INCREASED OIL RECOVERY FROM DISTILLERS DRIED GRAINS WITH SOLUBLES AND WHOLE STILLAGE

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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota State University's regulations and meets the accepted standards for the degree of

MASTER OF SCIENCE

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ABSTRACT

Finding a viable method to recover oil from the corn ethanol industry's co-products has considerable economic prospects for ethanol bio-refineries. This study examined the effects of enzymes and ethanol on oil recovery from dried distillers' grains with solubles (DDGS) and oil distribution in the whole stillage (WS). Protease and cellulase enzymes were tested either individually or in combination with the heavier fractions of DDGS and resulted in 18-20% more oil than the original DDGS. More than 90% of the oil was recovered from the heavier fraction of DDGS using ethanol at 30ºC with 30% solid loadings. Ethanol addition also improved oil partition in WS's liquid fraction by 17–20%. Overall, enzymes and ethanol treatments showed a positive effect on oil recovery from DDGS and WS. Ethanol bio-refineries may use these findings to recover oil as no significant changes are required in the ethanol plant's design.

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I am pleased to extend my gratefulness to all close friends and well-wishers who helped me directly and indirectly and made my stay here homey during the entire period of study. My deep gratefulness goes to all of my family members for their encouragement and invaluable support.

DEDICATION

This dissertation is dedicated to my lovely wife, Shamma Tasneem Chowdhury, who flew across the sea in this pandemic to be with me.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

DDGS.....Dried Distillers Grains with Solubles.

db.....Dry Basis.

CDS.....Condensed Distillers Solubles.

CDO .....Corn Distillers Oil.

TS.....Thin Stillage.

WS.....Whole Stillage.

WDG .....Wet Distillers Grains.

RFA.....Renewable Fuels Association.

USDA.....United States Department of Agriculture.

NASS .....National Agricultural Statistics Service.

CP.....Crude Protein.

ADF.....Acid Detergent Fiber.

NDF.....Neutral Detergent Fiber.

LIST OF APPENDIX FIGURES

CHAPTER 1. GENERAL INTRODUCTION

1.1. Background

Corn is a valuable crop for ethanol production that is produced in large quantities globally, and the United States is the largest corn producer in the world. According to the United States Department of Agriculture (USDA) and National Agricultural Statistics Service (NASS), the United States farmers planted 91.7 million acres in 2019, which is 3% more than in 2018. The proportion of the USA corn harvest used for fuel ethanol has reached 35% and is expected to remain stable in the next decade by USDA Agricultural projections (O'Donoghue et al., 2017). However, due to the sharp decline in crude oil prices, the profits from corn ethanol have fallen sharply (Irwin and Good, 2015). Since the ethanol plants are still in the low margin period, the additional revenue from coproducts is becoming more important.

Recovering oil from dried distillers grain with solubles (DDGS), a valuable co-product from the dry-grind ethanol process creates an additional profit to ethanol plants. The high energy, protein, and phosphorus contents of DDGS provide excellent animal food product quality. Due to these characteristics, DDGS has become one of the most popular feed ingredients worldwide, partially replacing some of the expensive ingredients, such as whole corn, soybean meal, and phosphorus ingredients.

According to the USDA, the production of DDGS comprises 46% of the co-products of the dry-grind ethanol process. Increased oil recovered from DDGS can be a high-value product over the animal feed. This would result in two fuels, ethanol, and biodiesel, produced from a single feedstock. Typically, DDGS contains around 10–12% (w/w) of oil (Chatzifragkou et al., 2015). Oil removal leads to the production of DDGS with higher protein content, a valuable animal feed component. Due to its low residual oil content (5–7%, w/w compared to ~ 10–14%

w/w in DDGS), it can be marketed for non-ruminant diets (e.g., swine and poultry) (Chatzifragkou et al., 2015). Moreover, DDGS extracted oils were found to contain increased amounts of tocotrienols and carotenoids (1762 and 75 μg/g, respectively) compared to corn germ oil (235 and 1.3 μg/g, respectively) (Winkler and Breyer, 2011). The advantage of increased stability for crude DDGS oil compared to corn germ oil is due to the above-mentioned compounds' antioxidant activity.

1.2. Problem Statement

Most of the co-products from corn ethanol production are not well utilized. Almost all of the co-products are used as animal feed. The goal of this research is to find a viable method to recover the oil from the ethanol co-products. DDGS oil is either extracted from the germ of the grain prior to fermentation by means of a solvent/pressing-assisted method or post-fermentation from the whole or thin stillage (back-end extraction process). In the back-end case, oil is extracted by a series of centrifugation, heating, and condensation steps, yielding 60-75% of the total oil content (Veljković et al., 2018).

Some industry professionals assume that the recovery of oil from post-fermentation (at the back end) is more feasible and cost-effective. It is due to the absence of the germ separation stage after initial grinding. The endosperm is unbroken in the germ fraction, and the oil level is increased in the final co-product due to the absence of starch, making oil easier to separate. Figure 1 shows different streams (with their compositions) of the backend oil recovery from corn biorefinery processing.

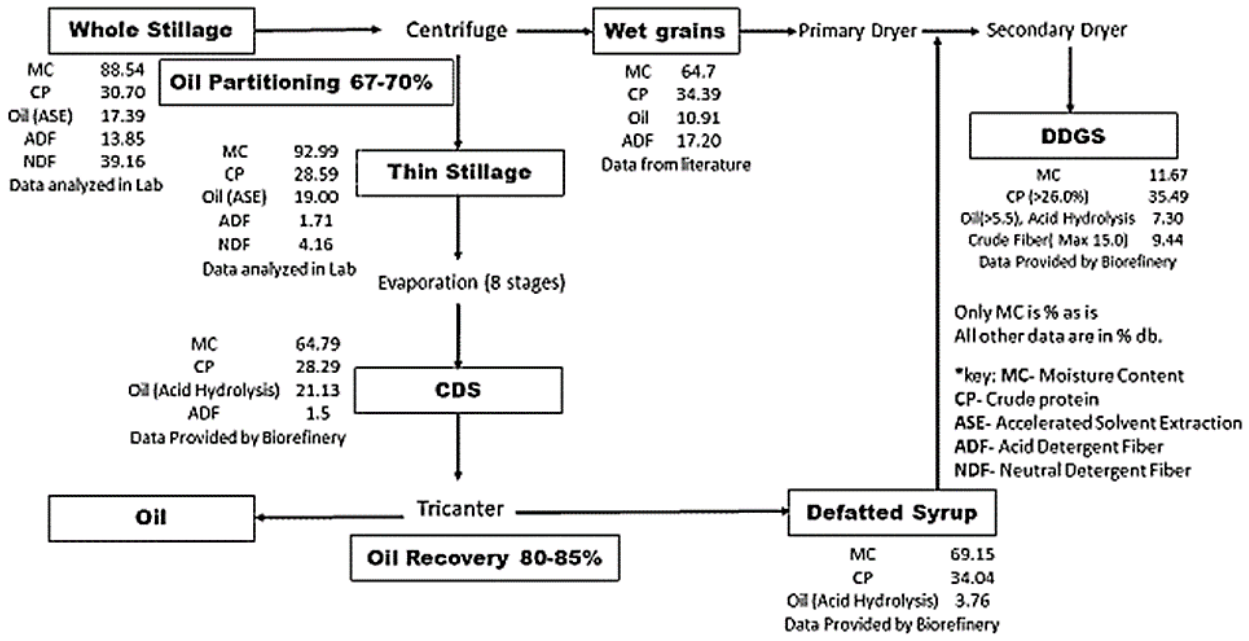

Figure 1. Back-end oil recovery from the dry-grind ethanol process

From Figure 1, it is seen that about 70% of the total oil goes to the liquid phase (thin stillage) and the remainder to the solids (wet grains or wet distiller's grains) (data from laboratory analysis and personal communication with industry people). One definite strategy to improve oil recovery from the dry-grind process is to shift more oil distribution to the thin stillage portion. The oil separation from the liquid phase is much easier, and it can be achieved by using existing centrifuges or decanters. Higher oil partitioning was achieved in thin stillage by using different methods to break corn kernels (such as grinding, flaking, extrusion, etc.) before fermentation (Wang et al., 2008) and using enzymatic treatments during or after fermentation (Luanthongkam et al., 2015; Yao et al., 2014; Wang et al., 2009a).

Enzymatic hydrolysis is a promising approach to oil recovery from DDGS without any improvements in the existing plant design compared to mechanical treatments. Various enzyme-assisted aqueous oil extraction processes have been investigated for different oil seeds, such as canola, soybeans, corn germ, etc. Enzymes have been used in dry-grind ethanol plants primarily

to boost ethanol production, but improved oil recovery from condensed distillers soluble (CDS) has also been claimed (Majoni et al., 2011a).

Small particles up to 0.40 mm favor subsequent and effective enzymatic hydrolysis of solid materials (Chang and Holtzapple, 2000). DDGS is a dry mixture of different particle sizes containing various complex organic macromolecules, such as carbohydrates, proteins, and oils. Individual DDGS particles differ significantly in their chemical composition, size, shape, and density (Bhadra et al., 2009). A bulk DDGS fractionation method for dividing bulk DDGS into high protein/oil and high fiber fractions could result in different applications for both fractions. The high protein/oil fraction could be used in the feed formulation (Belyea et al., 2004) and increased oil recovery. The high fiber fraction could potentially be used as a source for corn fiber gum (Singh et al., 2002). Belyea et al. (2004) reported that DDGS with high oil (13%) and high protein (33%) contents are worth $5–20 per ton more than original DDGS. Thus, the relative amounts of particles present, sorted according to size, would be a characteristic of a particular DDGS sample.

Studies have been conducted to evaluate the use of different solvents for ethanol corn oil extraction. Cheryan et al. (2012) evaluated the extraction of corn germ oil with absolute ethanol and observed the influence of raw material moisture content on oil yield. These authors also suggested that the process yield is affected in a positive way by the increase of temperature and solvent to solid ratio. Cheap ethanol is very much available to the ethanol plants. It will be easier and better economically to use ethanol for recovering oil from corn bio-refinery processing, as ethanol is non-toxic and a reusable demulsifier.

In this context, this thesis focuses on exploring the possibility of using different approaches (enzymatic hydrolysis, ethanol addition) to maximize oil recovery from DDGS and

oil partition in whole stillage. Experiments were conducted to achieve satisfactory oil recovery from various fractions of DDGS relative to the original DDGS and more oil partition from whole stillage into thin stillage. Outcomes of these experiments would complement ethanol plants to optimize their operations, recover higher value co-products (oil), and improve their profitability.

1.3. Objectives

The overall goal of this research was to maximize oil recovery from corn ethanol co-products. The specific objectives were; i) to examine the efficacy of different commercially available enzymes on oil recovery from fractionated heavy fractions of DDGS; ii) to investigate the effect of fractionated heavy fraction of DDGS on oil recovery using ethanol; and iii) to determine ethanol's effects on oil partitioning with whole stillage.

CHAPTER 2. LITERATURE REVIEW

2.1. Present Overview of US Ethanol Industry

Worldwide production of ethanol has increased from 4.49 billion gallons to 28.53 billion gallons in the last two decades (Iram et al., 2020). The chart in figure 2 shows global ethanol production by country or region from 2007 to 2019. Over this period, global production continues to increase. The main reason for this surge is the demand for more environmentally friendly fuels and a decrease in fossil fuel dependence. It is projected that ethanol production will increase to 35.53 billion gallons by 2024 (Bušić et al., 2018). Together, the United States and Brazil produce 84% of the world's ethanol. The vast majority of U.S. ethanol is produced from corn, while Brazil primarily uses sugarcane. The ethanol industry has become one of the most significant success stories of the past quarter-century in American manufacturing. The American ethanol industry is poised to produce nearly 16 billion gallons in 2019 from a cottage industry that produced 175 million gallons in 1980.

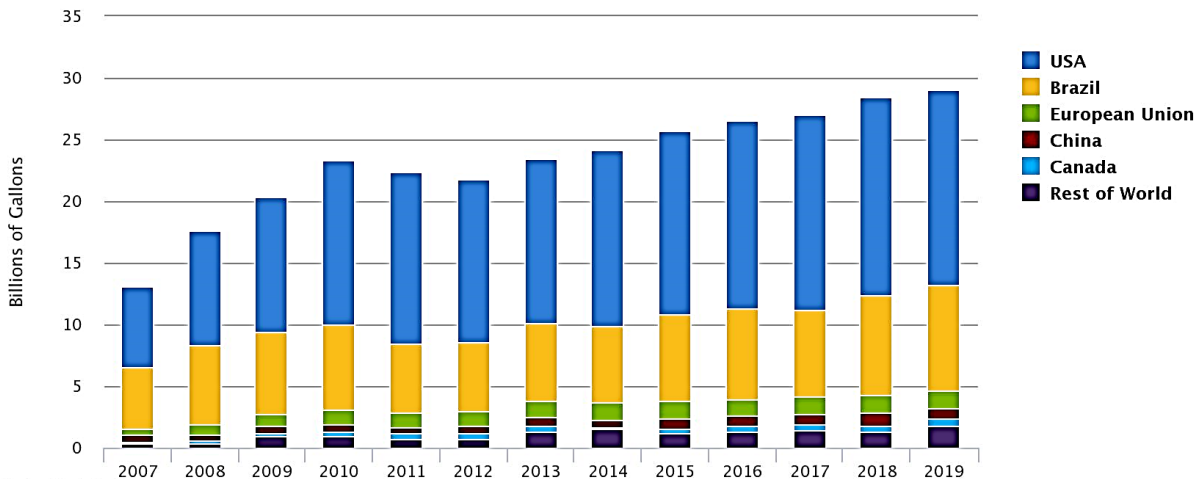

Figure 2. Global ethanol production trend from 2007 to 2019 (Source: Renewable Fuels Association, 2020)

Last year (2019), however, was the most challenging year for the United States ethanol industry in decades. It was due to the Environmental Protection Agency's indiscriminate use of

small refinery waivers that caused twenty plants to close either permanently or temporarily. In 2019, 15.8 billion gallons of ethanol were produced from 205 ethanol plants located across 27 states of the USA (Renewable Fuels Association, 2020). To produce these products, ethanol plants processed 5.52 billion bushels of corn, according to the USDA. Additionally, the industry produced nearly over 396 million tons of DDGS and 3.8 billion pounds of corn distiller's oil, which was a decrease of approximately 4% and 5%, respectively, from the previous year (Renewable Fuels Association, 2019). According to the report published by Renewable Fuels Association (2020), with the advancement of recent process technologies, one bushel of corn processed by a typical dry grind ethanol facility can now produce (on average):

- 2.9 gallons denatured fuel ethanol
- 15.9 pounds of DDGS (10 percent moisture)
- 0.8 pounds of corn distillers oil for animal feed and biodiesel production
- 16.5 pounds of biogenic carbon dioxide for food, beverage, and chemical manufacturing

Corn is the most expensive input for a dry grind plant, linking plant profitability tightly to its cost. The high volatility of corn prices (ranging from $2.00 to over $8.00 per bushel over the past ten years) has resulted in tight profit margins for the industry (Renewable Fuels Association, 2020). This has increased the importance of the co-products' contribution to the economic stability of ethanol plants. (Renewable Fuels Association, 2019). Based on average prices and yields of co-products in 2019, a typical dry mill ethanol plant added nearly 31% of additional value to every bushel of corn processed (Renewable Fuels Association, 2020).

2.2. Ethanol Production Process

The ethanol production process has retained similar steps for both beverage production and industrial production for fuel. The starch-based industrial fuel ethanol production process

has greatly improved yield and efficiency for ethanol production. (Hahn- Hägerdal et al., 2006). The corn-based ethanol process is one of the starch-based ethanol processes, and it is the most successful in the U.S. ethanol industry. This process developed a set of pathways to increase ethanol yield and minimize negative environmental impacts.

In the modern industrial production of ethanol, milling is an important process that affects the quality and quantity of ethanol production. In its 170 years of history, the corn milling industry has developed into the most diversified and integrated grain-processing industries. In the USA, corn is processed with two main distinct processes, wet milling or dry grind processing. Usually, each process generates unique coproducts. More than 90% of operational corn ethanol facilities in the USA are a dry grind (Renewable Fuels Association, 2019). The dry-grind ethanol production process is the most widely employed method used by fuel ethanol production industries because of its simplicity and low capital investments. A traditional ethanol plant converts corn starch into ethanol and carbon dioxide, while the non-starch portion is carried into different co-products. A schematic diagram showing the different steps of ethanol production in the Hankinson Renewable Energy ethanol plant in North Dakota is presented in Figure 3.

