PROTEIN ENHANCEMENT OF DDGS FROM CONVENTIONAL AND ENZYMATIC DRY GRIND PROCESSES

BY

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THESIS

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ABSTRACT

Conventional and enzymatic (E-Mill) dry grind processes were used to produce distillers dried grains with solubles (DDGS). In the conventional process, slurry used whole ground corn and fermented. In the E-Mill process, germ and pericarp fiber were recovered prior to fermentation. Protein concentrate, protein isolate and sieving processes were applied to increase DDGS protein and decrease fiber contents.

For conventional DDGS, protein contents of protein concentrate, protein isolate and sieved products were 33, 37 and 40%, respectively, compared to conventional DDGS (34%). The protein concentrate process yielded more protein (66%) compared to the protein isolate process (15%). Protein yield of sieving process (29%) was higher than the protein isolate process but lower than the protein concentrate process. For E-Mill DDGS, protein contents of protein concentrate, protein isolate and sieved products were 49, 52 and 51%, respectively. Compared to E-Mill DDGS (42%), the three processes increased E-Mill DDGS protein contents. The protein concentrate process yielded more protein (63%) compared to the protein isolate process (11%). Protein yield of sieving process (40%) was higher than the protein isolate process but lower than the protein concentrate process. Lower protein contents with conventional DDGS were due to higher oil and neutral detergent fiber (NDF) contents in conventional DDGS.

For the E-Mill process, NDF content of protein concentrate (3.8%) was higher than original DDGS (1.7%). However, NDF contents of protein isolate (1.3%) and sieved product

(1.8%) were not different from DDGS (1.7%). For the conventional process, NDF content of protein concentrate (27.1%) was higher than original DDGS (17.1%). NDF contents of protein isolate (11.6%) and sieved product (9.6%) were lower than DDGS.

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Chapter 1

Introduction

In the late 1970's, the U.S. ethanol industry was small and produced little ethanol. Increasing concerns about national energy security and the environment were major motivations for increased ethanol production. The U.S. government used to support and subsidy ethanol production on the basis that ethanol reduces dependence on imported oil and that it reduces greenhouse gas (GHG) emissions. The Clean Air Act Amendments (CAA) mandated blending oxygenates in gasoline in some states (EPA 2003). Blending gasoline with ethanol has become a popular method for gasoline producers to meet the new oxygen requirements. Presently, over 90% of all gasoline sold in the U.S. is blended with ethanol (RFA 2011). In 2011, ethanol production increased to 13.9 billion gallon from 1.63 billion gallon in 2000 (RFA 2012). The major feedstock for ethanol production in the U.S. is corn starch and the prevalent process for ethanol production is the dry grind process. By 2008, 86% of corn ethanol was produced using the dry grind process due to its lower capital cost and higher ethanol yield compared with the wet milling process, which is another ethanol production process (Mueller 2010).

In the conventional dry grind process, whole ground corn is mixed with water to form slurry. The slurry is liquefied in the presence of alpha-amylase, which breaks starch polymers into shorter chain molecules (dextrins). The liquefied slurry is cooled and transferred to a fermentor where simultaneous saccharification and fermentation (SSF) occurs with the

addition of yeast and glucoamylase. Glucoamylase breaks dextrins into mono or disaccharides such as glucose and maltose, while yeast converts these sugars into ethanol. Recently, a granular starch hydrolyzing (GSH) enzyme was developed to replace traditional alpha-amylase and glucoamylase. With GSH enzyme use, no liquefaction was needed. This modification resulted in ethanol concentrations and fermentation rates similar to the conventional process using traditional enzymes (Wang et al 2007) while simplifying the dry grind process and reducing energy consumption (Robertson et al 2006).

During the corn dry grind process, the starch component is converted into ethanol while residual components (protein, fiber, oil and ash) remain unfermented and are concentrated to form distillers dried grains with solubles (DDGS), a major coproduct of corn ethanol plants. Since starch accounts for approximately two thirds of the mass of corn, nonfermentable nutrients will be concentrated three times. With concentrated source of protein, amino acids and other nutrients, DDGS is used mainly as animal food, especially for ruminant animals due to relatively high fiber content. The DDGS supply has grown along with increased corn ethanol production. In 2011, 35.7 million metric tons of DDGS were produced, 80% consumed by the beef and dairy industry and 20% by the swine and poultry market (RFA 2012). The DDGS supply may affect its market price (Rausch and Belyea 2006). Variation of major nutrients in DDGS has reduced DDGS quality as animal food and thus limited its application (Liu 2011). Improvement of DDGS quality is critical to increasing DDGS value and corn ethanol plant profitability.

Fractionation can improve the dry grind process. Pericarp fiber and germ can be recovered before fermentation so DDGS composition is improved (higher protein and lower fiber contents). Recovered germ and fiber are valuable coproducts. Corn germ can be

extracted to obtain corn oil (Singh and Eckhoff 1997); corn fiber oil has been shown to reduce blood cholesterol level (Moreau et al 1996). Also, removal of germ and fiber prior to fermentation increases fermentation capacity and final ethanol concentrations (Singh et al 2005). Corn fractionation can be classified into wet and dry fractionation. Wet fractionation combines wet milling unit operations prior to the dry grind process while dry fractionation incorporates dry milling unit operations. For wet fractionation, modified processes such as quick germ (QG), quick germ quick fiber (QGQF) and enzymatic (E-Mill) processes have been developed (Singh et al 2005). Compared to the conventional dry grind process, higher protein and lower fiber contents in DDGS have been reported (Singh et al 2005).

Protein is an important nutrient and indicator of DDGS quality as an animal food. Increasing DDGS protein is a rational way to improve DDGS quality. Soybeans have a long history as a dietary protein source. A variety of soy protein products have been utilized, soy protein concentrate (SPC) and soy protein isolate (SPI) being two important products. SPC has a protein content of at least 65% on a dry basis (Lusas and Rhee 1995). SPI is the most refined form of soy protein with at least 90% protein content (Deak at al 2008). Typically, animal food market prices have an approximate linear correlation to protein contents of feed ingredients (Srinivasan et al 2006). The low digestibility of fiber in DDGS may result in increased quantities of manure from nonruminant animals (Stein and Shurson 2009). Reducing fiber content may increase the market share of DDGS as a food ingredient for nonruminant animals.

Finding methods to further increase protein content and decrease fiber content of DDGS is critical for increasing DDGS price and expanding DDGS market share for nonruminant animal food ingredients. The objectives were to:

1. Investigate and compare the effects of soy protein concentrate and isolate processes on increasing conventional and E-Mill DDGS protein contents.
2. Compare protein concentrate and isolate processes with a sieving process on DDGS protein content.

Chapter 2

Literature Review

2. 1 The Dry Grind Processes and its Development for Ethanol Production

The dry grind and wet milling processes are two main processes that convert starch into ethanol. By 2008, 86% of corn ethanol was produced using the dry grind process due to lower capital cost (Mueller 2010). The typical dry grind process includes grinding, cooking, liquefaction, saccharification, fermentation, distillation and recovery of coproducts. In the dry grind process, whole corn kernels are ground by hammer mill to reduce particle size and cooked at temperatures exceeding the starch gelatinization point of up to 120°C. The cooked corn is mixed with alpha-amylase, an enzyme that converts starch to dextrins, to form mash, and liquefied at 85°C. Mash is saccharified and fermented with the addition of glucoamylase, an enzyme that releases fermentable sugars, and yeast (*Saccharomyces cerevisiae*). This process is referred to as simultaneous saccharification and fermentation (SSF). After 48 to 72 hr fermentation, the resulting beer stream is distilled to produce 190 proof (95% v/v) ethanol, which is dewatered using molecular sieves to produce 200 proof (100% v/v) ethanol. The remaining stream after distillation is called whole stillage, which is separated using centrifuges to form thin stillage and wet grains. Thin stillage goes through evaporators to remove water and become condensed distillers solubles (syrup). Syrup and wet grains are mixed and dried to yield distillers dried grains with solubles (DDGS), which is typically the only coproduct from a dry grind ethanol plant.

