


Figure 2. Surface area-weighted mean,  $D_{3,2}$ , of the solid phase both before and after extraction using the milled and unmilled DG and the alkaline-ethanol and enzyme extractants. A multiple comparison test using Tukey's Procedure (Devore, 2004) was performed on the data. Bars with the same letter above them means there is no significant difference in the amount of protein extracted ( $\alpha=0.05$ ).

Interestingly, even without milling, the enzyme-assisted extraction decreased the  $D_{3,2}$  to the same value for both the milled and the unmilled material. Thus enzyme action was able to increase solvent access as well as potentially increasing protein solubility via hydrolysis. By comparing the unmilled DDDG with no extraction to that of unmilled DDDG after the alkaline-ethanol extraction, it can be concluded that the extraction process does reduce size somewhat but not nearly as much as milling does.

Comparison of Figures 1 and 2 for the unmilled and the milled alkaline-ethanol treatments demonstrates that increased surface area/volume is the primary

enabler of more effective protein extraction. Both protease treatment and milling reduced  $D_{3,2}$  and resulted in very similar protein extraction. This is not the case for the unmilled material, as the only process found to significantly reduce the protein content is the aqueous enzyme treatment. Light microscopy of the residual solids showed some intact cells at the core of the particles with disrupted and empty cells predominating closer to the surface. An economic analysis would need to be performed to determine if the cost of enzymes would be offset by the elimination of the milling step.

### **Size Distribution of Proteins from Alkaline-Ethanol Extract**

Approximately 0.2 g of precipitate/g DG (dry weight) was obtained from the alkaline-ethanol extract by pH adjustment. The crude protein content of the precipitate was found to be 70% by weight. At this point, the precipitate could be used as a high-protein feed supplement for cattle, similar to current DG use. However, characterization of the proteins could be beneficial, as they are believed to be zeins which have a much higher industrial value. Figure 3 shows a SDS-PAGE gel of the redissolved precipitate.

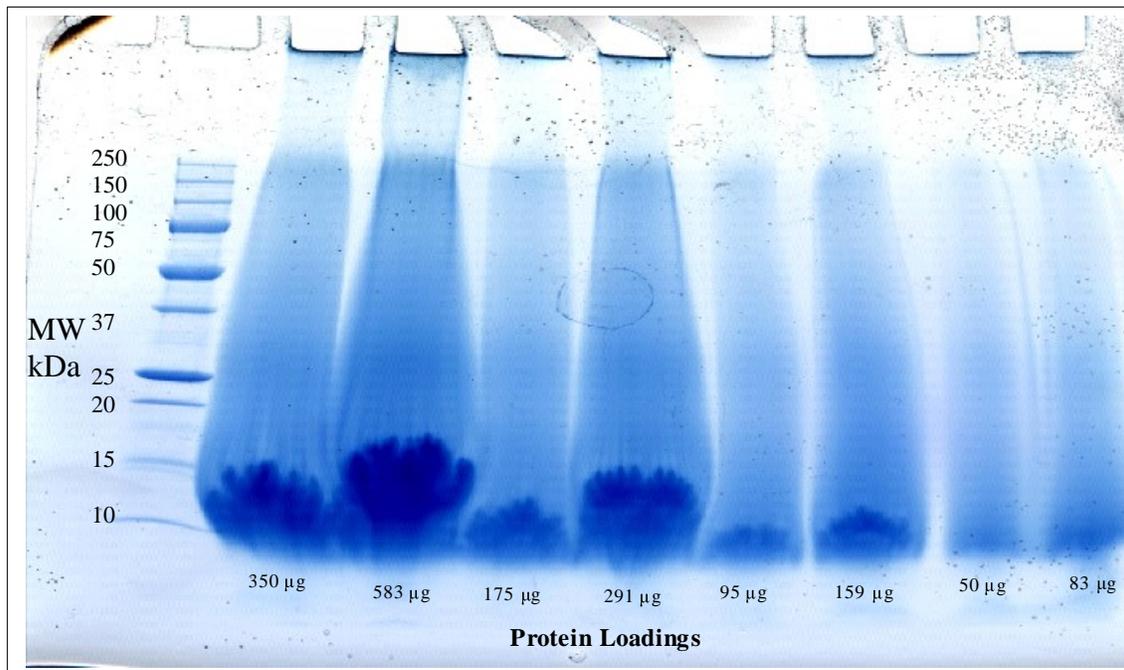


Figure 3. SDS-PAGE gel of the precipitate obtained from the alkaline-ethanol extraction. Lane 1 is the molecular weight marker. Lanes 2 through 9 have the same proteins with different loadings.