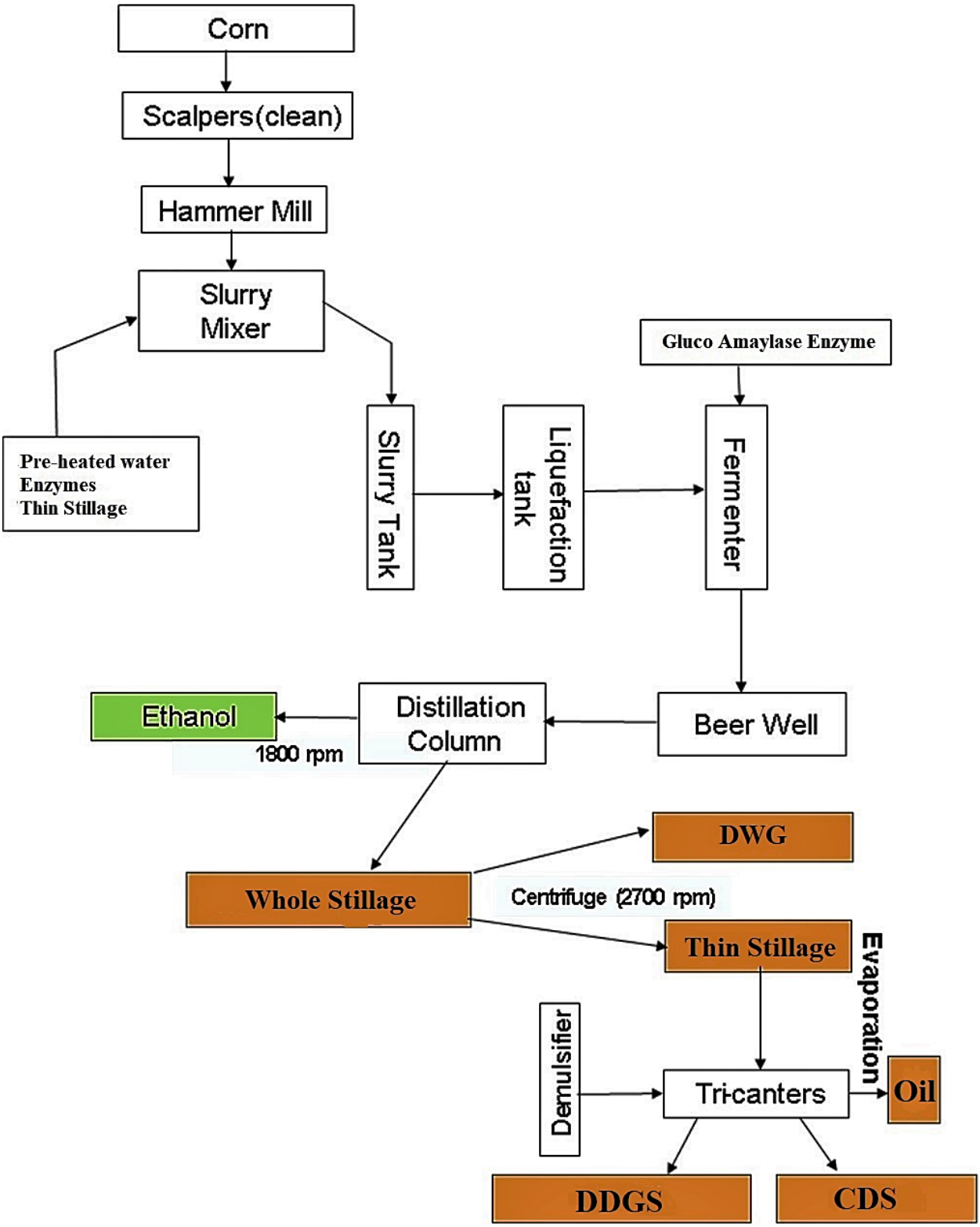

Figure 3. Different stages of ethanol production at Hankinson Renewable Energy

In the wet milling process, the germ and fiber (which become components of animal feed later) are separated before the corn kernel is subjected to starch saccharification and fermentation (Kwiatkowski et al., 2006). While dry milling is less capital intensive, it also yields less ethanol per bushel of corn than wet milling (Rajagopalan et al., 2005).

Wet grind ethanol plants soak whole corn kernels in water and dilute sulfurous acid for 24 to 48 hours. This soaking occurs before processing and acts to soften the kernel and promote the separation of the kernel into the components of starch, gluten, and germs (Nelson, 2015). After soaking, the corn slurry is processed by a series of grinders to isolate the corn germ. The remaining fiber, gluten, and starch components are further separated using the centrifugal, screen, and hydroclonic separators. The germ component is either extracted on-site or sold to crushers for the extraction of corn oil. The extraction of oil from the germ leaves the germ meal, which combined with the fiber portion of the kernel, produces corn gluten, a livestock feed ingredient. (O'Brien and Woolverton, 2009). The gluten component (protein) is filtered and dried for the production of feed ingredients in poultry broiler operations. The starch and any remaining water from the mash can then be processed in one of the three ways: fermented into ethanol, dried and sold as dried or modified corn starch, or processed into corn syrup. Starch fermentation uses a process similar to the dry mill process. The variety and value of co-products produced by wet grind ethanol processing are wide. However, there are relatively few wet grind plants operating in the U.S. because they require high capital investment and a very large scale to be efficient.

The traditional dry-grind ethanol process consists of five basic steps: grinding, cooking, liquefaction, saccharification, and fermentation. Firstly, whole corn is ground and combined with water and enzymes to create a slurry that goes through cooking and liquefaction. The hammer or

roller mill is used to grind corn, increasing the accessibility of starch. During cooking, the slurry goes through a jet cooker that heats the slurry and starts to break the starch polymers. The liquefaction step is partial hydrolysis that lowers the viscosity of cooked corn. It is essentially breaking up the longer starch chains into smaller chains with the help of α-amylase enzyme and certain temperatures (85-95°C) and pH (5.9-6.2) range. Glucoamylase enzymes are added to convert the starch in the kernel to glucose, a process referred to as saccharification. In the saccharification step, a temperature range of 55-65°C is maintained for enzyme activity, and ammonia is added both for pH control (4.5) and as a nutrient to the yeast. The fermentation by yeast (*Saccharomyces cerevisiae*) takes place in a tank at 30-32°C for 2-3 days in a batch, and the resulting glucose yields ethanol and carbon dioxide (Bothast and Schlicher, 2005). Non-fermentable materials are obtained as a whole stillage after the distillation of ethanol. Final ethanol concentrations are relatively low, around 12-15% (known as 'beer'), at the end of fermentation. All industrial fuel ethanol production uses continuous-feed distillation column systems, where the beer is heated to enhance separation from water. By the time the product reaches the final distillation column, it is 95 % ethanol or 190 proof. Molecular sieves are used to remove the remaining water after distillation. Then the ethanol is 200 proof and is referred to as anhydrous ethanol. Anhydrous ethanol is mixed with a small amount of gasoline to produce fuel-grade ethanol (Meredith, 2003).

2.3. Enzymes in Dry Grind Ethanol Process

Saccharomyces cerevisiae (generally known as yeast) is a fungus capable of consuming sugars such as glucose, fructose, and maltose to produce ethanol via fermentation. However, starch cannot be fermented directly into ethanol by yeast. The reason is the organism's inability to release simple sugars from starch (Power, 2003). Originally, starch depolymerization is

performed through a process involving enzymes, high temperatures, and pressures. The use of enzymes allows for a less hazardous and lower energy-consuming process. In addition, the enzymatic approach yields a higher quality product (Tester et al., 2006). In the following sections, different types of enzymes that are generally used in the dry grind process are described.

2.3.1. Alpha-Amylase

Alpha-amylases, 1,4-α-D-glucan glucanohydrolases, comprise of a family of starch degrading enzymes. In the dry grind process, the liquefaction step requires this enzyme to be active at high temperatures (80 to 110°C). Weemaes et al. (1995) tested three microbial sources of alpha-amylase, concluding that *Bacillus licheniformis*, a mesophilic bacteria, produced the enzyme with the highest thermo-stability. For this reason, alpha-amylase for starch liquefaction is manufactured primarily from this type of microorganism.

Alpha-amylases perform optimally at temperatures and pH values ranging from 85 to 95°C and 5 to 6, respectively. Ramchandran et al. (2016) reported the activities of alpha-amylase enzyme was 6,400 alpha amylase units (μmol maltose/min mL enzyme). This enzyme is endo acting, which means it attacks the inner region of amylose and amylopectin chains. It splits α-1-4 glycosidic bonds randomly, yielding water-soluble saccharides of varied lengths (van der Maarel et al., 2002). This enzyme's action pattern is limited to α-1-4 bonds; it cannot cleave α-1-6 glycosidic bonds and skips the branching points in amylopectin. Due to this limitation, the action of alpha-amylase on amylopectin results in branched products (Power, 2003).

2.3.2. Glucoamylase

Glucoamylases, amyloglucosidases, or 1-4-α-D- glucanohydrolases, are employed in the ethanol industry for saccharification of liquefied starches. Although glucoamylases can be

obtained from many fungal sources, they usually are produced from *Aspergillus niger* and *Rhizopus species* (Nigam and Singh, 1995). Fungal enzymes are less thermotolerant than those produced from bacterial sources (Power, 2003). Glucoamylase from *Aspergillus niger* has an optimum pH of 4.2 and is stable at 60°C (Crabb and Mitchinson, 1997). Ramchandran et al. (2015) reported the activities of glucoamylase enzymes was 8,951 gluco amylase units (μmol glucose/min mL enzyme).

Glucoamylase functions as an exo acting agent attacking both α-1-4 bonds and the α-1-6 bonds present at the branching points of oligosaccharides (Crabb and Mitchinson, 1997). It cleaves glucose monomers successively from the saccharide's non-reducing end. Thus, making them available for fermentation via yeast (Saha and Zeikus, 1989).

Pullulanases, pullulan-6-glucanohydrolases, are a group of debranching enzymes occasionally combined with glucoamylase for converting starch mashes into glucose. Pullulanases from microbial sources (e.g., from *Bacillus* species) are preferred due to their high specificity towards the hydrolysis of α-1-6 bonds (Hii et al., 2012). This enzyme's optimum temperature is 60°C, and the ideal pH is 4.5 to 5.5 (Norman, 1982). These optimum operational conditions match those of glucoamylase from *Aspergillus niger*, making these enzymes suitable for simultaneous performance.

2.3.3. Granular Starch Hydrolyzing Enzymes (GSHEs)

A combination of alpha-amylases, glucoamylases, alpha-glucosidases, and isoamylases are considered granular starch hydrolyzing enzymes (GSHEs) (Robertson et al., 2006). GSHEs are capable of hydrolyzing raw granular starches at sub gelatinization temperatures (30 to 48°C) and low pH (4.0 to 4.2) (Wang et al., 2007). This enzyme contained alpha-amylase from *A. kawachi* and a glucoamylase from *A. niger* and had an activity of ≥ 456 GSHU/g (Sharma et al.,

2007). Depolymerization of starch using GSHEs entails numerous benefits: performing hydrolysis at reduced temperatures can reduce process energy consumption (Robertson et al., 2006; Wang et al., 2007). Wang et al. (2007) tested GSHEs by conducting hydrolysis at 48°C. Subsequent fermentation resulted in ethanol concentrations comparable to those of samples liquefied at 90°C with conventional alpha-amylase. Uthumporn et al. (2012) conducted liquefaction with GSHE at 35°C, obtaining corn slurries with lower viscosities than heat-treated ones.

2.3.4. Cellulases and Hemicellulases

Cellulases and hemicellulases are typically used in advanced biofuels to obtain fermentable sugars by hydrolyzing pretreated lignocellulosic materials. At the same time, biomass degrading enzymes such as β-glucanases, cellobiohydrolases and xylanases have also been applied in the starch-based ethanol industry to improve plant efficiency and energy savings. Zhang et al. (2010) found cellulase or hemicellulase or their mixture has been added before or after liquefaction to decrease the viscosity of the slurry. Cellulases and xylanases may also help in releasing starch bound to the corn fiber, and induce cost and energy savings by decreasing viscosity and reducing water binding to grains, thus facilitating centrifugation and drying steps (Harris et al., 2014). New equipment, pretreatment technology and cellulase cocktails have been combined to convert corn fiber into fermentable sugars for additional ethanol.

2.3.5. Proteases

Like cellulases and hemicellulases, proteases also are typically used in advanced biofuels. It was shown to increase fermentation rate (Vidal et al., 2009) and ethanol yield by liberating free amino acids for the yeast (Perez-Carrillo et al., 2012; McAloon and Johnston, 2014). Proteases may benefit fermentations by changing the chemistry of the grain by dismantling

starch–gluten complexes, thereby making the starch more accessible for hydrolysis by amylase enzymes (Alvarez et al., 2010). Another benefit of proteases was recently discovered by Novozymes, which is changing the native chemistry of the corn kernel by hydrolyzing specific proteins named oleosins. Huang (1996) assumed proteins provide structure to the oil-encapsulating bodies called oleosomes. Extensive work has been done to show that specific proteases will hydrolyze oleosin better than other enzymes, resulting in the release of more oil (Majoni et al., 2011a). Under the right conditions of dose, temperature, time and pH, the use of a protease of an appropriate family greatly improves process conditions, yields more ethanol and oil, and saves energy during the drying of DDGS because the insulating properties of oil are removed, thereby allowing for more efficient heat transfer (Harris et al., 2014).

2.4. Co-Products of the Dry Grind Ethanol Process

Generally, the whole stillage is centrifuged into thin stillage (TS) and wet distillers grains (WDG). A portion of this thin stillage is recycled back into the slurry of the next batch (Kwiatkowski et al., 2006). The thin stillage is concentrated by removing water when passing through evaporators to produce the condensed distillers solubles (CDS) of about 30 % solids. The WDG from whole stillage can be dried to produce dried distillers grains (DDG) or mixed with CDS and dried to produce dried distillers grains with solubles (DDGS) (Kim et al., 2008). The proportion of various types of co-products produced by dry grind ethanol plants is shown in Figure 4.

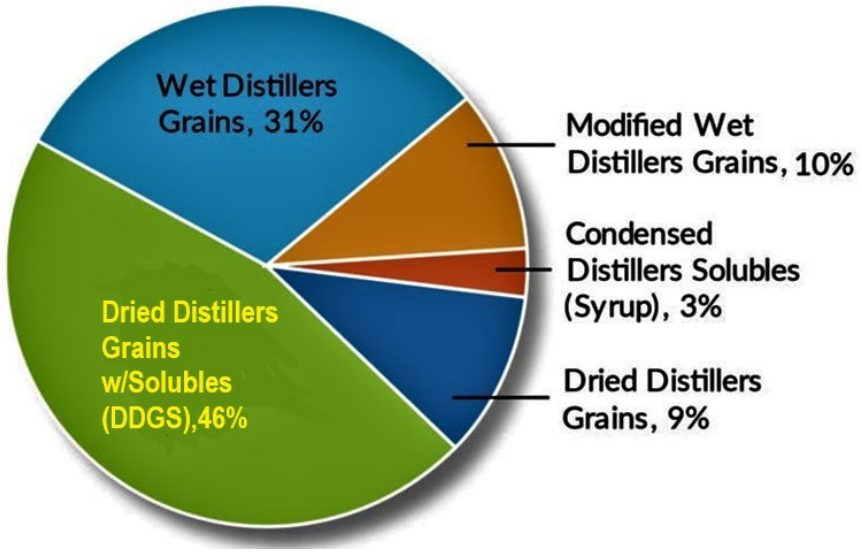

Figure 4. Production of various types of co-products in dry grind ethanol (Source: U.S. Dept. of Agriculture)

As the industry continues to grow, there will be an increased need to find additional uses for ethanol co-products. The physical and chemical properties of coproduct streams are becoming important, as these characteristics affect many aspects of utilization. These aspects of utilization include target species, optimal dietary substitution rates, transportation, flowability, and behavior during storage. Additionally, much interest lies in recovering oil from ethanol co-products. If the oil is recovered from these co-products, the resulting chemical and physical properties of the remaining constituents may be substantially changed. For example, higher levels of oil in DDGS are sometimes undesirable and affect feed quality negatively by interfering with milk production in cattle and bacon texture in DDGS-fed swine (Wang et al., 2009b). After removing oil from DDGS, the de-fatted DDGS with low-oil content potentially solved this problem. Recovery of oil from the thin stillage can be another option that will create a higher-value product stream than DDGS. In this context, the oil level of different ethanol co-products can help to decide which portion will be convenient for oil recovery.

Many researchers investigated in the laboratory setting to monitor concentrations and composition of various nutrients during the entire dry-grind process, from corn to the final product. These studies provided some information about changes in oil levels during the process. Moreau et al. (2011b) used sets of samples from three commercial dry-grind ethanol plants in Iowa. The oil level of a different fraction is presented in Figure 5.

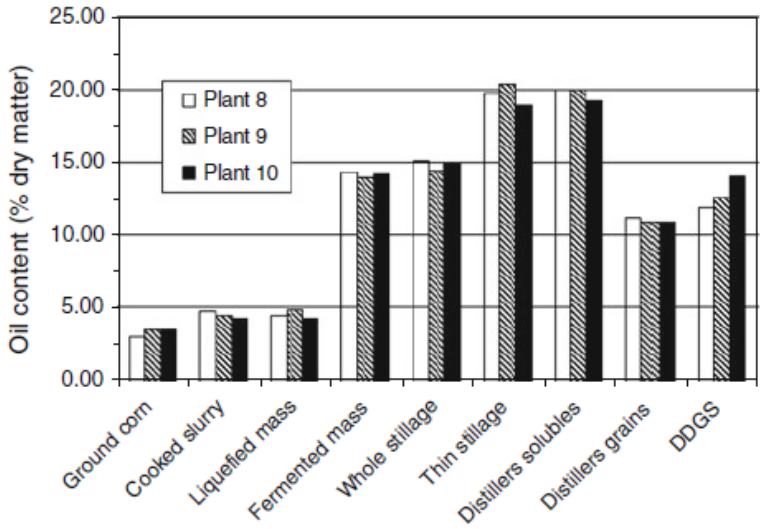

Figure 5. Oil yields from nine fractions of ethanol plants. Extractable oil levels were measured by hexane extraction with Ankom Model XT 10 Fat Extractor (Image retrieved from Moreau et al., 2011b)

The oil yields were low (<5%) in the pre-fermentation fractions. Oil yields increased nearly three-fourfold in the next four fractions due to the depletion of starch upon fermentation. A slightly higher percentage of the oil partitions into the liquid thin stillage fraction was seen compared to the solid distiller's grain. The final oil yield in the DDGS fraction is slightly lower than in the whole stillage. Belyea et al. (2004) found the fat content of DDGS has significant correlations, among other components. Fat content was significantly correlated with protein and ADF (0.82 and 0.63, respectively). Kim et al. (2008) reported crude oil (DM basis) of DDGS, wet cake, and thin stillage measured as ether extractives are 11.6%, 10.6%, and 1.3%, respectively.

2.5. Current Use of Different Ethanol Co-Products

The recent growth of fuel ethanol production resulted in increased availability of ethanol co-products, especially DDGS. The recovery of co-products is one of the key drivers for the ethanol industry's economic sustainability, accounting for nearly 25% of the total revenue for some ethanol plants (Hill et al., 2006). However, few studies have been conducted on the alternate use of upstream products, including the whole stillage, thin stillage, and CDS, that makeup DDGS. A large amount of these products can be converted into a value-added product (e.g., oil and feed ingredients) and then sold in the market to make them essential towards ethanol profitability. Moreover, increasing the use of intermediate products can reduce environmental impacts such as greenhouse gas emissions. Additionally, the drying process of thin stillage could significantly reduce or even eliminate overall energy consumption (Rosentrater and Lehman, 2008). In a typical dry-grind plant, coproduct recovery starts with whole stillage. In the following paragraphs, the current use of different ethanol co-products will be described.