In the dry grind process, nonfermentable fractions interact with enzymes, which reduce enzymatic activity and thus ethanol yield. Also, removing these fractions can decrease the amount of DDGS and diversify the market value of coproducts. Recently, new process modifications have been developed to address these issues. Wet fractionation and dry fractionation are two main developments. In wet fractionation, the quick germ (QG) process was developed to recover germ using corn wet milling degermination method (Singh and Eckhoff 1996) and quick germ quick fiber process (QGQF) to remove germ and pericarp fiber before fermentation (Singh et al 1999). In addition, an improved process called the enzymatic dry grind (E-Mill) process was developed including treatment of slurry with starch degrading enzymes and proteases. E-Mill process begins with soaking corn kernels for 12 hr, followed by coarse grinding in a blender so pericarp and germ can be released undamaged. Ground corn slurry is incubated with starch hydrolyzing enzymes and protease for 2 hr. Due to the increase in slurry specific gravity, germ and pericarp fiber from the slurry can be removed by gravity separation. The remaining slurry is subjected to grinding, SSF and screening (0.15 mm opening) to separate endosperm fiber (Wang et al 2005). Besides recovering germ and pericarp fiber as coproducts, the E-Mill process increases fermentation rates and ethanol yields compared to the conventional dry grind process, and other nonenzymatic wet fractionation such as QG and QGQF. The E-Mill process was reported to increase ethanol concentrations by 27%, reduce DDGS fiber content from 11 to 2% and increase protein content from 28 to 58% (Singh et al 2005).

Dry fractionation is based on corn dry milling. This method, which also is called dry degerm defiber (3D) process, uses particle size separation (screen) and air to separate different

corn fractions without soaking corn in water. In the 3D process, corn is tempered to increase the moisture to more than 20%, which prevents the germ from breaking under mechanical pressure when the tempered corn passes through a degerminator mill. Degermed corn is passed through a roller mill and a series of screening operations so that endosperm and germ fractions can be obtained. Fiber can be separated from both germ and endosperm fractions by aspiration. Endosperm fractions are processed by the conventional dry grind process to produce ethanol. Dry fractionation has been applied at a commercial scale to produce ethanol due to diversified coprodcts and lower costs compared to the E-Mill process. However, dry fractionation leads to lower fermentation rates compared to the conventional dry grind process and the E-Mill process, probably due to the loss of germ derived nutrients beneficial for yeast growth (Murthy et al 2006a). Supplementation with germ soak water and B vitamins (Murthy et al 2006a) and lipid supplementation (Murthy et al 2006b) can improve fermentation rates and glucose utilization, leading to higher final ethanol yields. Germ quality is poor because separation with endosperm is incomplete and soluble components from the germ fraction are not removed (Johnston et al 2005).

2.2 Utilization and marketing of DDGS

In 2010, 4.65 billion bushels of corn were utilized as a feedstock to produce 13 billion gallons of fuel ethanol and 32.5 million metric tons of coproducts in more than 200 ethanol plants across the U.S. (RFA 2011). Of the total production of grain based fuel ethanol, 85% was produced in dry grind plants; the rest was from wet mill refineries (RFA 2007). The Renewable Fuel Standard (RFS) mandated that 36 billion gallons of renewable fuel should be produced per year for transportation fuel by 2022, of which 15 billion gallons could be from corn starch

ethanol (USDA 2010). In 2011, more than 90% of gasoline sold in the U.S. was blended with ethanol, which means ethanol demand averaged 860,000 barrels per day in 2010, for a total demand of 13.1 billion gallons (RFA 2011). Driven by demand mandated by the RFS, economic value of ethanol as a blend in gasoline, and increasing awareness of limiting greenhouse gas (GHG) emissions, the construction of ethanol plants, especially dry grind ethanol plants, was rapid from 2000 to 2008. DDGS is the major coproduct from dry grind plants, so the growing market in dry grind ethanol industry increased the total amount of DDGS, which may affect the price of DDGS as animal food. Today, DDGS is used mainly in animal diets due to its high energy and protein contents but is limited primarily to ruminant diets because of high fiber content. It was estimated 80% of DDGS was consumed by dairy and beef industry, 10% by swine and 9% by poultry in 2010 (RFA 2011). Another factor that affects utilization and price of DDGS is its variation in nutritional elements. There is notable variation in protein, fiber, oil and minerals content in DDGS from difference sources (Belyea et al 2010; Liu 2011; Spiehs et al 2002). Possible reasons are the effects of raw material, processing methods, yeast performance and analytical methodology (Liu 2011). Further utilization and market diversification of DDGS is necessary for increasing the value of DDGS and thus crucial for profitable operation of dry grind ethanol plants.

Traditionally, DDGS is used as an ingredient in animal diets. A variety of investigators have focused on utilizing DDGS for beef and dairy cattle (Kelzer et al 2010; Mjoun et al 2010a; Nuez-Ortín and Yu 2010), poultry (Lumpkins et al 2004; Świątkiewicz and Koreleski 2008) and swine (Stein and Shurson 2009; Whitney and Shurson 2004; Whitney et al 2006). Additionally,

inclusion of DDGS in aquaculture and pet food is a potentially profitable market (Rosentrater 2007).

One possible improvement is to increase DDGS protein content. Liu (2009) used a combination of sieving and winnowing to enrich protein concentration of DDGS. Wind winnowing is an agricultural method developed by ancient cultures for separating grain from chaff. Protein content of DDGS was increased from 30.66% to 49.12% with the sequence of sieving and winnowing for one DDGS sample. Srinivasan et al (2007, 2008) combined sieving and elutriation (elusieve process) to remove fiber from DDGS and used it for ethanol production. Original DDGS had NDF of 36.7% (db) while elusieve fiber would have NDF of 57.3% (db). The ethanol production rate from elusieved fiber was similar to that from the quick fiber (QF) process. The elusieve process is a relatively simple process and requires low capital investment, so this process can benefit dry grind ethanol plants without changing the existing dry grind process equipment (Srinivasan et al 2006). DDGS typically contains 40% neutral detergent fiber (Liu 2011). There is no lignin in corn fiber; therefore, it is a good feedstock for producing cellulosic ethanol (Dien et al 2005). Corn fiber oil has been shown to lower plasma cholesterol, which makes it a healthy human food ingredient (Ramjiganesh et al 2002). Further work was done to increase protein content by converting residual starch and fiber into ethanol. Tucker et al (2004) pretreated distillers grain with 3.27% H_2SO_4 at 140°C for 20 min; this yielded 77% of available carbohydrates, which was used for fermentation for additional production of ethanol. The residual hydrolyzed distillers grain was high in protein level (58 to 61%) and fed to turkeys, nonruminant animals, since fiber was converted to ethanol and protein was concentrated during pretreatment and fermentation.