The irregularity of the bands could be due to precipitation of the proteins as the gel was running or degradation during extraction. This feature persisted over the range of protein loadings. However, all the proteins from the alkaline-ethanol precipitate appear to be in the range of 10-25 kDa consistent with the expectation that the proteins in DG are mainly zeins. The  $\alpha$ -zeins have molecular weight between 20 and 24 kDa, and reduced  $\beta$ -zeins have bands around 17-18 kDa (Esen, 1986).

### Size Distribution of Proteins from Aqueous Enzyme Extract

Integration of the SEC chromatograms over size-range increments gave the protein size distribution shown in Figure 4.

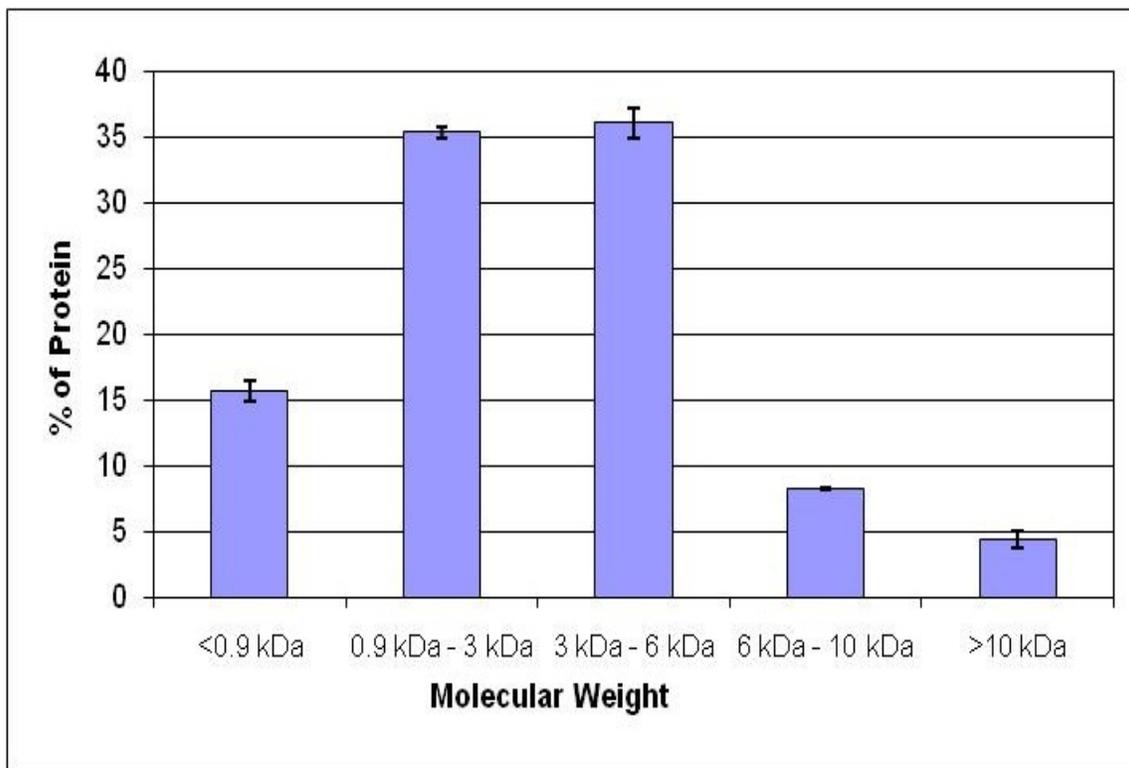


Figure 4. Protein molecular weight distribution from SEC of the aqueous enzyme extract. Results are averages of three replicates.

Over 95% of the protein was below 10 kDa. Most of the protein hydrolysates used in pet foods are 6-12 kDa and most allergenic proteins are typically 10-70 kDa (Ludlow, 2005). Since the vast majority of the proteins from the DG were below 10 kDa this product would meet these market specifications.

### Amino Acid Analysis

The amino acid contents of the precipitate from the alkaline-ethanol extract and from the enzyme extract were high in glutamic acid and leucine and low in lysine, as is the case for zeins (Lee *et al.*, 1976; Chui and Falco, 1995). The amino acid percentages of the precipitates are very similar to the reported amino acid

percentages for DG from the same source (Kim *et al.*, 2008). Figure 5 shows the similarity of the amino acid composition of the precipitates from the alkaline-ethanol extract and the enzyme extract with the results of others for DG and zein.