Whole Stillage (WS): Stillage handling is one of the major limitations of the corn to ethanol process since DDGS drying and stillage evaporation account for approximately 30.3% and 16.5% of total energy consumption of a bioethanol plant, respectively (Eskicioglu et al., 2011). Each liter of ethanol produced can generate up to 20 L of stillage (Wilkie et al., 2000). Currently, nutrient-rich (fiber, protein, lipids, and starch) whole stillage is used as animal feed.

Thin Stillage (TS): A significant portion of TS is used as the source of water and nutrients in the cooking step of the dry-grind ethanol process, which helps in water and thermal energy savings. This step is known by the term backsetting. TS is produced at scales up to 15 times the volume of ethanol produced (Reis et al., 2017), and concentrating TS to CDS is one of

the most energy-intensive processes in the corn ethanol biorefinery. TS processing is problematic, and it has a significant negative impact on the DDGS as animal feeds. Researchers found evaporator condensate from TS is the largest wastewater contributor (Schaefer, 2006).

Wet Distillers Grains (WDG): WDG is considered an alternative animal feed ingredient with 30%-35% moisture content but is less efficient to transport due to its short shelf life (Dooley et al., 2008). Therefore, WDG is usually utilized by the farms close to an ethanol production plant, with the ability to be delivered rapidly.

Condensed Distillers Solubles (CDS): CDS is a viscous liquid that resembles syrup. It is an excellent source of fat, minerals, and protein (Maisch, 2003). CDS is typically added back to WDG, and the resulting product is marketed as WDGS (wet distillers grain with solubles). The WDG can also be dried and sold as DDGS. CDS has been used as a feed ingredient for beef cattle and lactating dairy cows. In some plants, CDS is offered as a separate liquid product. This syrup-like product can be used to control dust and condition dry rations. CDS works well as a supplement for low-quality forage diets.

Dried Distillers Grains with Solubles (DDGS): DDGS is used for animal feed and is considered an inexpensive, high-protein feed ingredient for animal nutrition (Belyea et al., 1989). Currently, 30% and 44% of the DDGS used in feed ingredients in dairy and beef cattle feed, respectively. In contrast, swine and poultry represent 16 and 9%, respectively, of the total DDGS utilized as a feed ingredient (Renewable Fuels Association, 2020). The high fiber content limits the use of DDGS in monogastric livestock (Kim et al., 2008). The sale of DDGS to the feed industry contributes to the economic viability of the ethanol-producing industries. (Ganesan et al., 2006).

2.6. Corn Oil Extraction

In dry grind ethanol production, oil can be extracted in the front-end (before fermentation) or at the back-end (after fermentation). Moreau et al. (2014) focused on the implementation of new technologies to increase the performance and profitability of the ethanol plant. They identified two of these newly developed technologies, which are backend oil extraction and front-end corn fractionation. Backend oil extraction takes oil out of the DDGS product after fermentation. Front-end fractionation separates the corn kernel into different streams. In this section, most of the discussion will be the existing oil extraction technologies and studies to recover oil from different ethanol co-products.

Different corn oil extraction technologies are available commercially to the ethanol industry. Most of the USA ethanol plants are using various oil extraction technologies to remove varying amounts of oil before producing DDGS. Several commercial proprietary processes are used to extract corn oil from thin stillage after the distillation of ethanol. The most widely used method in the dry-grind ethanol process is separating the oil from the condensed distillers' solubles (CDS) by centrifugation (Liu and Barrows, 2013). The oil recovery procedure was described in U.S. Patent 7,601,858 (Cantrell and Winsness, 2009). In general, the whole stillage is separated by decanting into thin stillage and wet cake. Thin stillage contains approximately 30% of the oil available in corn whole kernel, and the rest is left in the wet cake after decanting. The thin stillage is further evaporated to produce CDS with 60 - 85% water content, and the oil is separated from the CDS using a disk stack centrifuge (Winsness and Cantrell, 2009). This process includes heating the TS at a temperature100 ºC at a pressure greater than its vapor pressure, followed by a cooling phase that helps to separate the oil from thin stillage. The traditional use of continuous centrifuges and three-phase decanter centrifuges is to remove oil,

but it does not provide yields as high as those by disk stack centrifuges. However, the three-phase decanter centrifuges provide higher centrifugal forces and can remove bound and emulsified oil (Moreau et al., 2011a). In general, these processes can separate 30 to 70% of the oil in this co-product stream.

Corn oil extraction systems have been added to existing ethanol plants to increase the energy efficiency of the plant. However, the installation of oil extraction equipment in existing ethanol plants facilitates the production of oil without affecting ethanol production. Not all of the corn oil produced through these processes is suitable for human food use. However, solvent (hexane) extraction is routinely used to extract corn oil from corn germ to produce high-quality corn oil for human consumption in wet mills (Moreau, 2005). The application of n-hexane is especially useful for the oil extraction from whole stillage with low oil content. Oil extraction from whole stillage and CDS at an optimal n-hexane-to-substrate ratio of 0.20 g/g provided oil yields of 9.8 ± 0.6% and 12.0 ± 0.8%, respectively. (Noureddini et al., 2009). Hexane extraction is very effective in capturing the corn oil in DDGS, but the high capital investment costs for constructing a hexane extraction facility has limited the adoption of this technology in the ethanol industry. Currently, only one facility (Novita, Brookings, SD) is using hexane extraction to remove corn oil from DDGS. This facility produces feed grade corn oil and a low-oil (3.5% crude fat) DDGS (U.S. Grains Council, 2018). Additional oil extraction may occur in the future because the remaining ethanol plants not currently extracting corn oil may adopt these technologies. Additionally, new technologies have been developed and are being implemented to extract additional oil in ethanol plants.

2.6.1. Front End Recovery of Corn Oil

Most of the front-end corn oil is obtained from corn germ, a by-product of the wet-milling industry (Moreau, 2005). Approximately 10% of the edible corn oil in the United States is obtained from corn germ produced by a process called dry milling. The corn germ fraction from the dry milling fraction represents about 10% of the mass of the kernel and it contains about 15-25% oil (Moreau et al., 1999). Corn germ can be removed prior to fermentation, and it is pressed or extracted with hexane to produce corn oil. It is reported that some dry grind ethanol plants contain the fractionation equipment to remove germ before fermentation. However, very little corn oil is produced via this route, mainly due to the current economics (Moreau et al., 2014). It is possible to remove corn germ in a dry grind ethanol plant before fermentation using 'wet' techniques. The first wet fractionation process was called the 'quick germ' process (Singh & Eckhoff, 1996). It involved soaking kernels in water, gently grinding, and separating the fiber and germ by floatation. Other wet fractionation methods have been developed using enzymes to improve the separation and purify the germ, fiber, and endosperm fractions (Johnson et al., 2005; Singh et al., 2002).

Karlovic et al. (1994) reported an 80% yield of corn oil from corn germ using an aqueous enzymatic method with a cellulase enzyme. An aqueous enzymatic oil extraction process was reported to extract corn oil from both dry and wet milled corn germ (Moreau et al., 2004; Moreau et al., 2009). The process involved milling the germ, incubating it with enzymes, and then centrifugation to float the free oil. Maximum oil yields were 80% and 65% from wet and dry milled corn germ, respectively. The enzymes used were cellulase (GC 220) in the wet process and a combination of cellulase (GC 220) and alkaline protease (Alcalase) in the dry process. Using a combination of cellulase (GC 220) and acidic protease (GC 106) enzymes, 60-

70% of the oil was recovered from corn germ, which was dry fractionated before the dry-grind ethanol process. The highest corn oil yield (about 90%) for any type of corn germ was found when the germ was wet fractionated before the dry-grind ethanol process and hydrolyzed with cellulase (GC 220) and alkaline protease (Alcalase) with no heat treatment (Moreau et al., 2009).

Some additional processes have been developed to produce corn oil over the year. One of the methods is called “corn kernel oil”, and it is produced by the extraction of ground corn with ethanol. The low levels of oil in most kernels (3-5%) have indicated that this method is not economical (Moreau et al., 2014). Hojilla-Evangelista et al. (1992) developed a “sequential extraction” process that involved an initial extraction of corn oil from flaked corn with 100% ethanol, followed by zein protein extraction with 70% ethanol, and finally extraction of the remaining proteins and starch. Kwiatkowski & Cheryan (2002) developed a similar two-step corn oil and protein extraction (COPE), but it included membrane filters to process both oil and protein. However, no commercial corn oil is currently processed in the United States using these methods. Moss (2012) proposed that two technologies for the recovery of corn oil could theoretically be used in tandem in a corn dry-grind ethanol process. Firstly, corn germs could be extracted by having a dry de-germination process and corn oil recovered by traditional hexane extraction (estimated oil yield of 0.25-0.50 lbs of oil per corn bushel). Secondly, the residual corn oil may be removed at the back end by heating and centrifuging (estimated oil yield of 1.3-1.5 lbs per corn bushel). Therefore, in terms of oil yield and economic feasibility, back-end oil recovery is a promising option for the biorefinery industry.

2.6.2. Back End Recovery of Corn Oil

Downstream corn oil recovery refers to the separation of oil after fermentation and the various intermediate co-products of the dry-grind ethanol process. Utilizing intermediate ethanol

co-products has many limitations. The composition of these coproducts has a long-range of variation. The shelf life of some coproducts is short due to high moisture content, and the nutritional profile is not favorable for many animals. Overall, these products cannot be sold for high values. Oil is a higher-value coproduct of the corn dry-grind process and is concentrated from 4% in corn kernel to about 14% in DDGS (Wang, 2008). Research on oil recovery from these coproducts (whole stillage, thin stillage, CDS, and DDGS) can be a feasible way to keep the ethanol industry profitable. Various US patents on back-end corn oil recovery have been issued to GreenShift Corporation (Cantrell and Winsness, 2009, 2011a, b, 2012; Winsness and Cartnell, 2009; Winsness, 2012), ICM (Gallop et al., 2012), Primafuels (Woods et al. 2012) and POET (Bootsma 2013). Some of the information on how to achieve the oil separation of these patents have been described earlier.

2.6.2.1. Oil recovery from dried distillers grains with solubles (DDGS)

There have been few published studies on oil recovery from corn fermentation co-products. Current industry practices on oil recovery from corn fermentation co-products give approximately 300 g of oil per 25 kg (1 bushel) of corn (Renewable Fuels Association, 2019), which is equivalent to approximately 30% oil recovery. Winkler et al. (2007) reported oil yields of 12.7% and 12.5% when it was obtained from DDGS by n-hexane extraction for 24 h, and SC-CO_2 extraction at 55 MPa, 80°C, and 2 L CO_2/min for 60 min, respectively. Ethanol extraction of corn oil from DDGS at the optimum ethanol-to-DDGS ratio of 6 mL/g, 50°C, and under agitation for 30 min has been shown to yield 66 mg oil/g DDGS, which corresponds to 50% of the total oil content in DDGS (Singh and Cheryan, 1998). The largest oil yield from DDGS reported by Ciftci et al. (2012) was 9.2% (82% of oil yield obtained by Soxhlet extraction with petroleum ether for 5 h) using SC-CO_2 oil extraction at 49.6 MPa and 70°C for 340 min.

Bruinsma et al. (2012) reported at least 77% oil recovery from DDGS using hexane to extract the oil. Fang et al. (2018) studied the effects of using acid-stable protease (Fermgen), pectinase, and cellulase enzymes during the fermentation step on the chemical and physical characteristics of DDGS. They found the control DDGS had significantly lower crude fat content (8.55%) than Fermgen (10.84%), and pectinase and cellulase (10.70%) treated DDGS.

2.6.2.2. Oil recovery from condensed distillers solubles (CDS)

To obtain higher oil yield from CDS, some efforts have been made, including physical, chemical, and enzymatic treatments on CDS. The oil yield from centrifugation, described in the previous section, has not been satisfactory for recovering oil from CDS. This is because oil presents in different forms in CDS, including oil-in-water emulsion, surface adhering oil, oil in oil bodies, and oil in unbroken cells (Majoni and Wang, 2010). Enzyme treatments have been tested on CDS for improving oil recovery, and an increase of 45% more oil recovery from CDS was observed when a protease was added in CDS (Majoni et al., 2011b). However, no studies were found on how the enzyme hydrolysis of non-fermentable matters affects oil recovery from CDS. Majoni et al. (2011a) also reported a sharp increase in oil recovery from CDS when the temperature was increased to 60°C, and no more increase was found when the temperature was higher than 60°C. Winsness et al. (2009) suggested using a high temperature (100-121°C) and pressure up to 552 kPa (80 psi) on CDS to free most of the bound oil. Randhava et al. (2008) patented a process in which the milled corn is extracted of oil before the corn ethanol fermentation process, such that oil-free coproducts are produced, and oil recovery from CDS was found approximately 99%.

Fang et al. (2015) used different surfactants (Tween® 80 and Span® 80) and silica nanoparticles (hydrophilic and hydrophobic) on oil recovery from commercial CDS. A surfactant

mixture with (1:1) led to the highest oil recovery compared with a control. Tween® 80 with silica, and surfactant mixture (1:1) with silica recovered 5-10 % more oil compared with the control groups. The distribution of free oil was significantly increased by centrifugation conditions (4,000g for 30 mins), heating (83ºC), and shaking (100 rpm for 10 mins). The synergistic effect between the surfactant and hydrolyzing enzymes (protease, pectinase, and cellulase) was also reported. Surfactant added to corn slurry before fermentation, combined with pectinase and cellulase showed a significant increase in oil recovery from CDS.

Many other methods were tested to improve oil recovery from CDS, including adjusting pH, using polar solvents, applying high temperature and pressure, and churning. (Majoni et al., 2011a). However, many of these treatments are non-practical for industry-level processing since potential time and money cost could be high for doing these treatments on CDS. To date, the acceptable method for ethanol plants to improve oil recovery is adding chemical aids into CDS before centrifugation. Numbers of chemical aids have been designed for oil recovery from CDS, including FoodPro SA9843 corn oil yield improver (General Electric, Trevose, PA, USA), PTV M-5309 corn oil extraction aid (Ashland Chemical, Covington, KY, USA), Ashland DPI-428 (Ashland Hercules Water Technologies, Wilmington, DE, USA), and Hydri-Maize Demulsifier 300 (Hydrite Chemical Co., Waterloo, IA, USA). However, the detailed composition of commercial aid packages and mechanism of action are missing. (Fang et al., 2015)

2.6.2.3. Oil recovery from thin stillage (TS)

Thin stillage in ethanol processing has its solids concentrated during the evaporation steps, including the oil phase. After decanting, there is still a large portion of oil remaining in the wet cake. One approach reported is the separation of oil before the evaporation stage using a centrifuge (Prevost and Hammond 2007), which is not a commercial success. It has been

reported that this approach does not produce usable oil but an undesirable emulsion phase that requires further processing. Moreover, the thin stillage volume is generally up to ten times greater than CDS, which requires considerable capital to acquire the number of centrifuges required. Another patented approach describes the use of filters for removing nearly all solids and recovering lactic acid and glycerol from TS without the need for evaporation (Bento and Fleming, 1993).

The utilization of separation additives, such as precipitated and hydrophobic silica, has been described as an alternative to decrease the need for additional separation units, which can be added on the inlet or outlet of an evaporator (Lewis and Shepperd III, 2016). However, they found that hydrophobic silica decreases oil yield if the treatment temperature is above 90ºC.

On another approach, breaking cell structure methods might be efficient in improving oil partition in thin stillage. Wang et al. (2008) examined the effects of corn grinding methods on oil distribution in thin stillage and found the flaked and then extruded corn meal released the highest amount of free oil, which was 25% compared to 7% for the average of other treatments. The effect of non-ionic surfactants (Tween® 80 and Span® 80) on oil partition in thin stillage has been reported. The use of Tween® 80 at the concentration of 500 ppm in corn slurry produced up to 10% more oil than control experiments (Fang et al. 2015). This group also explored the use of hydrolyzing enzymes, i.e., protease, pectinase, and cellulase during fermentation, and found significantly increased oil partition in thin stillage without hampering ethanol production. Luanthongkam et al. (2015) reported an increase of oil partition in thin stillage from 32% to 78% when protease, phytase, and non-starch hydrolyzing enzyme were used during fermentation. Yao et al. (2014) observed significantly higher oil partition (60%) in thin stillage when a blend of polysaccharide hydrolyzing enzymes was used, compared to 56% in control. Wang et al. (2009a)

used protease and cellulase during fermentation, and 70% oil partition in thin stillage was achieved, compared with 50% in control. However, the high oil partition in thin stillage cannot be converted into a high oil recovery from CDS.

2.6.3. Corn Oil Recovery Using Ethanol

Hexane has been traditionally implemented in the industry for vegetable oil extraction. It is a highly efficient technique; however, its main drawback is the toxicity of the solvent. On the other hand, ethanol is short-chain alcohol, which has gained interest in recent years as an alternative solvent to extract a high-quality oil due to its high polarity, bio-renewability, and low toxicity (Capellini et al., 2019, 2017; Sawada et al., 2014).

Kwiatkowski and Cheryan (2002) measured the oil yield in batch extraction from whole ground corn using ethanol as the solvent as a function of different temperatures (25, 50, and 70°C), time of extraction (15, 30, and 120 min), solvent-to-solids ratio (2, 4, 6, and 8 ml/g corn), and ethanol concentration (70, 90, 95, and 100 %v/v). The highest oil yield (70%) was found with a solvent-to-solids ratio of 4 mL/g corn, an ethanol concentration of 100%, 30 min of extraction time, and a temperature of 50°C. Later, a three-stage extraction resulted in a yield of 93% recovery of the oil in corn. When anhydrous ethanol was used, moisture was absorbed linearly by ethanol from the corn in successive stages, which, in turn, decreased oil yield and increased non-oil components in the extract.