Ammonia fiber expansion (AFEX) pretreatment has been employed to release fermentable sugar in DDGS, so additional ethanol production could be achieved. Typically, 8 to 12% glucose was recovered from DDGS, which was a low yield. Protease, alkaline solution and biobased solvent (ethyl lactate, d-limonene and distilled methyl esters) were tested to extract protein either before or after pretreatment. Only 20% protein was recovered from DDGS (Bals et al 2009; Brehmer et al 2008; Datta et al 2010). Even though the yield was low, they developed a new option for producing high valued product simultaneously with additional ethanol in an integrated method. Improved process engineering and operating conditions are needed to make the process more economically viable.

2.3 Protein extraction from corn, soybeans and their coproducts

In the corn kernel, protein mainly exists in germ and endosperm. Major proteins in corn germ are albumins and globulins while prolamins and glutelins are located primarily in endosperm (Lawton and Wilson 2003). Protein in corn is classified based on solubility. Albumins dissolve in water while globulins are soluble in saline, which is a dilute salt solution; prolamins are soluble in 70% ethanol and glutelins dissolve in dilute acid or alkali solution (Osborne 1924).

Corn germ, which makes up 11% of total weight of dent corn, contains 18 to 22% (dry basis, db) crude protein, which is equal to 29% of total kernel protein (Lawton and Wilson 2003). Nielsen et al (1973) reported that dry milled corn germ was used to produce protein isolate, which has similar amino acid composition to defatted germ meal. Protein also was extracted

and recovered from wet milled corn germ by using saline as the extracting solvent (Hojilla-Evangelista 2011) because the main proteins in corn germ are albumins and globulins.

Zein, which mainly consists of prolamins, is found only in corn endosperm. Zein composed 60% of endosperm proteins and 52% of total kernel proteins (Lawton and Wilson 2003). Zein is a family of similar proteins; α-zein, the major zein protein, makes up about 80% of total zein (Esen 1987).

Corn gluten meal (CGM) is used for commercial zein extraction because CGM protein contents are 61.5 to 74% (db) with 60 to 71% zein proteins (Wu et al 1997b). Early method used 95% aqueous ethanol to extract zein from CGM, but this method was never commercialized as the method was batch-oriented and 95% aqueous ethanol is not the best solvent for commercial extraction of zein. The extraction process developed by Carter and Reck (1970) is the common commercial method. They extracted zein from CGM using 88% (w/w) aqueous 2-propanol with 0.25% NaOH for 1 hr at 1:4 solution to solvent ratio and 55 to 65°C. Extracted zein was freeze dried after double precipitation. Zein yield was 22%. Recently, Anderson and Lamsal (2011) modified α-zein extraction procedure from corn gluten meal using different solvents. The modification typically increased alcohol concentration in the solvent and separated non α-zein by centrifuge. Among five organic solvent mixtures, 70% 2-propanol, 55% 2-propanol and 70% ethanol gave the best results, which showed yields of 35% zein from CGM with protein recovery of 44%. Protein contents of zein were from 87% to 91% using the three solvents.

DDGS can be used as a source of zein since corn protein is concentrated during the formation of DDGS. Early extraction methods typically used ethanol or propanol coupled with reducing agents. Wolf and Lawton (1997) showed 3.2 to 6.6% of crude zein was recovered by using 60% ethanol at 60°C with 0.1% dithiothreitol (DTT). Although yields were low, they showed zein extraction from DDGS was possible.

Xu et al (2007) extracted zein from DDGS under acidic conditions after recovering oil. Zein was extracted with 70% (w/w) ethanol in the presence of sodium sulfite. The optimized condition can obtain high quality zein with 90% protein and a yield of 44% of DDGS protein. However, nonprotein compounds may be extracted although SDS-PAGE showed zein extracted had similar protein profile to commercial zein.

Paraman and Lamsal (2011) extracted α-zein from DDGS using 70% (v/v) aqueous ethanol containing 0.5% sodium hydroxide and 1% sodium bisulfite. Centrifugation was employed to enrich the α-zein fraction. The yield of crude α-zein was 5.9 to 7.3% with 71.5 to 81.2% protein content. Compared with zein extracted from corn gluten meal, α-zein from DDGS showed lower yield and protein content, but held functional properties such as alcohol solubility and film forming.

Further work on zein extraction from DDGS was done by Anderson et al (In review). The modified method recovered α-zein using 70% (w/w) aqueous 2-propanol with 14% yield. Cellulase pretreatment had no effect on improving α-zein yield but led to clear and smooth films of α-zein.

Soy protein is an important plant protein source due to low cost compared to animal protein, excellent nutritional quality and multiple functions in food system. Soy proteins were classified into three groups, namely 2S, 7S, and 11S protein by their sedimentation coefficients using ultracentrifugation (Deak et al 2008). β-conglycinin is a storage protein, known as 7S globulin. It accounts for 85% of the 7S protein fraction (Deak et al 2008). Glycinin is another storage protein, comprising most of the 11S protein fraction (Deak et al 2008). Glycinin and β-conglycinin comprise 65 to 80% of protein in soy proteins and account for protein recovered in soy protein products.

Major soy protein products include defatted soybean meal, soy flours and grits, soy protein concentrate and soy protein isolate. Soybean meals are material resulting from extracting oil from soybean flakes. Soybean meal contains protein of 44% with hulls and 46.5% to 50% without hulls (Lusas and Rhee 1995). Soy flours and grits are produced by grinding and sieving defatted flakes and contain 52 to 54% protein (Lusas and Rhee 1995). Soy protein concentrate (SPC) and soy protein isolate (SPI) are two important soy protein products. A soy protein product is categorized as SPC with at least 65% protein content on dry basis (Deak et al 2008). Soluble solid is separated so protein is concentrated. SPI contains minimum 90% protein on dry basis (Deak et al 2008). Fiber was removed before soluble solid was removed in the final step. The conventional method uses defatted soy flakes as starting material. Wang et al (2004) also used extruded expelled soybean meal to produce SPC and SPI, which showed lower yield but similar functional properties (mulsification capacity and dispersibility).

For the production of SPC, dilute acid precipitation and aqueous ethanol leaching are two preparation methods (Lusas and Rhee 1995). For acid precipitation, ratios of water to soy flour were 10 to 20:1. The material was adjusted to pH 4.5 using 2N HCl and incubated at 40°C for 30 min followed by centrifuge at 14,000 × g at a temperature of 15°C for 30 min. For aqueous ethanol leaching, 20 to 80% aqueous ethanol was mixed with soy flour at a ratio of 10:1 and incubated at 40°C for 40 min. After centrifugation, the pellet was desolventized overnight in fume hood at 40°C and vacuum dried at 50°C for 8 hr. Soy protein concentrate contains at least 65% but less than 90% protein; protein concentrate yield is about 75% of defatted flour (Lusas and Rhee 1995).

For the production of SPI, defatted soy flour was extracted with water at a 10:1 water-to-flour ratio. The pH was adjusted to 8.5 with 2 N NaOH; the resulting slurry was stirred for 30 min at 60°C. After centrifuging at 14,000 × g for 30 min, the protein extract was adjusted to pH 4.5 with 2 N HCl and cooled to 4°C, followed by centrifugation at 14,000 × g for 30 min. The isolated protein curd was redissolved in deionized water, with sufficient 2 N NaOH added to achieve pH 7.0 and freeze dried. Four extraction temperatures (25, 40, 60 and 80°C) were used to produce SPI but protein yield and protein content were not affected by temperatures tested except for 80°C at which SPI yield was decreased (Deak and Johnson 2007). Higher extraction temperature increased water solubility and emulsification capacity; made better surface hydrophobicities, emulsification activities and stabilities, dynamic viscosities and improved foaming properties.