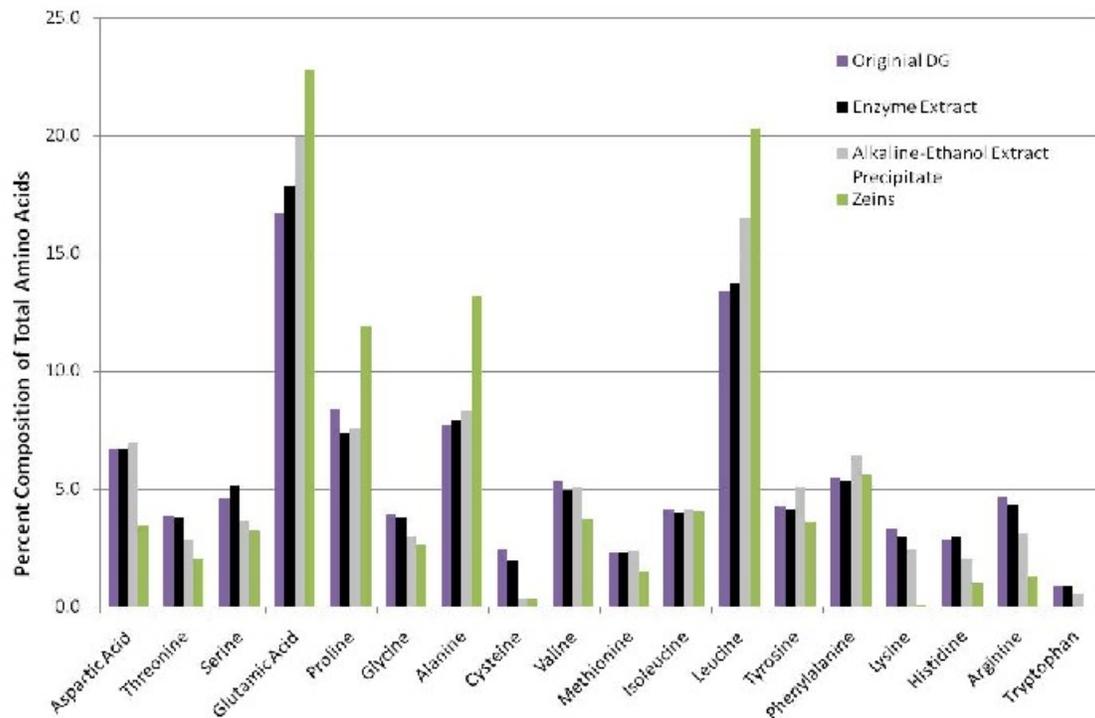


Figure 5. The amino acid composition, based on percent of total amino acids present, of the precipitate from the alkaline-ethanol and the enzyme extract. Original DG values from Kim *et al.* (2008)

The low lysine concentration would be of some concern for some feed applications. However, it has been reported that a concentration of 1.2% lysine is sufficient for male turkey growth (Lehmann, 1996).

## Conclusions

Two methods have been developed capable of successfully extracting proteins from DDDG, following oil recovery from DG. These methods are an

aqueous enzyme extraction and an alkaline-ethanol extraction. The latter required milling of the DDDG to reduce the particle size, while the aqueous enzyme extraction required no further processing of the DDDG. The alkaline-ethanol solvent likely extracted intact zeins while the aqueous enzyme extracted hydrolyzed proteins. Both processes provided concentrated protein byproducts that would have higher value than the DG if they prove applicable as coatings or fibers (the non-hydrolyzed protein) or as a replacement for soybean concentrate in turkey feed (the hydrolyzed protein). While this work does cover removal of the protein and some recovery/characterization of that protein, further development would require more complete examination of the carbohydrate losses from the residue and application evaluation of the recovered protein.

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## **Chapter 3 General Conclusion**

### ***General Discussion***

Two methods have been developed to successfully extract the proteins from distiller's grain. An aqueous enzyme extraction reduced the protein content in the solid phase for both the milled and unmilled DG from 38% (dry weight) to approximately 8% (dry weight). The alkaline-ethanol extraction was similarly successful for the milled material but not for the unmilled material. The aqueous enzyme treatment resulted in hydrolyzed proteins; whereas, the alkaline-ethanol extraction resulted in intact zein proteins. Both processes provided a concentrated protein byproduct that would have higher values than the DG

### ***Recommendations for Future Work***

After developing two techniques capable of successfully extracting the proteins from DG, a further analysis of the proteins should be conducted. Future research should focus on using the enzyme treated extract as a replacement for soybean concentrate. A feeding trial should be conducted using the enzyme treated extract as a food source for animals other than turkeys to see if any variation in the weight gain or the health of the animal is observed. The precipitate from the alkaline-ethanol extract should also be analyzed further. This would ensure the zeins could be used as films or fibers and have not been altered in any way during the ethanol production process. The final area that should be researched further is

to determine if enough protein has been extracted from the DG as to avoid poisoning of the catalysis used to convert the residual carbohydrates to ethanol.

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