Ni et al. (2016) found oil recovery of 93.74% when ground steam-exploded corn germ (1.3 MPa, 30 s, 30–35 μm particle size) was treated with 30 % (v/v) aqueous ethanol for 2 h, at 60 °C, and pH of 9.0. This batch experiment was conducted with the solid-liquid ratio set at 1:7 (w/v, kg/L), and the ethanol concentration was selected from 0 to 30 % (v/v). Moreau and Hicks (2005) found that oil yields were significantly affected by the solvent type and temperature in

Accelerated Solvent Extraction (ASE). The extractor was programmed to extract at a pressure of 1000 psi (69 bar) and extracting ground corn kernels, ground corn bran, ground wet-milled corn germ, and ground dry-milled corn germ with three solvents (hexane, isopropanol, and ethanol), at either 50 or 100°C, with a total of 22 mL of solvent, delivered in three 10-min extractions (3 × 7.3 mL). High temperature and ethanol significantly improved the amount of oil extraction. For instance, the yield of oil extracted from ground kernels with ethanol at 100°C was 65% and 72% higher than yields using isopropanol and hexane, respectively, at 50°C. Again, the yield of oil extracted from ground kernels with ethanol at 100°C was 69% and 10% higher than yields using isopropanol and hexane, respectively, at 100°C.

Espinosa-Pardo et al. (2020) investigated corn germ oil extraction was performed in batch at room temperature (24 °C) and 45 °C with Supercritical Fluid Extraction (SFE) using carbon dioxide and Soxhlet extraction using ethanol and hexane. The highest yield of extraction (37.7 g oil/100g) was obtained by the Soxhlet ethanol method (after continuous extraction of 4h), where the Soxhlet hexane method yielded 23.8g/100g. The discrepancy between hexane and ethanol extraction yields is attributed to the co-extraction of any polar compounds, lipidic or not, that increased the mass of extracted oil. They also found the ethanol-extracted oil was more turbid than the hexane-extracted one, indicating the presence of components at their solubility limit.

2.7. Fractionation

Research has found that the chemical composition of DDGS can be related to particle size, shape, and density (Bhadra et al., 2009). In this context, the fractionation of DDGS can be an important factor in terms of higher oil recovery. Fractionation can be divided into wet fractionation and dry fractionation. Most studies have focused on dry fractionation because it

requires less investment and simpler equipment. Several investigators experimented with various methods of dry fractionation for enhancing DDGS values, including dry milling accompanied by sieving (Wu and Stringfellow, 1982), sieving alone (Wu and Stringfellow, 1986; Liu, 2008), air aspiration (Singh et al., 2002), and a combination of sieving and elutriation (Srinivasan et al., 2005). However, all of them have their limits. Some studies did not get improved nutrients, while others had to use complicated equipment. The combination of sieving and winnowing is known as the elusive process, and so far, it is the most promising of different dry fractionation processes (Srinivasan et al., 2009). In this process, DDGS was first sieved into several fractions and then blown by air. The resulting elimination of small-sized non-fibers can be effective in separating fiber (Srinivasan et al., 2008). After elusive processing, DDGS protein can increase by 2.3% (Srinivasan et al., 2013). However, it required three air classification unit operations, which made the process complex.

Sieving can be a method to separate the various components of DDGS. Sieving was effective in producing fractions with varying compositions. The study by Liu (2008) on particle distribution of DDGS by sieving found that oil content in sized fractions of DDGS samples had an upper trend with an increase in particle size and positively correlated with particle size. This was in contrast to the change of protein content in sized fractions, which had a decreasing trend. Winnowing the sieved fractions was also successful in shifting composition, particularly for larger classes of particles. Liu (2009) recommended a combination of winnowing and sieving as a better choice because it required less time. However, this method still required an air fractionation unit for each size fraction. Aspiration is another method, which has been attempted by researchers (Garcia and Rosentrater, 2012). They sieved DDGS first using screening, and then the oversized fraction was milled into small particles. The milled DDGS was separated using an

aspirator into different fractions. The mixture of the undersize fraction and the low terminal velocity fraction were found substantially enriched in protein. This process was thought to be simpler and less complicated because it needed only one air fractionation unit. However, it still had to use a mill to produce a single stream with a narrow particle size distribution.

Zhang and Rosentrater (2013) conducted a study of fractionation by a pressure destoner to separate DDGS into a light and heavy fraction. When it ran with an air deflection angle of 8° and air-flow rate of 27.5%, they found that the light fraction resulted in 28.2% protein and 10.5% oil, while the heavy fraction had 31.3% protein and 17.2% oil. They also found that particle size distribution showed a positive correlation coefficient (0.93) with oil parameters and a negative correlation coefficient (-0.96) with moisture parameters. Primary sieving connected with aspiration used by Cheng and Rosentrater (2014) has been found to be efficient for condensing protein and oil contents of DDGS. Their research revealed that fractions with higher density, higher airflow rate, and smaller particle sizes improved the efficiency of separation of protein and oil, about 29.7% and 68.2%, respectively. However, Wu and Stringfellow (1982) reported that the protein and oil-rich fractions of DDGS have particles with high-density profiles due to their molecular structure property.

It is evident that fractionation has an impact on DDGS composition and processing. This fractionation technique can be implemented in whole stillage processing as well. Valicor, Inc. developed a unique technology for whole stillage processing dividing it into various fractions. The key purpose of introducing this technique was to improve the quantity of corn oil and other co-products. This technology was defined as Valicor Stillage Fractionation Technology (VFrac) and referred to in US patent No. 8,722,911 (Bleyer et al., 2014). In this VFrac process, the whole stillage was divided into a heavy and light fraction. The light fraction undergoes a hydrothermal

treatment that enables separation of the oil from the protein. Valicor used its patented centrifuge technology designed explicitly for three-phase separation: corn oil, a clarified liquid called slickwater, and a solids fraction termed VFrac solids. The heavy fraction was milled in a disc mill then rinsed with slickwater from the processing of light fraction. Then this mixture was centrifuged using the existing decanter centrifuges. This method washed starch and oil into the liquid fraction. The liquid fraction was used as a backset in the front end of the facility. Next, the oil in the backset was retrieved and returned to the front end of the facility in the VFrac process. A part of the stick water was evaporated, mixed with the wet decanter cake, and dried to form the DDGS. This method resulted in oil yields greater than 1.1lb. /bushel of corn.

CHAPTER 3. OIL RECOVERY FROM DDGS USING ENZYMES

3.1. Abstract

Oil recovered from DDGS (dried distillers grain with solubles) can be a high-value product over animal feed to provide an additional profit to ethanol plants that are currently operating at slim profit margins. To improve the oil recovery from DDGS, enzymatic hydrolysis, in addition to fractionation was considered in this study. A combination of sieving and then air aspiration was used to divide the original DDGS into three different fractions: small, medium, and heavy. Heavier fraction showed up to 24% increased oil content than original DDGS. Different commercial enzymes protease, cellulase, and hemicellulase were tested separately and in combinations at 55°C for 3 hours at 130 rpm to determine their effect on oil recovery from the original and fractionated DDGS. More than 90% of oil recovery was achieved by using a combination of cellulase and protease enzymes. Following enzyme hydrolysis of the sieved aspirated heavy fractions of DDGS, oil recovery was significantly improved by around 20% than original DDGS. Increasing the temperature above 55°C without any enzyme did not impact oil recovery using the heavy fraction DDGS. Overall, fractionation and enzyme hydrolysis showed promise in increasing oil recovery from DDGS. There is a need for optimization using the same approaches with DDGS and other different co-products of the dry-grind process to improve oil recovery without any current ethanol plant design changes.

Keywords: DDGS, Oil recovery, Enzyme, Fractionation, Temperature

3.2. Introduction

Dried distillers grain with solubles (DDGS) is a co-product of ethanol production fermentation that uses dry milling technology. DDGS is generally used as feed for livestock and poultry (Renewable Fuels Association, 2019). However, the ethanol industry has recently

centered on increasing the value of DDGS by manufacturing on-site value-added products. One of the most convenient ways to do this is by recovering DDGS oil. Chrenková et al. (2012) reported that DDGS could be higher in fat content than other feedstock grains. The oil content in the DDGS can range from 9.1% to 14.1% based on different processing methods and corn variety (Liu, 2011). The oil content in the DDGS is a justification for considering the production of corn oil. It was worth mentioning that the production of oil as a by-product of corn ethanol plants would help to sustain and increase the revenues of the ethanol industry (Jessen, 2013). According to the Renewable Fuel Association (RFA) report, more than 2 million tons of corn oil was generated from DDGS in the USA in 2019. This corn oil is commonly used to produce biodiesel (Renewable Fuels Association, 2020).

During dry-grind ethanol processing, the corn is ground, hydrolyzed and fermented, releasing much of the oil and oil from the corn (Rosenthal et al.,1996). Free oil released can be emulsified in the aqueous environment. The dispersed hydrophobic protein could stabilize the oil in the oil-in-water emulsion. Oil bodies released into the aqueous medium can only release free oil when mechanically disrupted, or enzymes are used to hydrolyze the protein and phospholipid layer of the oil body membrane, which protects and maintain the integrity of the oil bodies (Jacks et al.,1990). In aqueous oil extraction, enzymes have been used to increase oil yield by breaking the cell wall and membranes and hydrolyzing the emulsifying proteins (Moreau et al.,2004).

Numerous efforts have been made to develop enzyme-based oil processing technologies from oilseeds, but the high cost of biocatalysts has slowed down technological adoption in the industry (Gaur et al., 2007). Enzyme-assisted aqueous extraction methods have been used to recover edible oil, reduce the use of organic solvents and recover oil from ranging from 53-97% (Rosenthal et al., 1996; Nobrega de Moura et al., 2008; Nobrega de Moura and Johnson, 2009).

Some researches focused on the extraction of oil from CDS (condensed distiller grain) by using different enzymes and showed some effectiveness when enzymes were used in combination and particle size reduction of CDS (Majoni et al., 2011a). The same methods can be implemented to increase oil recovery from DDGS.

The efficiency of enzyme hydrolysis is expected to depend on the size of particles and cell distortion. Particle size reduction enhances the enzyme diffusion rates efficiently act on the substrates (Rosenthal et al.,1996). DDGS particle size ranges from 0.11 to 3.66 mm (Liu, 2009). This high variability in particle size creates a common problem with the even distribution of nutrition in DDGS. Often grinding or sieving can be used for decreasing the particle size and making it uniform. However, grinding alone is not believed to be an efficient form of size reduction for DDGS (Chatzifragkou et al., 2015). Fractionation is an efficient way of dividing DDGS into different fractions of specific particle sizes to condense the oil content. Srinivasan et al. (2005) found that sieving the DDGS into various size categories and then elutriating sieved fractions of larger size classes at appropriate airflow velocities was more effective than sieving alone in separating the fiber from DDGS. They also reported that fractions with smaller particle size had reduced fiber and increased protein and fat contents relative to the original DDGS.

In this context, this research explores the possibility of using commercial enzymes separately and in combination to increase oil recovery from a different fraction of DDGS. The objective of this study was to examine the effect of different commercial enzymes on oil recovery from fractionated heavy fractions of DDGS. The commercial enzymes will be tested for achieving high oil yield on heavy fractions of DDGS produced from the laboratory setting. This experiment will provide insight into how the enzymes influences oil recovery from the DDGS.

Based on oil recovery performance, a fraction of DDGS will be selected for further experimentation.

3.3. Materials and Methods

3.3.1. Raw Materials and Chemicals

The DDGS were collected from NDSU Beef Cattle Barn (3559 19th Ave N #3501, Fargo, ND 58102) and stored at 4-6°C until used in the experiment. To reduce the variability of the DDGS composition, the same batch of DDGS was used throughout the study. Hexane (95% by volume) was purchased from Fisher Scientific (Fairlawn, NJ, USA).

3.3.2. Fractionation of DDGS

Fractionation of DDGS was completed by using a standard sieve and lab aspirator. Initially, the original DDGS was sieved, and then different sieve sizes were aspirated separately. The sieving procedure was conducted based on the American Society of Agricultural Engineers (ASAE) standard method (ASAE Standard, 2003). A 1.0 kg representative sample of DDGS, without any additional processing and moisture adjustment, was sent through selected US standard sieves of Nos.10, 20, 40, and 60, and a pan fitted into a testing sieve shaker (Ro-Tap W. S. Tyler, Mentor, Ohio) with shaking for five-minutes. The mass of material retained on each sieve, as well as on the pan was weighted and recorded. The yield (%) for material retained on each sieve size was also calculated. The sieving procedure was repeated three times for each sample to produce enough sieved fractions for subsequent aspiration. Aspiration was performed using a KICE laboratory aspirator unit, as shown in Figure 6 (Model 6DT4-1, KICE Metal Products Co. Inc., Wichita, KS).

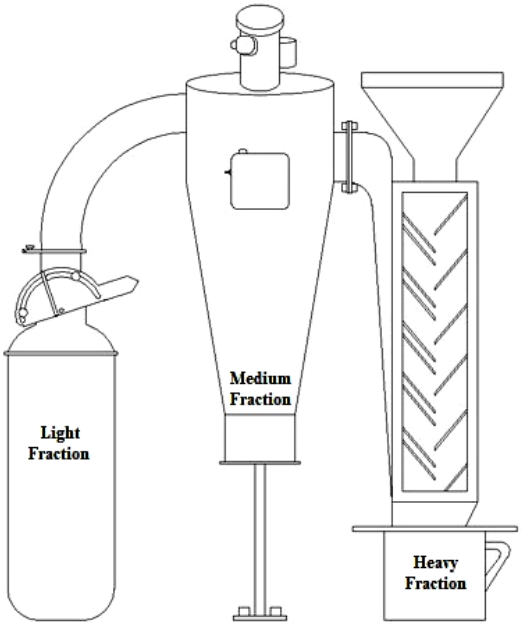

Figure 6. Schematic diagram of the laboratory aspirator (Source: KICE intl.)

The aspirator divided the sieved sample into three fractions based upon density, weight, and particle size. The product was fed by gravity into an open feed hopper. The discharge of the feed hopper was equipped with an adjustable slide gate to regulate product flow. The air baffle was set at a 75-degree angle so that pressure at the inlet of the cyclone was 6.35 mm (0.25-inch) water column.

3.3.3. Compositional Analysis of DDGS

Compositional analyses of fractionating DDGS and original DDGS were conducted to see the effect of fractionation. The original DDGS is termed "original" fraction, in contrast to sieved/aspirated fractions. The original DDGS samples and all fractions (sieved/aspirated) were measured for moisture, ash, protein, and oil content. Moisture contents of DDGS samples were determined by drying in an oven at 105°C for 3 h. (AOAC, 2002). A muffle furnace was set at 550°C for 24 hours to determine the ash content (AOAC, 2002). The combustion method was followed for determining protein contents using a factor of 6.25 to convert percent Nitrogen to

percent protein (AOAC, 2002). To measure the oil content of the sample, an accelerated solvent extractor (ASE200 solvent extractor, Dionex, Sunnyvale, CA, USA) was used according to the official methods (AOCS, 2005). In this AOCS method, 6 g of DDGS samples were mixed with approximately 2 g of diatomaceous earth and grinded it in a small grinder. Then the ground sample placed in an 11–mL sample of extraction cells. The extraction conditions in the cells were as follows: a pressure of 6895 kPa (1000 psi), temperature of 100°C, heat time of 5 min, start time of 10 min, 3 static cycles, 100% flush volume, and purge time of 60 sec. This oil quantification was used as the base to calculate oil recovery. The moisture content was used to convert concentrations of other components (ash, crude protein, and oil content) into a dry matter basis.

3.3.4. Enzymes

In order to investigate the influence of different enzymes on the oil recovery from DDGS, commercial enzyme solutions, NS50013 (cellulase complex), Cellic HTec (hemicellulase), and protease (AP1+) were used. Novozymes North America, Inc. (Franklinton, NC, USA) provided NS50013 and Cellic HTec. The protease was collected from a local bio-refinery. According to the manufacturer information sheet, the optimum temperature for cellulase complex NS50013 and Cellic HTec is in the range of 45-50ºC and 50-55ºC, respectively. AP-1+ has a broad operating range for enhanced process flexibility and performs well within the temperature ranges typical in whole ground corn batch fermentations. Regarding operating pH, the range for NS50013 is from 4.5 to 6.5, protease is from 3 to 5, and Cellic HTec is from 4.75-5.25.

3.3.5. Design of Experiment

The original DDGS sample was experimented with protease, cellulase, and hemicellulase to explore the effect of different enzymes. All enzymes were used both as single and

combination. Two heavy fractions DDGS, which were rich in oil content (selected based on its yield and composition) experimented with protease and cellulase, applied as single and combination. In both experiments, the temperature was set at 55ºC, and the control treatment had no enzyme but treated with the same temperature condition. Enzyme dosage was fixed at 5% (v/w). A follow-up experiment was conducted to explore the temperature effect on oil recovery. Original DDGS and one heavy fraction DDGS (showed better oil recovery in the previous experiment) were experimented with no enzyme condition but varying the operating temperature from 55-75ºC at 5ºC interval.

3.3.6. Enzymatic Hydrolysis

Enzyme hydrolysis was carried out at the optimum conditions for each of the enzymes, and each treatment was done in three replicates. Treatments were conducted in a 50-mL centrifuge tube using the calculated amount of DDGS to have at least 10g of dry matter DDGS in the tube. The dry matter content of the DDGS was adjusted to 30% by adding the calculated amount of de-ionized water. The enzyme dosage was calculated based on the solids content (dry basis) of the DDGS. After adding the enzyme in the tube, the pH of the solution was checked. If the pH was less than 4.0, the buffer solution was used. Then, the 50-mL centrifuge tubes were heated up to a specific temperature (as described in section 3.5) in a shaker water bath (MaxQ 7000, Thermo Scientific, Dubuque, Iowa), and the tubes were kept in the water bath for 3 hours at 130 rpm.

3.3.7. Oil Separation

Following enzyme hydrolysis, oil separation was carried out by centrifugation (8500 x g) at 10,000 rpm for 10 min (Allegra X-15R Benchtop Centrifuge, Beckman Coulter, Fullerton, CA, fitted with a FX6100 Fixed-Angle Aluminum Rotor, 25 degrees fixed angle,6.65 cm radius

at 11,200 rpm, 11,400 x g). Then, the top layer was washed several times using hexane. Some oil with solid residues were placed in the micro centrifuge tube and centrifuged at 10,000 rpm for 5 min (Galaxy 16 micro centrifuge, VWR International, Bristol, Conn.) to remove the solid residues. The hexane and oil mixture was then transferred to pre-weighed round-bottom vials with the help of transfer pipettes. The solvent was removed by an evaporation system equipped with a water bath (Microprocessor Controlled 280 Series, Thermo Electron Corporation) at 60ºC. Any residual solvent remaining in the mixture was removed using a vacuum oven. The weight of the oil was then determined gravimetrically. Oil recovery was calculated based on the oil content determined by the accelerated solvent extraction method for the specific fractions of DDGS used. Different steps of oil separation described in this section is illustrated in Appendix Figure A1.