Zhang and Liu (2005) prepared SPC directly from full fat soy flour with 90% aqueous ethanol with hexane. The ratio of hexane to 90% ethanol was 9:1 (v/v), and SPC protein content was 64%, which is comparable to standard SPC (65%). This procedure can recover protein and oil simultaneously, so the volume of recovery solvent was reduced.

Enzyme assisted aqueous extraction processing (EAEP) is considered to be an environmentally friendly process in which oil and protein can be extracted simultaneously (Rosenthal et al 2001). De Moura et al (2011) used countercurrent two stage EAEP to extract protein from full fat extruded soybean flakes. Countercurrent two stage EAEP was set at a 1:6 solids to liquid ratio, 50°C, pH 9.0 and 120 rpm for 1 hr. Three enzyme usage strategies were conducted: protease in both extraction stages; protease only in the second extraction stage; no enzyme in either stage. The three strategies led to protein yields of 96, 89 and 66%, respectively. The conventional process typically recovers 60 to 70% of protein in soy flake in industry for both SPC and SPI (Alibhai et al 2006).

In recent years, new technologies such as ultrasound, isoelectric precipitation and nanofiltration have been applied to extraction of soy protein (Alibhai et al 2006; De Moura et al 2011; De Moura et al 2011; Karki et al 2010). These technologies increased protein yield and improved protein functional characteristics.

Chapter 3

Increasing DDGS Protein Content Using Protein Concentrate and Isolate Processes

3.1 Introduction

DDGS is a major coproduct in corn dry grind ethanol plants. Increasing protein content and decreasing fiber content of DDGS can improve quality and thus benefit ethanol plants. In this project, protein concentrate, protein isolate and sieving processes were used with conventional and E-Mill DDGS as process input. The objectives were to evaluate and compare effects of protein concentrate, isolate and sieving processes on effecting DDGS protein, fiber and oil contents.

3.2 Materials and Methods

3.2.1 Materials

Yellow dent corn hybrid (P1184XR) harvested in 2010 was obtained from DuPont Pioneer (Champaign, IL). Each corn sample was cleaned by sieving over a 12/64'' (4.8 mm) round hole sieve to remove broken corn and foreign material (BCFM). Cleaned corn was stored at 4°C. Urea (99.6% ACS grade) was obtained from Fisher Scientific (Fair Lawn, NJ). Active dry yeast (Ethanol Red, Fermentis, Lesaffre Yeast Corporation, Milwaukee, WI) was used for fermentations. Sulfuric acid (5.0 N, ACS reagent grade), hydrochloric acid (2.0 N, ACS reagent grade) and sodium hydroxide (2.0 N, ACS reagent grade) used for pH adjustments were obtained from Fisher Scientific (Fair Lawn, NJ).

Enzymes used in the conventional and enzymatic (E-Mill) dry grind processes were granular starch hydrolyzing (GSH) enzyme (STARGEN 002) and protease (GC 212), obtained from Genencor International (Palo Alto, CA). STARGEN 002, derived from genetically modified strains of *Trichoderma reesei*, was an optimized blend of amylases to hydrolyze granular starch. GC 212 was obtained by fermentation of a selected strain of *Aspergillus niger* and was able to hydrolyze peptide bonds along a protein chain at low pH. Protease activity was 2,000 SAPU/g (SAPU = spectrophotometer acid protease units).

3.2.2 Methods

The E-Mill Process

Corn was processed according to the procedure developed by Singh et al (2005) (Figure 1). Kernel moisture content was measured by drying at 103°C for 72 hr in a convection oven (Method 44-15, AACC International 2000). Cleaned corn (1000 g) was soaked with 2 L tap water for 12 hr at 55°C. Soaked corn was ground for 8 min in a Waring blender attached to a variable speed controller and a tachometer for speed measurement at 3500 rpm. Slurry was adjusted with sulfuric acid to pH 4.2 and incubated for 3 hr in a water bath maintained at 48°C with 2.8 mL granular starch hydrolyzing enzyme and 0.7 mL protease. Skimming was applied to recover germ and pericarp fiber, which were dried and separated by aspiration (6DT4, Kice Metal Products, Wichita, KS). Mash was ground using a disk mill (Model 4-E, Quaker City Mill, Philadelphia, PA) with water to reach 25% solids mash. Prior to fermentation, mash was adjusted to pH 4.0 using sulfuric acid.

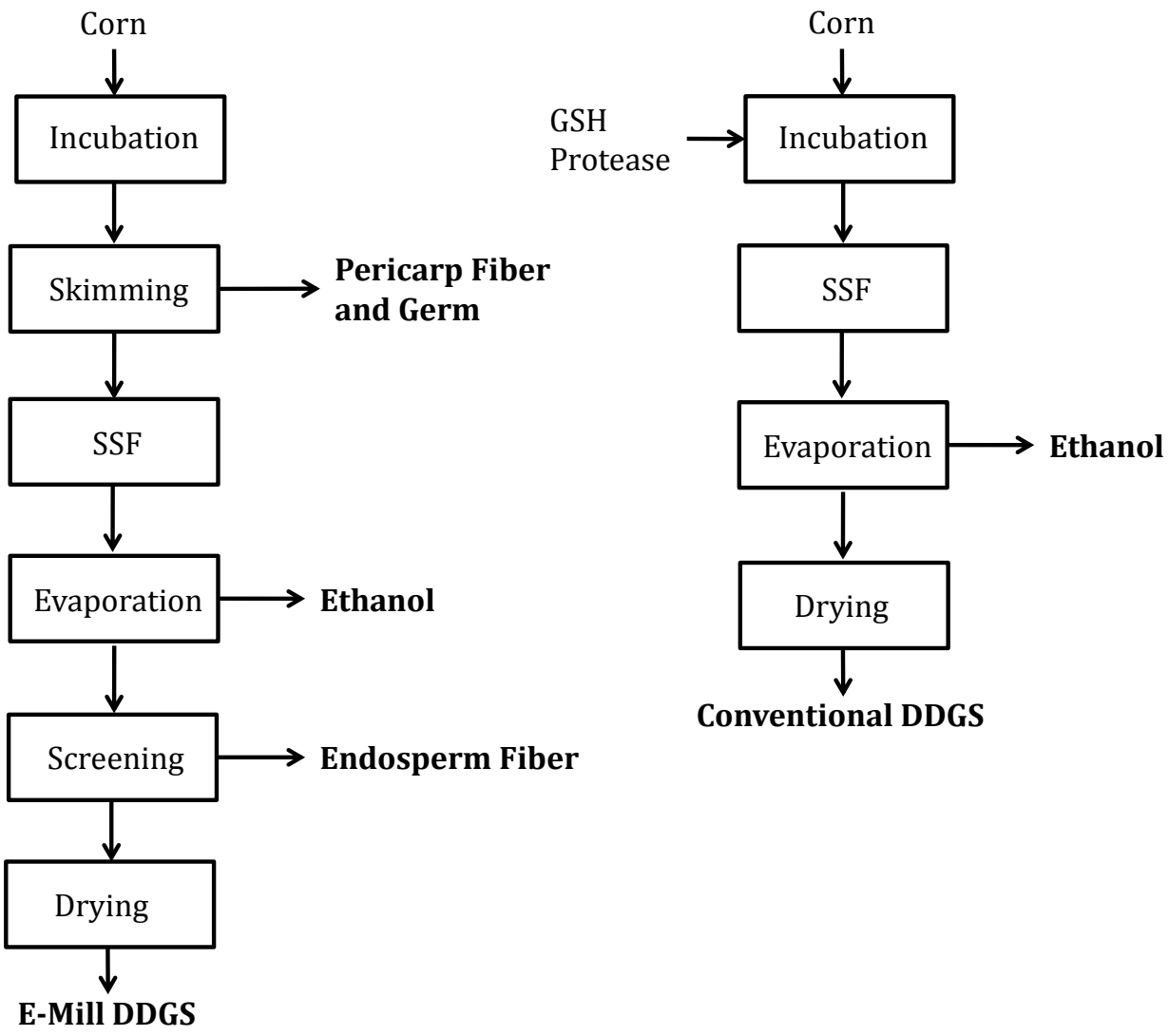

Figure 1. Laboratory enzymatic dry grind (E-Mill) process and conventional dry grind process.

The Conventional Dry Grind Process

Cleaned corn was ground at 500 rpm in a laboratory hammer mill (1100 W, model MHM4, Glen Mills Inc., Clifton, NJ) equipped with a 0.5 mm sieve (Figure 1). A two stage oven method (Method 44-19, AACC International 2000) was used to determine ground corn moisture content. Ground corn (1000 g, as is) was mixed with tap water to prepare 25% (db) solid

content slurry. Slurry was adjusted with 5.0 N sulfuric acid to pH 4.2 and incubated for 3 hr in a water bath maintained at 48°C with 5.6 mL STARGEN 002 (Genencor International, Palo Alto, CA) and 1.05 mL GC 212 (Genencor International, Palo Alto, CA). The conventional process used α-amylase and glucoamylase. Applying granular starch hydrolyzing enzyme and protease replaced liquefaction. The process is called conventional process is to differentiate it from the E-Mill process. Prior to fermentation, mash was adjusted to pH 4.0 using 5.0 N sulfuric acid.

Fermentation

Yeast culture was prepared by dispersing 5 g yeast in 25 mL distilled water at 30°C for 20 min in a 100 ml flask with shaking. Urea (45% w/v) was added to fermenter as a nitrogen source for the yeast. For the E-Mill process, 2.8 mL Stargen 002 and 0.35 mL GC 212 were added. Fermentation was maintained at 30°C for 72 hr with agitation at 50 rpm controlled by overhead drives (model DHOD-182, Bellco Glass, Vineland, NJ) in 3 L flasks. Fermentation samples (5 mL) were collected at the beginning and end of fermentation for the E-Mill process and only at the end for conventional process. After 72 hr fermentation, beer was heated to 90°C for 2 hr to evaporate ethanol. To produce DDGS, stillage left after boiling was poured into 2 L flat bottom, open aluminum pans and dried in a convective oven for 24 hr at 49°C. For the E-Mill process, endosperm fiber was recovered using 100 mesh sieve before drying the mash to produce DDGS.

Protein Concentrate Process

The protein concentrate process with DDGS (Figure 2) was conducted using modified protocol described by Lusas and Rhee (1995). Conventional and E-Mill DDGS were ground using

a blender (6694B, Sunbeam Products, Inc., Boca Raton, FL). Ground DDGS (20 g) was mixed with water to 10% (w/v) solids mash, adjusted to pH 4.5 using NaOH (2.0 N) and incubated at 60°C for 60 min using a Labomat reactor (BFA 12, Werner Mathis AG, Concord, NC) equipped with a digitally controlled infrared heating system with a temperature range of 20 to 200°C. During the incubation, protein was extracted. Mash was centrifuged at 15°C for 30 min at 14000 × *g* (RC5C Sorvall Instruments, Thermo Fisher Scientific, Asheville, NC). Supernatant contained soluble solids and ash. The pellet was called DDGS protein concentrate. Supernatant and protein concentrate were dried in a convective oven for 24 hr at 49°C. Sample moisture content was determined using a two stage oven method (Method 44-18, AACC International 2000).

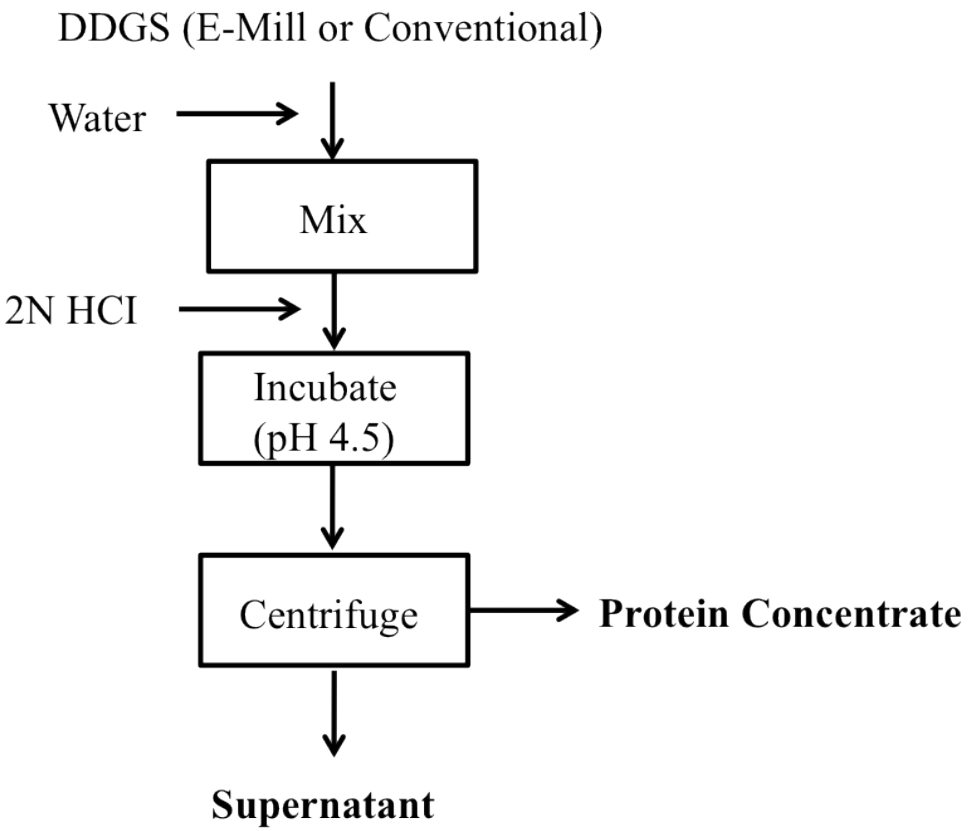

Figure 2. Protein concentrate process.

Protein Isolate Process

The protein isolate process (Figure 3) was conducted using modified protocol described by Lusas and Rhee (1995). Ground DDGS (20 g) was mixed with water to 10% (w/v) solids mash. Mash was adjusted to pH 8.5 using 2N NaOH to solubilize proteins in DDGS and incubated in a Labomat reactor (BFA 12, Werner Mathis AG, Concord, NC) at 60°C for 60 min. During the incubation, protein was solubilized. Mash was centrifuged at a temperature of 15°C for 30 min at 14000 × *g* (RC5C Sorvall Instruments, Thermo Fisher Scientific, Asheville, NC). Supernatant pH was adjusted to 4.5 using 2N HCl. The sample was held at 20°C for 2 min to precipitate the protein and refrigerated at 4°C for 1 hr and centrifuged a second time at 15°C for 30 min at 14000 × *g* (RC5C Sorvall Instruments, Thermo Fisher Scientific, Asheville, NC). All products were dried in a convective oven for 24 hr at 49°C. Sample moisture content was determined using a two stage convection oven method (Method 44-18, AACC International 2000).