3.3.8. Analysis of Defatted DDGS

After oil separation, samples of defatted DDGS were analyzed for CP (crude protein), NDF (neutral detergent fibre), and ADF (acid detergent fibre). All samples were analyzed in the Nutrition Laboratory, Department of Animal Sciences, North Dakota State University. The combustion method was followed for determining protein contents using a factor of 6.25 to convert percent Nitrogen to percent protein (AOAC, 2002). Percent NDF and ADF were calculated using standard methods. (AOAC, 2002.04; AOAC 973.18). The moisture content of the samples was used to convert concentrations of these components (CP, NDF, and ADF) into a dry matter basis.

3.3.9. Statistical Analysis

Each experiment was treated as an individual trial with a completely randomized treatment design. Statistical analysis was performed using the general linear model procedures of SAS 9.1 (Cary, NC, USA). Analysis of variance (ANOVA) was used to determine significant

differences among the different treatments within an experiment. The least significant differences (LSD) test was also conducted for pair-wise comparisons when there was a significant effect at $p < 0.05$ based on the ANOVA. All treatments were carried out in replicates, and results are reported as the means of replicates ± standard deviation (SD).

3.4. Results and Discussion

3.4.1. Effect of Fractionation

The fraction of DDGS was completed with the help of standard sieving and a laboratory aspirator. Two primary points that need to be discussed in this aspect. First, the yield of different DDGS fractions. Second, the composition of a different yielding fraction. The result of these fractionation operations and the composition of different yielding DDGS helped us decide which portion is suitable for oil recovery.

3.4.1.1. Yield percentage of sieving and aspiration

Table 1 presents the yield percentage of DDGS in different sieves. DDGS was divided into four fractions, excluding the pan (above No. 10, between No. 10–20 mesh, between No. 20–40, and between No. 40–60) by sieving. The particle sizes from the 0.42–0.84 mm sieve had the most occurrence, approximately 55%, followed by the particle sizes from the 0.84–2 mm sieve, with an occurrence of approximately 22%. The average particle size of the original DDGS was found to be about 0.34 mm.

Table 1. Yield percentage of different fraction of DDGS in sieving operation

Sample	Us Sieve Size	Sieve Opening (mm)	Yield Percentage (%)*
1	No.10	>2.00	1.80±0.40
2	No. 10-20	0.84-2.00	22.22±4.47
3	No. 20-40	0.42-0.84	55.44±11.58
4	No. 40-60	0.15-0.42	19.68±6.61
5	<No. 60 (Pan)	<0.15	0.73±0.03

*Mean of triple measurements ± standard deviation

This result contradicts the findings of Cheng et al. (2014), where the highest yield was found in the particle size with 0.84–2.00 mm sieve. However, the DDGS used for their experiment was much bigger (average size 0.75mm) than the DDGS used in the present experiment. This is also an indication of the variability of DDGS particle size that makes DDGS a challenging feedstock for oil recovery.

Particle sizes for DDGS samples 2 and 3 (Table 1), which had the first and second highest yield from the sieving operation, were chosen for aspiration. The aspirator fractionated the original DDGS, Number 20-sieved DDGS (sample 2), and Number 40-sieved DDGS (sample 3) into three fractions: heavy, medium, and light fractions. Table 2 indicates the yield percentage of different fractions of DDGS with the air baffle of the aspirator set at a 75° angle with 6.35 mm (0.25-inch) water column pressure.

Changing this air pressure had an impact on the yield and composition of different fractions of DDGS. The stated air pressure was selected from a preliminary experiment, mainly focusing on the fiber and oil content of different fractions with different air pressure to have a reasonable yield of all three fractions. We did this because it was convenient to use the other fractions (mainly medium and light fraction for fiber separation). The target was to have the highest yield (%) in the medium fraction and the lowest in the light fraction. This target was set prior to the preliminary experiment results, which showed the heavy fraction was rich in oil content, and the medium fraction was rich in fiber content. Srinivasan et al. (2009) reported that a large sample of DDGS is required to have a right amount of fiber (only 4-15% of fiber, depending on particle size, can be separated from the whole sample).

Table 2. Yield percentage of different fraction of DDGS in aspiration operation

Aspiration Sample	Fraction	Yield (% Whole Fraction)*
Original DDGS	Heavy	12.42±3.67
	Medium	77.40±5.67
	Light	9.36±4.79
Number 20-sieved DDGS Sample 2 (No. 10-20)	Heavy	41.41±6.05 (9.29)
	Medium	44.36±4.15 (9.86)
	Light	12.84±8.67 (2.85)
Number 40-sieved DDGS Sample 3 (No. 20-40)	Heavy	8.10±4.39 (4.44)
	Medium	70.75±2.80 (39.23)
	Light	18.54±7.40 (10.28)

*Mean of triple measurements ± standard deviation

The desired result was achieved when the original DDGS was aspirated using the stated air pressure setting (aspirator's air baffle was set at 75° angle with 6.35 mm water column pressure)**.** The heavy fraction yield of original DDGS was about 12%, whereas the medium fraction yield was about 77% (Table 2). An interesting result was found when DDGS sample 2 and 3 from sieving operation were aspirated. In both cases, the medium fraction had the highest yield of 44% and 70%, respectively. For number 40-sieved DDGS (sample 3), the heavy fraction had the lowest yield. This shows that the DDGS sample had a smaller particle size in number 40-sieved DDGS (sample 3) than the original DDGS. The same trend followed when smaller particle size DDGS was aspirated (number 60-sieved DDGS [sample 4]; not documented). However, a considerable yield of heavy and medium fractions was found in the number 20-sieved aspirated sample (41 and 44%, respectively).

3.4.1.2. Composition variation in different fraction of DDGS

The variations of moisture, ash, oil, and crude protein are illustrated as a bar graph in Figures 7, 8, 9, and 10, respectively. Changes in moisture, ash, protein, and oil content of each DDGS fraction were affected by the sieving and aspiration operations. The composition of these

fractions shifted because the aspiration process separates the nutrients depending on their different densities. The straight-line in each figure represented corresponding Y-axis properties such as moisture, ash, oil, and protein content of the original DDGS sample, with whom the fractionation process was operated.

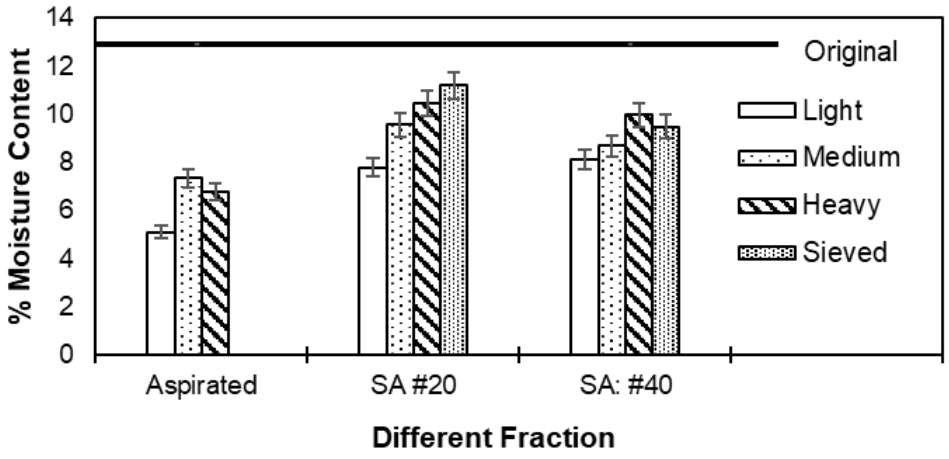

Figure 7. Moisture content variation of different fraction

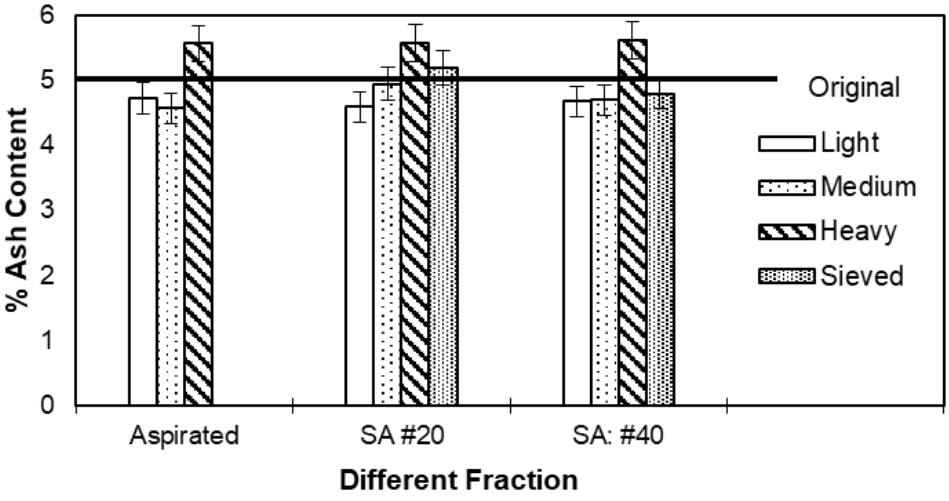

Figure 8. Ash content variation of different fraction

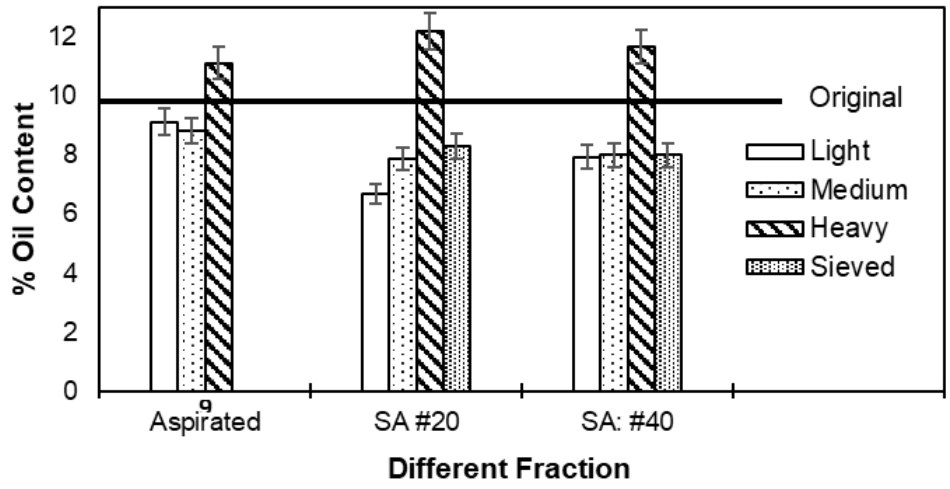

Figure 9. Oil content variation of different fraction

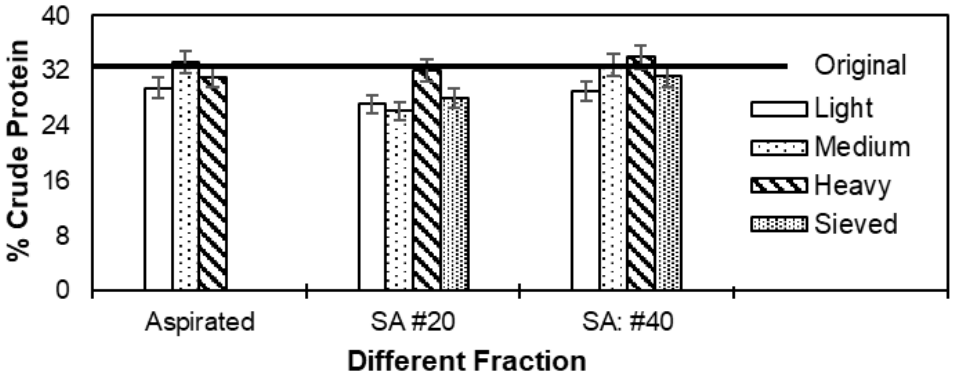

Figure 10. Crude protein variation of different fraction

Figure 7 shows that moisture content is reduced in the different fractions compared to the original because of the fractionation process. This is likely to exposure to dry air in the lab during fractionation. Cheng et al. (2014) explained that during aspiration, the lighter DDGS was blown further, and the time DDGS was in contact with air was quite long, which suggests that aspiration can be regarded as a partial drying process. For ash content (Figure 8), the heavier fractions of aspirated, number 20, and 40 DDGS has more ash content than the original. It was evident from Figure 8 that the process of aspiration increased ash content, which may lead to higher oil concentration. However, the oil content of the fraction also follows the same trend as the ash content (Figure 9). The heavier fraction of aspirated, number 20 (sample 2), and number

40 (sample 3) DDGS had more oil content of 14, 24, and 19%, respectively, compared to the original DDGS. This finding was quite similar to Liu's study (2008). He found that oil content in sized fractions for most DDGS samples had an upper trend with an increase in particle size. Crude protein increased in number 40 heavy fraction by 4%, but reduced by 1.5% in number 20 heavy fraction (Figure 10) compared to the original. However, Srinivasan et al. (2005) reported that fractions with smaller particles increased protein contents relative to the original DDGS, supporting present findings.

After analyzing the yield and composition of different fraction of DDGS, two fractions were selected for further experimentation. They were Number 20 (sample 2) and 40(sample 3)-sieved aspirated heavy fraction DDGS (Figure 11). The differences in the physical appearances of the three different samples are visible from Figure 11. Number 20 sieved aspirated heavy fraction DDGS had the darkest color, referring to the presence of more oil than other samples. Number 40 sieved aspirated heavy fraction DDGS had the smallest particle size among the three samples showing finer fraction than other samples.

Figure 11. Samples used for different experiments

3.4.2. Effects of Different Enzymes on Oil Recovery

Original DDGS was used to experiment with the effect of different enzymes in single and combination. The enzyme concentration was fixed at 5% (V/W) for all the treatments. The effect of different enzymes (in single and combination) on oil recovery from original DDGS is shown in Table 3. The highest oil of 94% was recovered with the combined treatment of 5% protease, cellulase and hemicellulase. However, it was not significantly different from the combined treatment of 5% protease and cellulase with 93% oil recovery.

Table 3. Effects of different enzymes (in single and combination) on oil recovery from original DDGS

Treatment (% Enzyme, v/w)*	% Oil ± SD**	% Oil Recovery
5% Protease	7.57 ±0.38$_b$	77.34
5% Cellulase	7.35 ±0.11$_b$	75.03
5% Hemicellulase	6.79 ±0.24$_c$	69.39
5% Protease +5% Cellulase	9.13 ±0.08$_a$	93.19
5% Protease +5% Cellulase +5% Hemicellulase	9.23 ±0.13$_a$	94.29
Control	6.44 ±0.23$_c$	65.76

*Enzyme dosage was based on the solids content of DDGS.
**Means with different letters are significantly different ($P< 0.05$)

Oil recovered using only protease and cellulase is not substantially different from each other. Only the hemicellulase had a low impact on oil recovery. It had almost the same effect as the control sample. The cell wall of the corn kernel is comprised of hemicelluloses and celluloses, but no pectin (Karvolic et al., 1994); therefore, it was reasonable to use the cellulase and hemicellulase enzymes for oil recovery.

However, Moreau et al. (2004) observed that increasing levels of some cellulases in the hydrolysis of corn germ cell wall components resulted in no obvious trend for increased oil recovery. This suggests that it may be necessary to use cellulases in combination with proteases to increase oil recovery significantly. Proteases hydrolyze the proteins and destabilize the oil-in-

water emulsion, releasing free oil that can be separated and recovered. Since cellulases are effective in breaking down the cell wall polysaccharides and facilitate oil body release (Rosenthal et al., 1996), oil may not have been freed without protease treatment. The control samples (no-enzyme treatments) had around 65% oil recovery, due to the incubation of samples in high temperatures that may break some of the DDGS oil-in-water emulsion that freed a small amount of oil. The combination of protease and cellulase gives 28% more oil than no enzyme treatment (Table 3). However, there will be debate over the economic benefit of purchasing the enzymes and revenue from the additional oil.

3.4.3. Effect of Different Fraction of DDGS on Oil Recovery

Sieved aspirated heavy fractions of DDGS (number 20 and number 40 sieve size) were used to experiment with the effect of different enzymes (excluding hemicellulose from the last experiment) in both single and combination on oil recovery. Table 4 shows the result of oil recovery from different fractions of DDGS using different enzyme treatments. The enzyme concentration was fixed at 5% (V/W) for all the treatments like the previous experiment. From the following table, we can see that a combination of protease and cellulase did achieve 100 percent oil recovery from both fractions.

Table 4. Effects of different fractions of DDGS on oil recovery using different enzymes

Treatment (% Enzyme, v/w)*	% Oil Recovery± SD** (Sieved Aspirated Heavier fraction of DDGS)	
	Sieve 20 (0.841mm)	Sieve 40 (0.420mm)
5% Protease	$87.23\pm1.03_{bc}$	$89.69\pm4.87_{ab}$
5% Cellulase	$95.19\pm3.61_{b}$	$93.57\pm5.36_{ab}$
5% Protease + 5% Cellulase	$109.86\pm2.99_{a}$	$98.59\pm1.49_{a}$
Control (No enzyme)	$85.67\pm4.91_{c}$	$83.61\pm4.76_{b}$

*Enzyme dosage was based on the solids content of DDGS.
**Means with different letters are significantly different ($P< 0.05$).

From Table 4, it was evident that protease did not work well on fractionated DDGS, having oil recovery not significantly different from the control sample. This was likely due to protease liberating the oil attached to solid surfaces such as cellular debris or proteins and emulsified oil droplets. However, the protease did not make the oil more available for separation. On the other hand, cellulase worked very well on both fractions recovering around 95% of the oil. Because adding cellulase opens up the cellular debris, releasing oil trapped in the complex cellulosic structure results in higher oil recovery. Comparing the control treatments with the previous experiment with the original DDGS showed that fractionation improved oil recovery significantly. Oil recovery was almost 20% higher in the control treatment of sieved aspirated heavy fractions than the original DDGS (Tables 3 and 4). This was probably due to the specific particle size of fractionated DDGS, which condensed the oil from the original DDGS by the eliminating small and medium-sized fibers and other particles. The aspirated heavy fractions has more oil content as shown in Figure 9. This may have contributed to the high oil recovery. Previous literature also showed that oil contents are positively correlated with particle size (Liu, K. 2009), which supports findings of this study.

3.4.4. Effect of Different Enzyme Treatments on Composition of Defatted DDGS

Sieved aspirated heavy fractions of DDGS (number 20 and number 40 sieve size) were used to experiment with the effect of different enzymes in both single and combination on oil recovery. After recovering oil, the defatted DDGS was analyzed for crude protein (CP), natural detergent fibre (NDF) and acid detergent fibre (ADF). The variations of CP, NDF, and ADF are illustrated as a bar graph in Figures 12, 13, and 14, respectively.

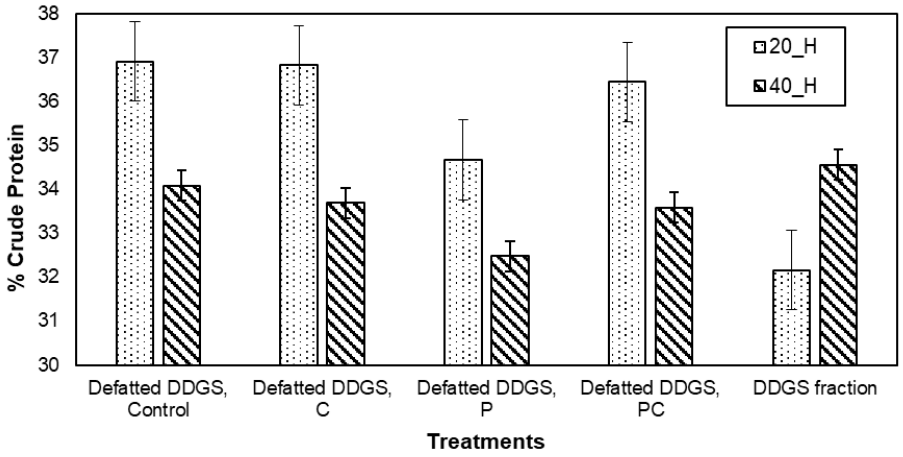

Figure 12. Effect of different enzymes on the crude protein variation in defatted DDGS (The error bars represent standard deviations; C- 5%Cellulase, P-5% Protease, PC-5% Protease + 5% Cellulase, Control: No enzyme; Fractions, 20_H: Number 20 sieved aspirated heavy fraction and fractions, 40_H: number 20 sieved aspirated heavy fraction)

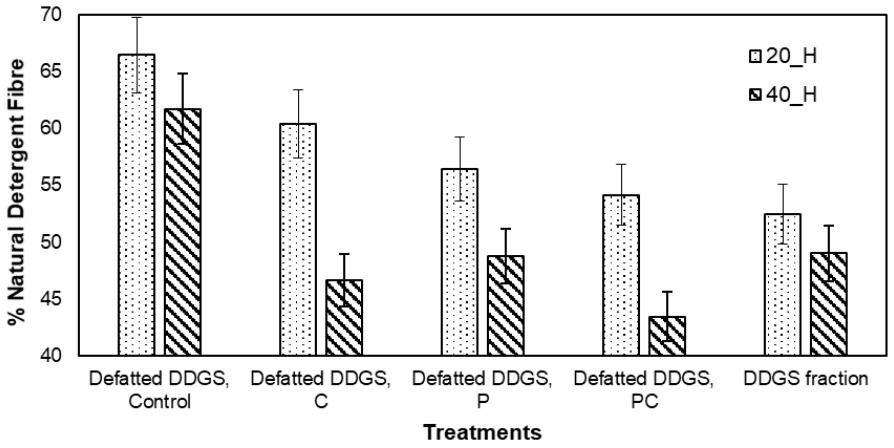

Figure 13. Effect of different enzymes on the NDF variation in defatted DDGS (The error bars represent standard deviations; C- 5%Cellulase, P-5% Protease, PC-5% Protease + 5% Cellulase, Control: No enzyme; Fractions, 20_H: Number 20 sieved aspirated heavy fraction and fractions, 40_H: number 20 sieved aspirated heavy fraction)

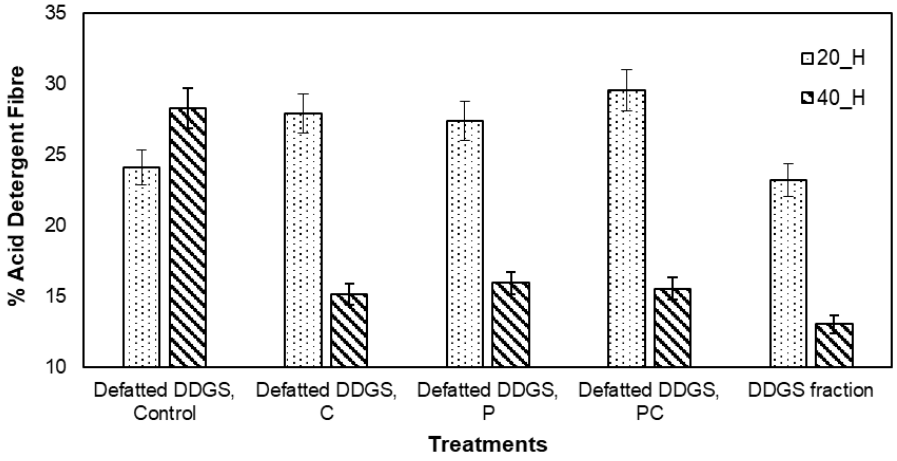

Figure 14. Effect of different enzymes on the ADF variation in defatted DDGS (The error bars represent standard deviations; C- 5%Cellulase, P-5% Protease, PC-5% Protease + 5% Cellulase, Control: No enzyme; Fractions, 20_H: Number 20 sieved aspirated heavy fraction and fractions, 40_H: number 20 sieved aspirated heavy fraction)

Figure 12 shows that crude protein is reduced in the heavy fractions of number 40 sieved aspirated sample compared to the number 20 sieved aspirated heavy fraction. Percent crude protein amount ranges from 32-37% regardless of the treatments. Due to enzymes activity, more crude protein is observed in the number 20 sieved aspirated heavy fraction than number 40 sample. Particle size plays an important role here. More protein may be hydrolyzed in the small particle sized DDGS compared to large particle sized DDGS. However, this does not have any effect on the oil recovery from these fractions. Proper crude protein levels are essential for the many types of livestock that rely on them for nutrition. When the percentage of CP is low, the bacteria responsible for digestion cannot sustain adequate levels to process forage. Ultimately, the animal's intake and digestibility are reduced. Therefore, enzyme treatments (including control; heat treatment only) in number 20 sieved aspirated heavy fraction DDGS did improve crude protein percent of the defatted DDGS.

For NDF content (Figure 13), the heavier fraction of aspirated, number 20, and 40 DDGS has less NDF than the control treatment. Reduced NDF is observed (except control) in the number 40 sieved aspirated heavy fraction after the enzymatic hydrolysis. However, opposite

trend is followed in the number 20 sieved aspirated heavy fraction after the enzymatic hydrolysis. No enzyme treatment (control) leads to a very high NDF content. As the NDF percent increases, generally the dry matter intake, and metabolic and digestive energy decreases; which is not favorable for a livestock feed requirement.

It was evident from Figure 14 that enzymes did work for decreasing ADF in number 40 sieved aspirated heavy fraction and increasing in number 20 sieved aspirated heavy fraction. However, increasing ADF reduces the ability to digest the feed. The difference between NDF and ADF is also a good indicator of feed product; reflecting the amount of hemicellulose in the feed product. From this analysis, it was found that (Figures 13 and 14) enzyme treatments with combination of cellulase and protease resulted in lower hemicellulose than other treatments for the both fractions. As hemicellulose content increases in animal feed, the voluntary feed intake typically decreases.

From the discussions of the Figures 13, 14, and 15, enzyme treatments illustrates a considerable impact in the variation of percent CP, NDF and ADF value of the defatted DDGS. This outcome will certainly help the biorefinery to produce both oil and livestock feed product from DDGS to improve the economics of the ethanol plant.

3.4.5. Follow-up Experiment (Effect of Temperature)

Based on the last experiment results, higher oil recovery (83-85%) was found in control treatments of sieved aspirated heavy fraction. Control treatment had no enzymes, only treated at the temperature at 55°C. Therefore, we hypothesized that increasing temperature may increase the oil recovery from the original and sieved aspirated heavy fraction. Because heating provides the energy required to break emulsion and possibly weaken physical interactions between protein and lipid or carbohydrates and lipid such that oil recovery may be increased (Xu et al., 2007). A

follow-up experiment was set to determine the effect of temperature on oil recovery from sieve aspirated (SA#20) heavy fraction and original DDGS. Temperatures were increased with 5°C interval from 55°C up to 75°C.

Figure 15 presents the findings of this follow-up experiment. It was evident that increasing temperature over 55°C did not increase the oil recovery from any of the DDGS samples used. The decrease in oil recovery with increasing temperature, however, did not decrease significantly. The lowest oil recovery were found at 65°C and 70°C, for sieve aspirated (SA#20) heavy fraction (77%) and original DDGS (55%), respectively. Another interesting point to be noted that as the temperature increases after 65°C, more deviations in the oil recovery were found. It indicated that the oil recovery process was very unstable as the temperature increases.

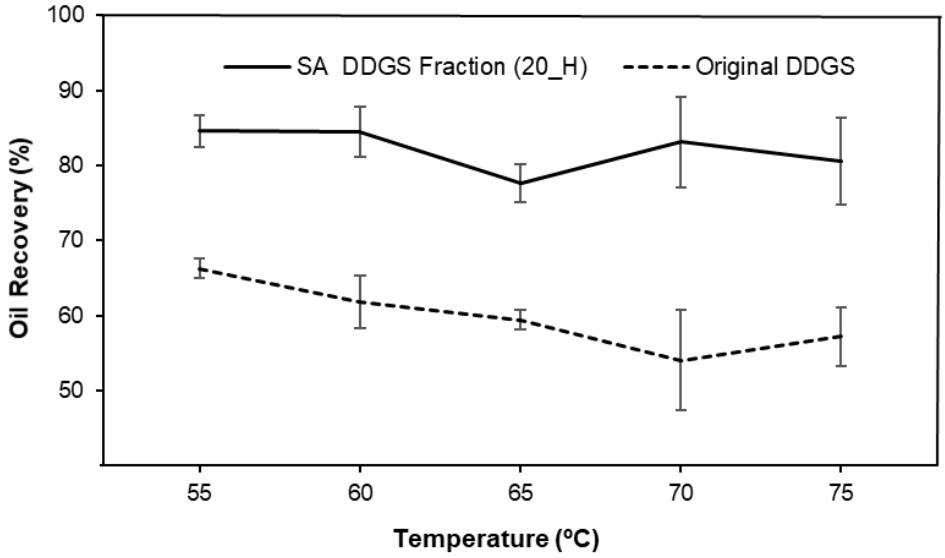

Figure 15. Effect of temperature on oil recovery from Sieve Aspirated (SA #20) Heavy fraction and original DDGS
(The error bars represent standard deviations)

These results were almost identical to those of Majoni et al. (2011b). They found a rise in temperatures from 25°C to 59°C increased oil recovery from CDS. Most of the oil can be in the form of an oil-in-water emulsion containing proteins and phospholipids acting as emulsifiers. Heating is a functional way of demulsifying (Chabrand et al., 2008) when protein denaturation

occurs. Thus, a temperature of 55°C resulted in the maximum rupture of the oil-in-water emulsion. As temperature rises, free minute oil droplets trapped on the hydrophobic surface and free intact oil bodies or oil bodies in intact cells have not been damaged by heating. The free oil cannot be recovered. This may be the reason for not increasing oil recovery with an increase in the temperature.

3.5. Conclusion

Fractionation has a positive impact on the composition of the resulted fraction of DDGS, especially on the oil content of the original DDGS. Different enzymes were experimented with both in single and in combination to compare the oil recovery from original DDGS. At 5% enzyme concentration, a combination of protease and cellulase resulted in a high oil recovery similar to the combination of protease, cellulase and hemicellulase. Therefore, there is no need for additional hemicellulase, since it had a low impact on oil recovery when used alone. The combination of protease and cellulase increased oil recovery from original DDGS by 28% compared with no enzyme. However, the cellulase enzyme almost similarly to the combination of protease and cellulase by recovering more than 95% of oil from sieved aspirated heavy fractions. It will be more cost-effective for the biorefinery industry to use only one enzyme instead of combinations. When treated at 55°C without enzymes, the oil recovery in the fractionated sample were increased by 20% compared to original DDGS. Increasing the temperature after 55°C, however, did not help to improve oil recovery. For future experimentation, different concentrations of enzymes and different operating conditions can be used to optimize the amount of enzymes for maximum oil recovery from DDGS.

CHAPTER 4. OIL RECOVERY USING ETHANOL

4.1. Abstract

Bio-refineries are seeking efficient techniques for recovering oil from the corn ethanol co-products. Ethanol can be a useful solvent to recover the corn oil in the co-product of the dry-grind corn ethanol process, especially from distillers' dried grains with solubles (DDGS) and whole stillage (WS). This study determined the effects of 190 proof ethanol on oil recovery from DDGS and oil partitioning from WS on a laboratory scale. Ethanol was mixed with original and heavy fractionated DDGS in different operating temperatures (Room temperature, 30ºC, 40ºC, and 50ºC) and solid loadings (20%, 30%, and 40%), and their effects on oil recovery were evaluated. More than 90% of the oil was recovered from the heavier fraction of DDGS using ethanol at 30ºC with 30% solid loading. The WS was also mixed with ethanol at 20% solid loadings and incubated at room temperature (~20 ºC) and 50 ºC to evaluate the oil distribution in the liquid and solid phases from WS. Ethanol treatment resulted in an 8–10% higher wet yield of liquid fraction and a 17–20% oil increase in liquid fraction than the other treatments. It is also notable that both temperature and solid loading had positively impacted oil partitioning from WS. This study shows that ethanol can improve oil recovery from DDGS and oil partition in WS by varying different process conditions. This outcome is beneficial to ethanol plants by increasing corn oil yield using their existing setup and in-situ product (ethanol).

Keywords: DDGS, Whole Stillage, Oil Recovery, Ethanol, Solid Loadings, Temperature

4.2. Introduction

Corn distillers oil (CDO) is produced from the coproducts of the ethanol production process. One of the coproducts, called whole stillage (WS), is obtained after fermentation and separation of ethanol. After the fermentation of ethanol, corn oil is divided between the liquid

and solid fractions using a centrifuge. The liquid fraction is comparatively richer in oil than the solid fraction on a dry weight basis, so that the oil can be derived from the liquid fraction. The dried distiller grain with soluble (DDGS), among the different fractions of the whole stillage, is the most commonly used feedstock for oil recovery.

Considering the composition and annual production of DDGS, its value is underrated and is primarily used in the formulation of animal feed (YANG and Rosentrater, 2015). DDGS contains 8–10% of the oil (based on dry weight), which is higher than the amount required for animal feed (Nježić et al., 2018). Removal of corn oil affects the nutritional profile of the DDGS, primarily by reducing the crude fat content (around 4-5%) and make it favorable to use in animal feed formulation (US Grains Council, 2018). Technology of corn oil extraction prior to the manufacturing of DDGS is already available to the majority (over 90 percent) of U.S. ethanol plants. In this process the oil recovery generally ranges from 80-85% (data collected from Hankinson Renewable Energy, LLC). So, there is potential for recovering more 10-15% oil from DDGS that can provide an additional profit to the ethanol plants and improve feed quality of DDGS.

Regarding the environmental and economic aspects of corn oil, many laboratory-scale eco-friendly processes have been studied, including aqueous enzymatic processes (Dicky et al., 2008, 2010; Karlovic et al., 1994; Moreau et al., 2009) and supercritical fluid extraction (Rebolleda et al., 2012; Shi et al., 2015). The aqueous enzymatic approach, however, has economic concerns that have limited its large-scale industrialization. The usual high dosage (1~2 percent (w / w)) of enzymes caused high production costs, which were not economically feasible. Consequently, the research into the better recovery of aqueous corn oil to solve this issue appears to be reasonable.

Ethanol is a non-toxic and effective emulsion splitter used in vegetable oil separate emulsion to recover free oil (Chi et al., 2014). In the internal reaction mechanism, the diluted aqueous ethanol solution might adjust the local emulsion micro-environment, causing the structure of organized molecular water around emulsion drops to collapse (Farrell Jr. et al., 1990). Thus, before forming a stable oil-in-water state, all slurry emulsions have been demulsified into free oil, which improves the free recovery of oil and considerably shortens the incubation time. Besides, ethanol is readily available to the bio-refinery industry. Therefore, no additional cost is required for solvent storage and transportation that is beneficial for the industry to choose this option for a profit. However, there will be some additional cost for purification of the oil by separating the solvent (ethanol).

Traditional aqueous and aqueous enzymatic approaches used water as a medium of extraction. Water helps to dissolve soluble cellular materials (primarily proteins) and release oil into the water phase, from which the oil could be extracted by centrifugation. However, the recovered oil was found in the form of a cream emulsion (Campbell and Glatz, 2009). A stable emulsion must be broken to recover free oil; otherwise, the yield cannot reach the highest level. High concentrations of ethanol have been reported to be commonly used as a demulsifier (Chi et al., 2014).

The research of the recovery of oil from DDGS began at the end of the twentieth century and, as a result, there is currently little literature available. Approximately 50% of crude oil could be extracted from DDGS using a 6:1 ethanol-to-DDGS ratio in a single-stage extraction reported by Singh and Cheryan (1998). Randhava et al. (2004) described oil recovery from corn fermentation co-products using ethyl acetate and isopropanol acetate. Bruinsma et al. (2012)

described a method of using hexane to extract oil from DDGS to achieve at least 77% oil recovery.

The use of n-hexane, however, is limited to whole stillage with a lower oil content (Nježić et al., 2018). Extraction of oil with an organic solvent is the most useful process for a low oil content product such as whole stillage containing around 2–3% oil (17–18% on db). Hexane-type naphtha is the most commonly used solvent and is typically favored by the oil refining industry (Thiex et al., 2003). Noureddini et al. (2009) used hexane to optimize the conditions for the extraction of oil from the whole stillage samples. The percentage of the extracted oil for the whole stillage at the solvent/substrate mass ratio of 0.20 was 9.8 wt. % (db.) after 30 min, which increased to 10.5 wt. % (db.) after 240 min. Consequently, it is assumed that the inclusion of ethanol with whole stillage could enhance oil partition in thin stillage.