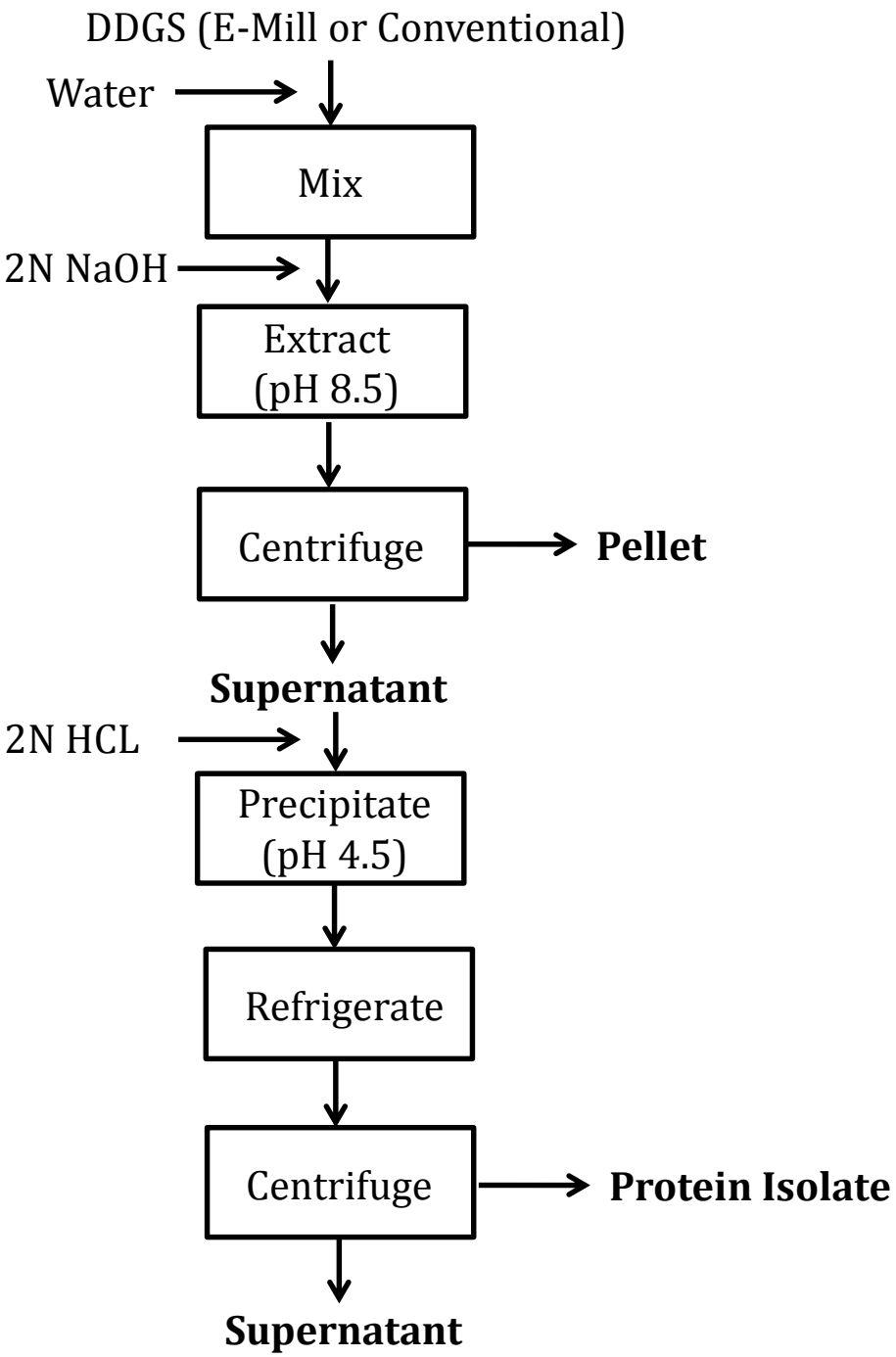

Figure 3. Protein isolate process.

Sieving Process

DDGS (20 g) was ground using a blender (6694B, Sunbeam Products, Inc., Boca Raton, FL) and mixed with water at a ratio of 1:5. Slurry was incubated using a Labomat reactor (BFA 12, Werner Mathis AG, Concord, NC) at 60°C for 60 min. A disk mill (Model 4-E, Quaker City Mill, Philadelphia, PA) was used to grind DDGS slurry and screened through 325 mesh (0.044 mm) sieve attached to a shaker. The material remaining on the 325 mesh sieve was called the insoluble solid fraction. The slurry that went through the sieve was centrifuged at 14,000 ×*g* at 15°C for 30 min. The pellet was called enhanced DDGS (Figure 4). Sample moisture contents were determined using a two stage oven method (Method 44-18, AACC International 2000).

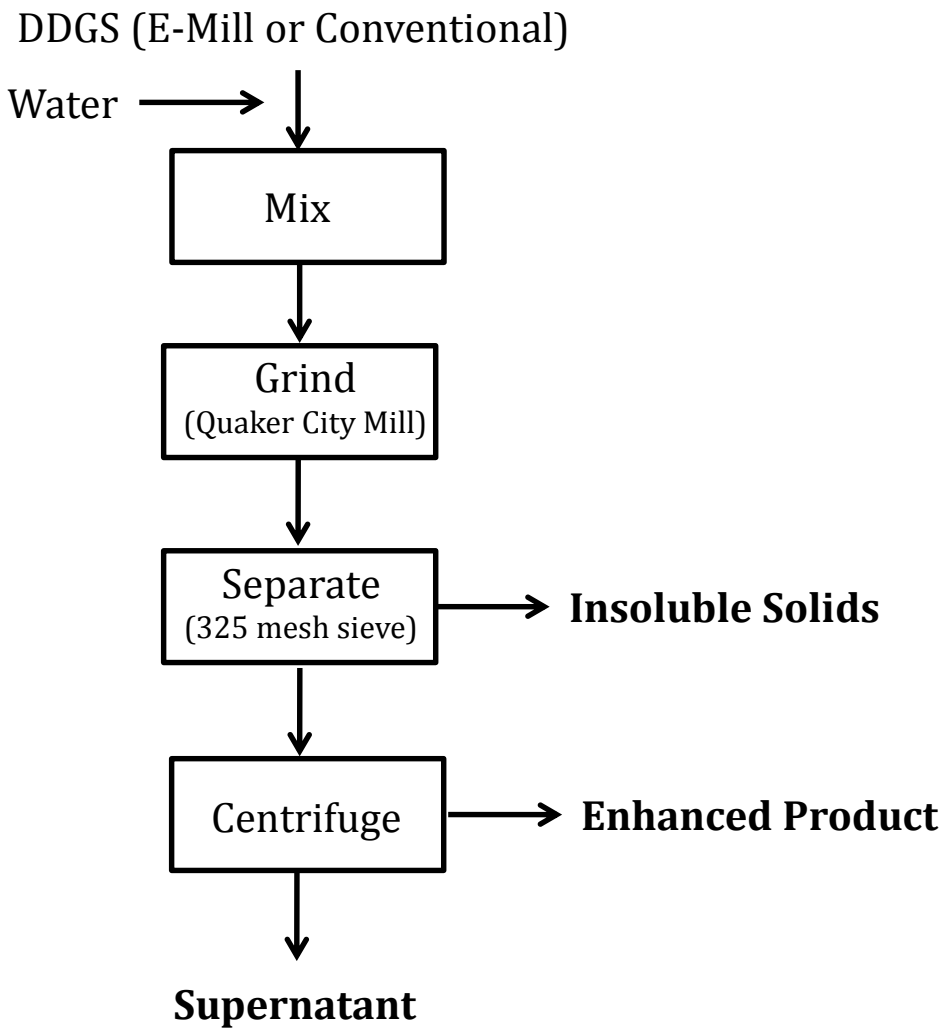

Figure 4. Sieving process.