Based on the aforementioned findings, this study investigates the potential to use ethanol in varying conditions to increase oil recovery from DDGS and oil partition from whole stillage to thin stillage (liquid fraction). The first objective was to analyze the effect of heavy fraction of DDGS on oil recovery using 190 proof (95% by volume) ethanol. The second objective of this study aimed to determine the effects of ethanol (190 proof, 95% by volume) addition with whole stillage on oil partitioning. A selected fraction of DDGS and whole stillage were tested with ethanol. Different operating conditions (e.g. type and degree of hydration of solvent and temperature) were examined to achieve higher oil yields compared to original DDGS and more oil partition in liquid fraction of whole stillage.

4.3. Materials and Methods

4.3.1. Raw Materials and Chemicals

The DDGS were collected from NDSU Beef Cattle Barn (3559 19th Ave N #3501, Fargo, ND 58102) and stored at 4-6°C until used in the experiment. To reduce the variability of the DDGS composition, the same batch of DDGS was used throughout the study. The original DDGS was fractionated by using a standard sieve shaker (Ro-Tap W. S. Tyler, Mentor, Ohio) and KICE laboratory aspirator unit (Model 6DT4-1, KICE Metal Products Co. Inc., Wichita, KS). Details procedure of sieving and aspiration were described in Section 3.4.2. Based on fractionation yield and their composition, 20-sieved aspirated heavy fraction DDGS were selected for this experiment.

Both whole stillage and ethanol (200 proof) were collected from a local biorefinery plant (Hankinson Renewable Energy, LLC). Ethanol was then diluted to 190 proof (95% by volume) using de-ionized water for the experiment. The whole stillage was stored in the room freezer at a temperature of (-18°C to -20°C). Prior to the experiment, the sample was defrosted to a normal room temperature. For homogeneity of the sample, it was mixed vigorously before use.

4.3.2. Proximate Analyses

The original DDGS is termed "original" fraction, in contrast to sieved/aspirated fractions. The DDGS and the whole stillage samples were measured for moisture, protein, and oil content. Moisture contents of the samples were determined by drying samples in an oven at 105°C for 3 h. (AOAC, 2002). The combustion method was followed for determining protein contents using a factor of 6.25 to convert % Nitrogen to % protein (AOAC, 2002). To measure the oil content of the sample, an accelerated solvent extractor (ASE200 solvent extractor, Dionex, Sunnyvale, CA, USA) was used according to the official methods (AOCS, 2005). In this AOCS method, 6 g of

dried samples were mixed with approximately 2 g of diatomaceous earth and placed in an 11–mL sample extraction cells. The extraction conditions in the cells were as follows: a pressure of 6895 kPa (1000 psi), temperature of 100°C, heat time of 5 min, start time of 10 min, 3 static cycles, 100% flush volume, and purge time of 60 sec. This oil quantification was used as the base to calculate oil recovery. The moisture content was used to convert concentrations of other components (oil and crude protein content) into a dry matter basis.

4.3.3. Oil Recovery from DDGS Using Ethanol

Oil was recovered from DDGS using aqueous ethanol as a solvent, according to Ni et al. (2016). Batch experiments were carried out in 125 mL Erlenmeyer flasks, which were kept in a constant temperature water bath (MaxQ 7000, Thermo Scientific, Dubuque, Iowa). Each treatment was carried out with 10 g of dry DDGS sample. Ethanol (95% by volume) was mixed with the DDGS fractions according to different solid loading of 20%, 30%, and 40%, respectively. The experiment was conducted for 2 h at 130 rpm with different temperatures (Room temperature, 30ºC, 40ºC, and 50ºC). The mixtures were added into Erlenmeyer flasks and both the time and shaker were started after it reached its desired temperature. To prevent any mass loss due to evaporation, the Erlenmeyer flasks were closed with the aluminum foil paper. After incubation, all mixtures were poured into 100 mL centrifuge tubes and centrifuged (Allegra X-15R Benchtop Centrifuge, Beckman Coulter, Fullerton, CA, fitted with a SX4750 swinging bucket rotor, 20.78 cm radius at 4750 rpm, 5250 x g) at 25°C with 4500 rpm for 20 min. To remove the solid residue, the centrifuged mixture was transferred to pre-weighed round-bottom vials using a Buchner funnel and 8-µm filter paper (Whatman No. 2). Free oil was measured by weighing after evaporation of the solvent (ethanol) from the vials. The solvent was removed by an evaporation system equipped with a water bath (Microprocessor Controlled 280 Series,

Thermo Electron Corporation) at 60ºC. Any residual solvent remaining in the mixture was removed using a vacuum oven. The weight of the oil was then determined gravimetrically. Different steps of oil separation described in this section is illustrated in Appendix Figure A2. Oil recovery was calculated based on the oil content determined by the accelerated solvent extraction method for the specific fractions of DDGS used. The yield of oil was expressed using equation (1):

$$\text{Yield (\%)} = \frac{\text{free oil weight (g)}}{\text{total oil in DDGS (g/10g DDGS)}} \times 100 \quad (1)$$

4.3.4. Oil Partitioning Experiment

Specified amount (87.50g) of whole stillage was transferred in Erlenmeyer flask, which were kept at a constant temperature water bath (MaxQ 7000, Thermo Scientific, Dubuque, Iowa). Before placing the whole stillage sample, vigorous mixing was performed in the storage bottle to ensure having a homogenous sample for the experiment. Each treatment was carried out for 2 h at 130 rpm with specified temperature. Two temperatures were selected, one was room temperature (~20 ºC) and other one was 50 ºC. Ethanol (95%) were added into flask according to selected solid loading (20%) respective to dry whole stillage solid content. To explore the effect of different solid content on oil partitioning from whole stillage, another treatment was introduced by mixing distilled water with the whole stillage sample. The amount of distilled water mixed was determined to have the same solid content of whole stillage in ethanol treatment. Control treatment had no ethanol and water, but was treated in different temperature conditions. To prevent any mass loss due to evaporation, the Erlenmeyer flasks were closed with aluminum foil paper. After incubation, liquid and solid portion were obtained using centrifugation (Allegra X-15R Benchtop Centrifuge, Beckman Coulter, Fullerton, CA, fitted with a SX4750 swinging bucket rotor, 20.78 cm radius at 4750 rpm, 5250 x g) of 3750 rpm for 20 min

in a 100 ml centrifuge tube. Different steps of oil separation described in this section is illustrated in Appendix Figure A3.

4.3.5. Sample Analysis and Yields

Representative solid samples from all treatments were dried at 65 ºC and analyzed in duplicate for oil recovery using ASE as described in section 4.4.2. The oil partition in the liquid was calculated from the difference between the oil in the whole stillage and the oil in the solid portion. The three fractions (whole stillage, solid and liquid portion after centrifuge) were considered to follow these relationships of mass balance:

$$Y_{sf}+Y_{lf}= 100$$

$$S_{sf}+S_{lf}= S_{ws}$$

$$Y_{sf}\times \%\ solid_{sf}+ Y_{lf}\times \%\ solid_{lf}= 100\times \%\ solid_{ws}$$

Where, Y = % yield on wet-weight basis,

S = solids on dry basis, and the subscripts sf, lf, and ws refer to solid fraction, liquid fraction, and whole stillage, respectively. The term % solids was the solid content in the material.

The wet and dry-matter yields, and the solid content of liquid and solid portion were quantified by measuring the wet- and dry matter weights relative to the original whole stillage using equation 2, 3 and 4:

$$\text{Wet yield of solid fraction (\%)} = \frac{\text{g of solid fraction, as-is}}{\text{g of whole stillage, as-is, before centrifugation}} \times 100 \quad (2)$$

$$\text{Percent solid in solid fraction(\%)} = \frac{\text{g of dry matters in solid fraction}}{\text{g of dry matters in whole stillage, before centrifugation}} \times 100 \quad (3)$$

$$\text{Oil partition in liquid fraction(\%)} = \frac{\text{1- g of oil in solid fraction, as-is}}{\text{g of oil in whole stillage, as-is, before centrifugation}} \times 100 \quad (4)$$

4.3.6. Statistical Analysis

Each experiment was treated as an individual trial with a completely randomized treatment design. Statistical analysis was performed using the general linear model procedures of

SAS 9.1 (Cary, NC, USA). Analysis of variance (ANOVA) was used to determine significant differences among the different treatments within an experiment. The least significant differences (LSD) test was also conducted for pair-wise comparisons when there was a significant effect at $p < 0.05$ based on the ANOVA. All treatments were carried out in replicates and results are shown as the means of replicates ± standard deviation (SD).

4.4. Results and Discussion

4.4.1. Effect of Ethanol on Oil Recovery from DDGS

In this research, aqueous ethanol was used with different fractions of DDGS as a solvent and chemical demulsifier. Two key parameters of concern that need to be addressed in this aspect are the process temperature and the solid loading of different fractions of DDGS relative to ethanol. Selected process temperatures were room temperature (RT~20ºC), 30ºC, 40ºC, and 50ºC, while the different solid loadings were fixed at 20%, 30%, and 40%. Considering its cost-effectiveness, ethanol concentration was chosen at 190 proof over 200 proof in this experiment.

4.4.1.1. Composition of original and fractionated DDGS

The composition of the original DDGS and number 20-sieved aspirated heavy fraction DDGS used in this study are tabulated in Table 5. The moisture and oil content were the essential criteria to be considered. The oil recovery in this experiment was determined based on the oil content determined by ASE as described in section 4.4.2.

Table 5. Oil, protein, and moisture contents of DDGS

Composition (%)	Original DDGS*	Number 20-Sieved Aspirated Heavy Fraction DDGS*
Oil (dry basis)	9.8±0.05	12.20±0.08
Moisture (wet basis)	11.5±0.66	9.50±0.73
Crude protein (dry basis)	32.6±0.29	32.10±0.47

*Mean of triple measurements ± standard deviation

The oil content in 20-sieved aspirated heavy fraction of DDGS increased 24% compared to the original DDGS as an impact of the fractionation process (Table 5). The moisture contents (wet basis) of the original DDGS and 20-sieved aspirated heavy fraction DDGS used in this study were determined to be 11.5% and 9.5 %, respectively. The low moisture level in fractionated DDGS relative to the original DDGS is due to the aspiration, which is considered a partial drying process. There was no significant change between the crude protein content of the fractionated sample compared to that of the original DDGS.

4.4.1.2. Effect of temperature

The change in the amount of oil recovered with the variation of process parameters in number 20 sieved aspirated heavy fraction and original DDGS are illustrated in Figures 16 and 17, respectively. With the increase of temperature from room temperature (~20ºC) to 50ºC (regardless of the solid loadings), a significantly increased amount of oil was recovered from both types of DDGS samples used. From Figures 16 and 17, it is apparent that the highest amount of oil was recovered at 50ºC. However, the increment of the amount of oil recovered with the increase in temperature differs for the type of DDGS fraction. The increment of the amount of oil recovered ranges between 45-70% in 20 sieve aspirated heavy fraction DDGS. On the contrary, a lower increment (25-45%) was noted when the original DDGS was used.

Johnson (1997) showed that the solubility of oil in alcohol is dependent on temperature, and oil solubility increases as temperature increases. This justifies the findings of getting high oil yield at high temperature. Moreover, heating is also considered as a practical means of demulsifying (Chabrand et al., 2008). Singh and Cheryan (1998) also found a reasonable amount (0.66 g crude oil/ 10g of DDGS) of oil from DDGS at 50ºC, when anhydrous ethanol was used as a solvent. Temperature is, however, a very critical input for the biorefinery industry. An

optimized temperature where a significant amount of oil is recovered, provides the biorefinery more oil output with possible savings on energy.

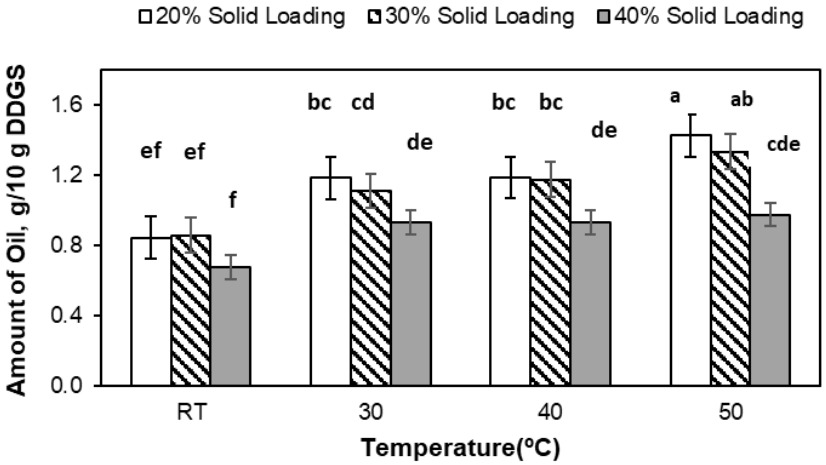

Figure 16. Effect of temperature and solid loading on the amount of oil recovered from 20 sieved aspirated heavy fraction

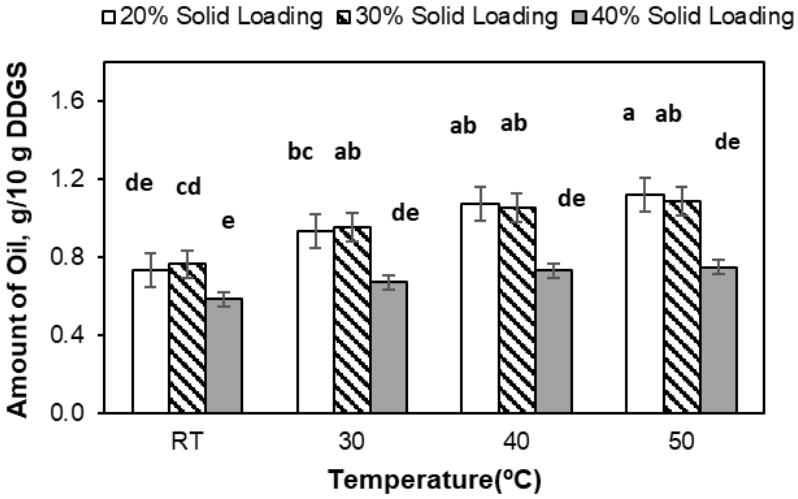

Figure 17. Effect of temperature and solid loading on the amount of oil recovered from the original DDGS

4.4.1.3. Effects of solid loading

Different solid loadings were evaluated to determine their impact on oil yields from different fractions of DDGS, and this is shown in Figures 16 and 17. The amount of oil recovered from the DDGS samples used decreased considerably at distinct temperatures when the solid loading increased from 20 % to 40 %. It is clearly seen from Figures 16 and 17 that the

amount of oil recovered from number 20 sieve aspirated heavy fraction DDGS and original DDGS was significantly different at 20% and 40% solid loading, at a higher temperature than room temperature. Unlike the effect of temperature, the amount of recovered oil did not vary with the type of DDGS fraction. For both types of DDGS samples, the decrease in the amount of oil ranged from 20-35% (regardless of the temperature).

The addition of ethanol at various levels of heavy loading increases the recovery of oil from DDGS. Kadioglu et al. (2011) stated that ethanol affects the interfacial tension (IFT) when applied to the oil extraction system. The IFT of ethanol is 22.39 mN / m (at 20°C), less than that of water 72.75 mN / m (at 20°C); thus, as the concentration of ethanol rises in solution, the interfacial tension and the polarity of the alcohol-water system will decrease. The reduction of IFT will facilitate pathways for the release of oil and increase the yield of oil. (Miñana-Perez et al., 1995). This could be the reason to have higher oil yields at lower solid loadings (20-30 percent).

4.4.1.4. Oil recovery from 20 sieved aspirated heavy fraction at different temperatures and solid loadings

It was apparent from the discussion of the previous sections that number 20 sieved aspirated heavy fraction DDGS yielded more oil than original DDGS. Figures 18 and 19 displayed the influence of temperature on oil recovery from sieved aspirated heavy fraction DDGS (number 20) at 20% and 30%, and 40% solid loading. As shown in Figure 18, the oil recovery increased significantly with the increasing temperature from room temperature. More than 90% of oil recovered at 20% and 30% solid loading when treated with the temperature 30ºC and 40ºC. However, oil recovery did not change significantly when the temperature increased

from 30ºC to 40ºC. It is worth mentioning that the oil recovery reached nearly 100% at 40ºC, so we did not present the data of oil recovery at 50ºC.