Data Analysis

Each process was conducted in triplicate. DDGS Composition (oil, protein, NDF) was determined by the Illinois Crop Improvement Association (Champaign, IL). Oil content was determined using ether extraction (Approved Method 30-25.01, AACC International 2010), protein content was determined using the combustion (Dumas) method (Method Ba 4e-93, AOCS 2009) and NDF content was determined using method developed by NFTA (NFTA 5.1

1993). Protein content was defined as percentage of protein contained in a product. Protein yield was defined as the ratio of protein in final product to protein in starting material. Yields of protein, oil and NDF for product and coproduct in each process were determined based on material balance. Conventional and E-Mill DDGS protein, oil and NDF contents also were determined. The experiment was arranged as a complete randomized design. Analysis of variance (ANOVA) and Fisher least significant difference (LSD) test (R version 2.14.1) were used to compare means of final protein contents and yields. The level selected to show statistical significance was 5% ($P<0.05$).

3.3 Results and Discussion

3.3.1 Comparisons of E-Mill and conventional DDGS

Protein contents of DDGS from the E-Mill and the conventional processes were 42 and 34%, respectively (Table 1). The increased concentration of protein in E-Mill DDGS was a result of recovering fiber and germ before fermentation.

DDGS oil content was 1 and 12% for the E-Mill process and conventional process, respectively, since germ was recovered before fermentation in the E-Mill process. With the E-Mill process, NDF content was reduced to 2% compared with the conventional dry grind process (17%) due to fiber recovery. Germ and pericarp fiber are additional coproducts from the E-Mill process.

Higher protein and lower fiber contents can expand DDGS markets since E-Mill DDGS could be included at higher inclusion levels in nonruminant animal diets. With increased ethanol production, DDGS supply has grown as a result, which may limit market value of

conventional DDGS as ruminant animal food (MacDonald et al 2003). Thus, E-Mill DDGS is important for DDGS market diversification and ethanol plants.

Table 1. Protein, oil, and neutral detergent fiber content of DDGS from conventional and E-Mill dry grind processes (means of three observations). [a]

	Protein (% db)	Oil (% db)	Neutral Detergent Fiber (% db)
E-Mill Process	41.96±0.78a	0.72±0.09b	1.69±0.05b
Conventional Process	34.3±0.17b	12.68±0.10a	17.07±0.51a

[a] Means followed by the same letter in a column were not different (P<0.05).

3.3.2 Effects of processes on DDGS protein concentration

The protein concentrate, protein isolate and sieving processes increased E-Mill DDGS protein contents (Table 2). Compared to E-Mill DDGS (42%), protein contents of protein concentrate, protein isolate and sieved products were 48, 52 and 51%, respectively. Differences existed among three processes in influencing DDGS protein content and yields. Protein content of protein isolate was higher than that of protein concentrate. However, the protein yield of protein concentrate from conventional DDGS was 63% compared to 11% yield for protein isolate obtained from the same source.

In the protein isolate process, most insoluble solids were removed during the first centrifuge step while most soluble solids were removed in the second centrifugation step, resulting in higher protein content in final protein isolate. In contrast, for the protein concentrate process, only soluble solids were removed so protein contents of final protein concentrates were lower due to presence of fiber. Typically, animal food price has a linear

correlation to protein content of food ingredient (Srinivasan et al 2006). Thus, higher protein content means higher value of DDGS. Sieving process also was effective in increasing DDGS protein content (Table 2). Protein content of final product from sieving process (51.2%) was similar to the protein isolate process (51.8%) and higher than protein concentrate process (48.7%). The difference between sieving process and the protein concentrate process with respect of protein content was due to fiber removal by sieving process. No difference was observed between sieving process and the protein isolate process in increasing DDGS protein content. Protein yield from sieving process (40%) was higher than the protein isolate process (11%) but lower than the protein concentrate process (63%). Apparently, grinding and sieving recovered more protein than centrifugation.

Table 2. Effect of processes on protein content and yield with E-Mill DDGS as starting material (means of three observations). [a]

	Protein Content	Yield (%, db)	
	(%, db)	Protein	Product
E-Mill DDGS	41.96±0.78c		
Protein Concentrate	48.74±0.3b	63.01±0.84a	54.24±0.59a
Protein Isolate	51.86±0.57a	10.96±1.54c	8.86±1.16c
Sieving	51.23±0.27a	40.23±5.58b	32.95±4.56b

[a] Means followed by the same letter in a column were not different ($P<0.05$).

The general trend of three processes on conventional DDGS was similar to that on E-Mill DDGS (Table 3). Protein contents of protein concentrate, protein isolate and sieved products were 33, 37 and 40%, respectively. Protein content of conventional DDGS was 34%.

Compared to results from E-Mill DDGS, protein contents of final products from conventional DDGS were lower. For the protein isolate process, protein content of protein isolate was 2.7% higher than starting DDGS. However, for the protein concentrate process, no difference was detected in protein content. The protein concentrate process yielded 66% of the original protein in conventional DDGS and a 15% protein yield was observed in the final product by the protein isolate process. These were similar to results from E-Mill DDGS. Furthermore, compared to protein yield from E-Mill DDGS (11% for protein concentrate and 63% for protein isolate), higher protein yield was observed from conventional DDGS for both protein concentrate and isolate processes. The sieving process resulted in 40% protein content in the final product; protein content of sieved product was 7.2 and 3.0% higher than protein concentrate and protein isolate, respectively. Similar to results from E-Mill DDGS, protein yield of sieved product (29%) was between protein concentrate and protein isolate.

Table 3. Effect of processes on protein content and yield with conventional DDGS as starting material (means of three observations)[a].

	Protein Content	Yield (%, db)	
	(%, db)	Protein	Product
Conventional DDGS	34.32±0.17bc		
Protein Concentrate	32.76±0.29c	66.19±0.72a	69.34±0.16a
Protein Isolate	37.02±0.52b	15.32±2.13c	14.22±2.17c
Sieving	40.01±0.45a	29.23±0.46b	25.07±0.28b

[a] Means followed by the same letter in a column were not different ($P<0.05$).

The protein concentrate and isolate processes increased E-Mill DDGS protein content; the protein concentrate and isolate processes had no or small effects on conventional DDGS

protein content (Table 5). Differences were a result of higher oil and NDF contents in conventional DDGS (Table 4). For example, total oil contents of conventional and E-Mill DDGS were 12 and 1%, respectively; whereas, protein contents of protein concentrate from conventional and E-Mill DDGS were 33 and 49%, respectively. Residual oil diluted the protein percentage. Our findings were similar to those published by Wang et al (2004), who reported the low protein contents in soy protein concentrate was caused mostly by residual oil content. For fiber content, NDF of E-Mill DDGS was lower than that of conventional DDGS; protein content of protein concentrate from E-Mill was higher than that from conventional DDGS. Same trend was observed for protein isolate from conventional and E-Mill DDGS. The protein concentrate and isolate processes led to higher protein yields with conventional DDGS. Germ and pericarp fiber were not recovered prior to fermentation for the conventional dry grind process, so protein located in germ was recovered in conventional DDGS. The protein concentrate and protein isolate processes typically are used to recover soy protein, which contains mainly globulins (Murphy 2008). Globulins also exist in corn germ mostly as an enzyme protein (Lawton and Wilson 2003). Thus protein yield increased for the protein concentrate and isolate processes with conventional DDGS even though protein content of final products decreased. Protein content of final product from the sieving process was the highest among three processes, but protein yield decreased from using E-Mill DDGS to conventional DDGS. With higher amounts of oil and fiber in conventional DDGS, more protein was left in these coproducts. In general, three processes can result in higher protein contents of final products with E-Mill DDGS while more protein can be recovered with conventional DDGS.

Table 4. Composition of conventional and E-Mill DDGS and protein concentrate and isolate (means of three observations). [a]

	Protein (%, db)	Oil (%, db)	Neutral Detergent Fiber (%, db)
Starting materials			
Conventional DDGS	34.32±0.17e	12.68±0.10c	17.07±0.51b
E-Mill DDGS	41.96±0.78c	0.73±0.09e	1.69±0.05e
Protein Concentrate			
Conventional DDGS	32.76±0.29f	15.41±0.11b	27.14±1.29a
E-Mill DDGS	48.74±0.35b	1.05±0.09de	3.82±0.76d
Protein Isolate			
Conventional DDGS	37.02±0.52d	21.67±1.16a	11.59±2.11c
E-Mill DDGS	51.86±0.57a	2.14±0.12d	1.33±0.31e

[a] Means followed by the same letter in a column were not different (P<0.05).

3.3.3 Effects of processes on DDGS fiber and oil content

For processes using E-Mill DDGS, the protein isolate process increased DDGS oil content while no differences were observed by the protein concentrate and sieving processes on DDGS oil contents (Table 5). Oil content of protein isolate (2.1%) was higher than E-Mill DDGS oil content (0.7%). Oil content of protein concentrate (1.0%) and sieved product (0.7%) was comparable to DDGS (0.7%). For processes with conventional DDGS, all three processes increased DDGS oil content (Table 5). Compared with conventional DDGS oil content (12.7%), the protein concentrate process (15.4%) and sieving process (14.8%) increased oil content to similar levels. Oil content of protein isolate process (21.7%) was higher than DDGS oil content. The protein isolate process, which featured two centrifuge processes, increased DDGS oil

content more effectively than the protein concentrate process and sieving process. Energy is often the major factor in determining the value of most food ingredients for poultry (Batal and Bregendahl 2011). Oil content is the best single predictor of true metabolizable energy (TMEn) content in DDGS; a high level of fat in DDGS is associated with a high gross energy (Batal and Dale 2006). Corn oil has a higher (2.25 times) energy value compared to starch, and is the main reason why DDGS is considered a valuable energy ingredient in swine foodstuff. However, there is concern in dairy cattle industry of the negative effects of oil in DDGS on milk fat synthesis creating milk fat depression; therefore, interest exists in feeding dairy cattle with reduced fat DDGS (Kalscheur et al 2011). Thus, there is tradeoff for amount of oil content of DDGS as an animal food ingredient. The E-Mill process recovered corn germ prior to fermentation, reducing DDGS oil content to 1%. The protein isolate process increased DDGS oil content to 2%. Feeding low oil content DDGS (3.5%) to dairy cows resulted in good milk and milk protein production (Mjoun et al 2010b). Lower DDGS oil content can be achieved by the E-Mill process and further by the protein concentrate or isolate process.

For processes using E-Mill DDGS, NDF content of protein concentrate (3.8%) was higher than DDGS (1.7%) (Table 5). However, NDF contents of protein isolate and sieved product were not different from that of DDGS. No difference was due to low NDF content of E-Mill DDGS. For processes using conventional DDGS, the protein concentrate process increased DDGS fiber content from 17.1% to 27.1%. NDF contents of protein isolate (11.6%) and sieved products (9.6%) were lower than DDGS (17.1%). Sieving process was more effective on fiber reduction than protein isolate process. Furthermore, sieving was more effective than centrifugation with respect to DDGS fiber reduction. Across both E-Mill and conventional DDGS, the protein

concentrate process increased DDGS fiber content since protein concentrate process did not separate fiber before removing soluble solids. The protein isolate and sieving process decreased or did not change fiber content of DDGS. The E-Mill process reduced DDGS fiber content compared with the conventional dry grind process. The protein isolate and sieving process had no effect on changing fiber content of E-Mill DDGS. Even though the protein concentrate process increased fiber content, NDF was still only 3.8%, which was lower than conventional DDGS NDF content (17.1 %). As a result, E-Mill DDGS and further processed DDGS can be further used for as nonruminants food ingredients. The low digestibility of fiber in DDGS may result in increased quantities of manure from nonruminant animals (Stein and Shurson 2009).

Table 5. Oil and NDF content of product from three processes (means of three observations). [a]

DDGS Type	Product	Oil (%, db)	Neutral Detergent Fiber (%, db)
E-Mill	Protein Concentrate	1.05±0.09de	3.82±0.76e
	Protein Isolate	2.14±0.12d	1.33±0.3f
	Sieved DDGS	0.73±0.19e	1.85±0.85f
	DDGS	0.73±0.09e	1.69±0.05f
Conventional	Protein Concentrate	15.41±0.11b	27.14±1.29a
	Protein Isolate	21.67±1.16a	11.59±2.11c
	Sieved DDGS	14.83±0.13b	9.58±1.00d
	DDGS	12.68±0.10c	17.07±0.51b

[a] Means followed by the same letter in a column were not different ($P<0.05$).

3.4 Conclusions

The protein concentrate and protein isolate processes increased E-Mill DDGS protein contents. For conventional DDGS, the protein concentrate process had no effect on protein content while the protein isolate process increased protein. Higher protein contents and lower fiber contents were detected for protein concentrate and isolate from E-Mill DDGS. This was attributed to lower oil and fiber content of E-Mill DDGS as a starting material for further processing. The protein concentrate process resulted in highest protein yield with conventional and E-Mill DDGS. Sieving process led to highest protein content with conventional or E-Mill DDGS; protein yield was between the protein concentrate and isolate processes. The E-Mill process reduced DDGS fiber content. The protein isolate process further reduced E-Mill DDGS NDF content while the protein concentrate process increased NDF content; NDF content of protein concentrate was lower than that of conventional DDGS. Thus, higher protein content and lower fiber content DDGS from the E-Mill and protein concentration (concentrate and isolate) processes has potential to be used at higher rates in nonruminant animal foodstuffs due to better nutrition and higher digestibility of DDGS. Considering both protein content and yield, the protein concentrate process was the preferred process with E-Mill DDGS and the sieving process was preferred with conventional DDGS.

Chapter 4

Recommendations for Future Work

From this study, processes were investigated to increase DDGS protein content and decrease fiber content. The protein concentrate and isolate processes were modified from procedures used to produce soy protein concentrate and isolate. These two processes can be modified further based on corn protein characteristics so protein contents and yields can be optimized. Two dry grind process methods were used in this project to obtain DDGS as starting materials. Other fractionation methods such as dry fractionation can be used to produce DDGS. Also, commercial DDGS is a good feedstock for further protein concentration. There are some potential issues that need further study.

A broader range of pH values can be tested for the protein concentrate process to precipitate protein components and for the protein isolate process to solubilize proteins. Typical pH value for producing soy protein concentrate is 4.5 (isoelectric point of globulin). Considering different protein composition between corn and soybean, a broader range (3 to 6) of pH values can be applied to DDGS for further protein enrichment.

Alcohol washing is traditional method to produce soy protein concentrate compared to acid leaching process, which was applied in this DDGS research. Ethanol concentration range is from 50 to 70% (w/w). It is another option for further DDGS protein concentration.

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