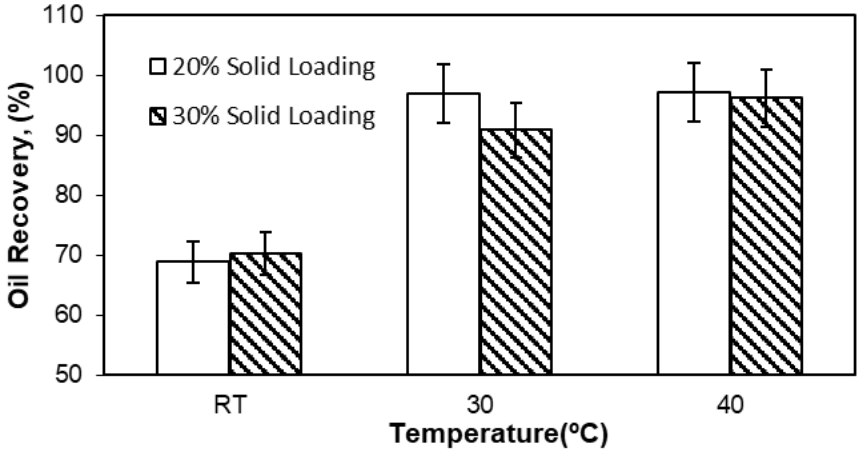

Figure 18. Effect of temperature on oil recovery from sieve aspirated (SA #20) heavy fraction at 20% and 30% solid loading

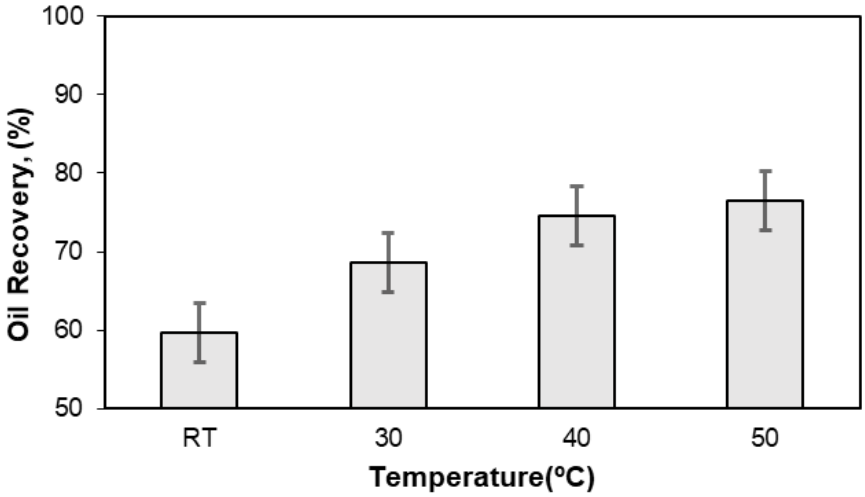

Figure 19. Effect of temperature on oil recovery from sieve aspirated (SA #20) heavy fraction at 40% solid loading

Based on the results, it was interpreted that the particle size of DDGS could also influence the oil recovery. Fractionation of DDGS provided fractions of a particular particle size that could expand the interface between the substance particles and the aqueous solvent or enzyme (Ni et al., 2016) and thus improve the free oil yield. Several particle size reduction

methods were developed such as, grinding and sieving (Liu, 2010), colloid milling (Dickey et al., 2010), dry milling (Ni et al., 2016), elutriation process (Srinivasan et al., 2005), etc. These methods were used for efficient oil recovery from various corn ethanol co-products. Some of these methods were complex, energy-intensive, and not easy to replicate. We used a simple sieving and aspiration technique to separate DDGS into specific sizes and used for higher oil recovery.

Figure 19 shows that oil recovery increased significantly with the increase of temperature at 40% solid loading. At 50ºC, only 77% of the oil was recovered, which is, however, not satisfactory. The industry would not be interested in high energy input for less than 80% oil recovery. Besides, it would be much more efficient for the industry if they could use higher solid loading for more oil. Considering these aspects, a temperature of 30ºC with 30% solid loading would be sufficient for maximum oil recovery from the number 20 sieved aspirated heavy fraction DDGS.

4.4.2. Oil Partitioning in Whole Stillage by Using Ethanol

The oil in whole stillage can be present in four different forms: oil-in-water emulsion, oil inside unbroken oil bodies (oleosomes), oil droplets attached to hydrophobic particle surfaces, and oil in unbroken cells of germs and endosperm (Majoni et al., 2011a). Luangthongkam et al. (2015) assumed that emulsified oil and oil in oleosomes would partition into thin stillage (liquid fraction); whereas, oil in unbroken matrices and adhering to larger particles partition with wet cake (solid fraction) after centrifugation of whole stillage. However, some emulsified oil could not partition into thin stillage without a demulsifier. A demulsifier, like aqueous ethanol, may dissolve soluble cellular materials and partition more oil in the liquid phase. This process of demulsification inside the whole stillage can be affected by process temperature and solid

loading. In this present study, 190 proof ethanol was used to treat whole stillage in different temperatures and solid loadings to have better oil partition in liquid fraction.

4.4.2.1. Composition of whole stillage sample

The composition of the whole stillage used in this experiment is listed in Table 6. A high moisture level (around 90%) is one of the distinguishing characteristics of whole stillage. The amount of protein and oil accounted for about 48% of the whole stillage sample composition. The moisture and oil level were the most essential criteria to be considered. The oil partition in this experiment was determined based on the oil content determined by using ASE as described in section 4.4.2.

Table 6. Oil, protein, and moisture contents of whole stillage used

Composition (%)	Whole Stillage*
Oil (dry basis)	17.45±0.02
Protein (dry basis)	30.70±0.52
Moisture (wet basis)	88.54±0.69

*Mean of triple measurements ± standard deviation

4.4.2.2. Effect of temperature on oil partitioning

Two different temperature conditions were selected to evaluate the effect of temperature on oil partition from the whole stillage. One is the room temperature (RT~20ºC), another is 50ºC. Table 7 shows the effect of temperature on wet yield (%wt.) and percent solid contents, and oil partitioning in solid and liquid fractions as a percent of whole stillage. The oil partition had a positive impact on increasing temperature. When the whole stillage was mixed with ethanol and the temperature was increased from room temperature (~20ºC) to 50ºC, the oil partition in liquid fraction increased by 19% (from 19.99% in RT to 24.71% in 50ºC). A similar trend also follows (oil partition increased from 11.59% in RT to 16.94% in 50ºC) when whole stillage is treated with water for the same solid content as ethanol treatment. However, no significant change was

found in oil partition in liquid fraction control treatments treated with different temperatures that have different solid content than other treatments (Table 7). It seems that the presence of ethanol may have helped solubilize the oil and break the emulsion that trapped the oil. A previous study conducted by Yao et al. (2014) showed that the removal of ethanol from whole stillage led to a decrease in free oil recovery from a liquid fraction (thin stillage) with heating treatment.

Table 7. Weight distribution and oil partitioning in liquid and solid fractions relative to whole stillage

Treatments		Liquid Fraction			
Temperature	Medium (%solid)	Wet Yield (%)*	Solids Content (%)*	Dry Matter Yield (%)*	Oil Partitioning (%)*
Room temperature (RT~20ºC)	With ethanol (7.5%)	$75.00\pm2.06_{a}$	$2.58\pm0.04_{c}$	$30.69\pm1.69_{a}$	$19.99\pm0.86_{b}$
	With water (7.5%)	$70.34\pm0.05_{b}$	$2.67\pm0.04_{c}$	$28.47\pm0.51_{b}$	$11.59\pm1.41_{c}$
	Control (11.5%)	$68.78\pm0.54_{b}$	$4.69\pm0.27_{a}$	$32.81\pm1.04_{a}$	$16.86\pm1.75_{b}$
50ºC	With ethanol (7.5%)	$76.20\pm1.32_{a}$	$3.42\pm0.29_{b}$	$29.67\pm1.14_{ab}$	$24.71\pm2.88_{a}$
	With water (7.5%)	$70.37\pm0.56_{b}$	$2.15\pm0.14_{c}$	$28.83\pm0.20_{b}$	$16.94\pm1.54_{b}$
	Control (11.5%)	$67.82\pm0.55_{b}$	$4.57\pm0.16_{a}$	$30.42\pm1.69_{a}$	$16.99\pm2.20_{b}$
LSD		2.53	0.52	2.88	4.21
		Solid Fraction			
Room temperature (RT~20ºC)	With ethanol (7.5%)	25.00_{c}	$22.44\pm1.77_{b}$	69.31_{ab}	80.01_{b}
	With water (7.5%)	29.66_{b}	$19.18\pm0.07_{c}$	71.53_{a}	88.41_{a}
	Control (11.5%)	31.22_{ab}	$26.60\pm0.21_{a}$	67.19_{b}	83.14_{b}
50ºC	With ethanol (7.5%)	23.80_{c}	$20.58\pm0.03_{bc}$	70.33_{a}	75.29_{c}
	With water (7.5%)	29.63_{b}	$20.27\pm0.69_{c}$	71.17_{a}	83.06_{b}
	Control (11.5%)	32.18_{a}	$26.22\pm0.03_{a}$	69.58_{ab}	83.01_{b}
LSD		2.53	2.10	2.88	4.21

*Within each column, means with different letters are significantly different ($P< 0.05$).

Increasing temperature with ethanol addition increased the solid content of liquid fraction significantly, which subsequently resulted in higher oil partitioning (Table 7). The increased temperature may evaporate some moisture and result in more oil in the liquid fraction. Wang et al. (2008) found that oil content in liquid fraction (thin stillage) strongly correlated with solid content and thus supported findings in this study.

The wet yield of liquid fraction ranged from 67.82–76.20%, with the highest yield for ethanol treatment with high temperature (Table 7). These values are close to the industrial liquid fraction (thin stillage) yield of 81% estimated by Wang et al. (2009b). However, the effect of temperature with ethanol addition is not significant both on the wet yield and dry matter yield of liquid fraction.

4.4.2.3. Effect of solid loading on oil partitioning

Mixing ethanol with whole stillage results in lower solid loading than the control treatment. To examine the effect of solid loading, water was added with whole stillage having the same solid loading as ethanol treatment. The control treatment percent solid was found to be 11.5, whereas the other ethanol or water treatment percent solid was 7.5. Table 7 illustrates the effect of solid loading on wet yield (%wt.), solid, and oil partitioning in solid and liquid fractions as a percent of whole stillage. It is evident from Table 7 that oil partition was higher in the liquid fraction from ethanol treatments compared to other treatments, regardless of the temperature effect. After comparing with the same solid loading of 7.5%, the ethanol addition improved oil partition significantly (ranges from 45-72% increase). Moreover, oil partition increased significantly with ethanol addition at high temperature (50ºC) relative to the control treatment (higher solid loading than others). However, ethanol addition did not change significantly in oil partition at room temperature with different solid loading (comparing to control treatment).

The liquid fraction dry matter contents in the control and other treatments ranged from 2.15-4.69% (Table 7), which is very low relative to typical industrial values 7.0-7.5% (Wang et al.,2009b). This was due to the decanting process of whole stillage. It was challenging to simulate the industrial decanting in a laboratory. However, this result will still give us the idea of how ethanol affected the oil partition in the whole stillage. Control treatments had higher solids

to start with and resulted in significantly higher solids in the liquid fraction compared to other treatments. There is no significant difference found in other treatments with same solid content, only exception for the ethanol treatment at higher temperature. Ethanol treatment at higher temperatures resulted in significantly higher solid content relative to other treatments. The same trend is also observed in the solid fraction, which explained the increase of percent solids in the liquid fraction.

The impact of ethanol addition with lower solids increases the wet yield of liquid fraction significantly compared to other treatments (Table 7). The greater yield of a liquid fraction means energy saving in the subsequent drying phases of the DDGS, which would be a desirable outcome for the biorefinery industry. Table 7 shows that the solid partitioned in liquid fraction (dry matter yield) ranged from 28.47% to 32.81%. The dry matter yield is highest in the control sample, which is reasonable because they had a higher amount of solids than others. Lowering solid content of whole stillage by ethanol mixing, however, did not significantly reduce the dry matter yield compared to the control.

Wang et al. (2008) reported that the dry matter yield and oil partition in liquid fraction were only generally correlated, which means that the oil in some samples was freer than in others (the oil was not bound to or stored in the solids following various treatments in the same way). Based on one batch of industrial decanting, the dry matter yield and oil partitioning in the industrial thin stillage (liquid fraction) were reported 48 and 53%, respectively (Wang et al., 2008), which were higher than findings in this study. This was attributed to reproducing the industrial process within a laboratory environment. Wang et al. (2009b) stated a need for accurate simulation device/methods to produce liquid fraction and solid fraction on a bench scale from whole stillage, as is the case in the dry-grind corn ethanol fermentation industry.

4.5. Conclusion

The impact of ethanol on oil recovery from DDGS and oil partition from whole stillage was worth mentioning. Ethanol was added with two different fractions of DDGS to find the impact of different solid loadings and temperatures on oil yield. Higher oil was yielded from the number 20 sieved aspirated heavy fraction of DDGS. The oil recovery was ranged from 92-95%. Increasing temperature over 30ºC, however, did not improve significant oil recovery for 20% and 30% solid loading. Therefore, the solid loading can be increased up to 30% without compromising the oil yield and considering economic benefit and handling advantage. Oil recovery increased with the increment of temperature, but it was not satisfactory at 40% solid loading. The whole stillage was experimented with ethanol by varying two different temperatures and solid loadings. The outcome of this experiment showed the positive effect of ethanol addition at certain level and increased temperature during the process on the oil partitioning of whole stillage. More research is required to scale up this study from laboratory scale to pilot scale. Different concentrations and amounts of ethanol can be used to optimize the amount of ethanol for maximum oil recovery for future works.

CHAPTER 5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

This research demonstrated that incorporating different commercial enzymes and ethanol at their optimized conditions, can enhance oil recovery from DDGS and oil distribution into liquid fraction from whole stillage. Fractionation also played an essential role in increasing oil recovery from DDGS. A simple fractionation method consists of subsequent sieving and aspiration to separate the oil-rich fraction efficiently. Satisfactory oil recovery was achieved by using a combination of cellulase and protease enzymes. However, the cellulase enzyme worked almost similarly to the combination effect. It will be more cost-effective for the biorefinery industry to use only one enzyme instead of combinations. A follow-up experiment revealed that the physical process alone (e.g., heating) is not enough to get higher oil recovery from DDGS.

The effects of ethanol on oil recovery from DDGS and oil partition in a liquid fraction from the whole stillage were in agreement. Positive impacts of ethanol addition were observed in different experiments. The highest oil recovery from fractionated DDGS was obtained with 30% solid loading at 30ºC. When ethanol was used with whole stillage at 50ºC, significantly more oil partitioned into liquid fraction than into the solid fraction compared to other treatments. These results suggest that ethanol can be used for back end oil recovery from the corn biorefinery. An additional benefit of using ethanol is that it is readily available and would not require any significant change in an ethanol plant's design.

5.2. Recommendations for Future Work

In the present study, all the enzymatic treatments were conducted with a fixed enzyme dosage (5%, v/w). There is a possibility to optimize this enzyme concentration with specific process parameters. Present work already established that enzymes had a positive effect on oil

recovery from DDGS. The same enzymatic hydrolysis approach can be adapted to treat the whole stillage. Despite the additional costs of enzymes, additional revenues from the oil can minimize those costs. In this context, economic analysis can be conducted to compare the oil recovery scenario using enzymes, ethanol, and traditional practices.

Air velocity and density of particles play a critical role in the fractionation process of DDGS. A more diverse fraction can be achieved by varying the air velocity of the aspirator. A different batch of DDGS also varies in composition and density. Therefore, there is a potential opportunity to conduct more optimization in the fractionation process.

This research focused on the oil-rich (heavy) fraction produced from the fractionation process of DDGS. More value-added (e.g. particle boards) products can be manufactured using fiber-rich fractions (medium and light). Besides, a considerable amount of de-oiled DDGS (residues) left after oil recovery from DDGS. This de-oiled or low-fat DDGS has a great prospect of using as animal and fish feed. DDGS is commonly used in ruminant feed primarily for the beef and dairy industry due to the high fiber content, which limits its use for non-ruminant feed. With application of enzymes, the amount of fiber in defatted DDGS can be reduced and more digestible to non-ruminants. This defatted DDGS can be used at higher concentration in non-ruminant feeds.

There is also the potentiality of ethanol production from the different DDGS fractions and de-fatted DDGS. Mixing of DDGS fractions or defatted DDGS into corn ethanol process as secondary feedstock might improve overall ethanol production. As DDGS is the byproduct of corn ethanol fermentation, the conversion of carbohydrates in DDGS would reduce the overall raw material cost. In this way, fractionation can help in producing streams of products from DDGS. The biorefinery industry can market these products to increase their profitability.

Future research work can continue to maximize oil recovery from the corn bio-refinery, mainly focusing on back end processing. Oil distribution in whole stillage is critical to have more oil yield from the various corn ethanol co-products. Oil distribution in the whole stillage experiment was limited, considering the effect of temperature and solid loading in this study. To maximize the oil partition, various concentrations and amounts of ethanol can be used to optimize the amount of ethanol. Moreover, variation in residence time can be explored for efficient handling of whole stillage.

Most of the present dry-grind ethanol plants use mesophilic hydrolytic enzymes, which get denatured at increased temperatures (while processing of whole stillage), resulting in consumption of large amounts of enzyme and thus, half of the process cost in biorefineries is attributed to the enzyme production process. Employment of thermostable enzymes for hydrolysis at higher temperatures may help in increasing oil distribution in the liquid fraction of whole stillage. Additionally, enzymes can be used for front-end recovery of oil and it may save energy and money for the processing of whole stillage.

Finally, all the experiments in this research were conducted in a laboratory-scale environment. Considerable efforts are required to scale this research up to the pilot scale/industrial scale so that it will give a more transparent idea of the effect of the different treatments. Additionally, it will be easier for the industry to apply the findings to gain more profit from ethanol plants.

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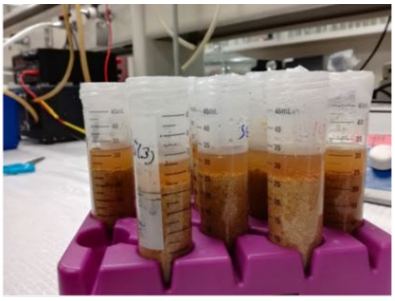
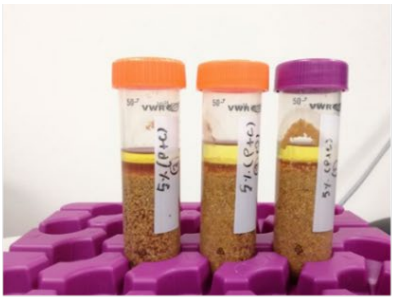
APPENDIX. OIL SEPARATION AND PARTITIONING STEPS

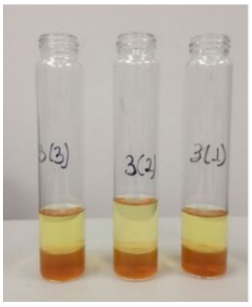
Experimental setup



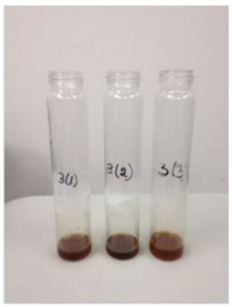
After enzymatic hydrolysis



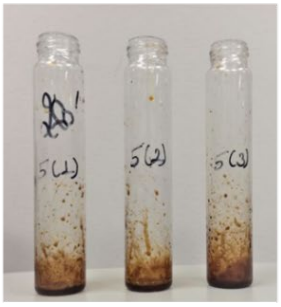
After centrifuging



After washing with hexane



After evaporation In water bath



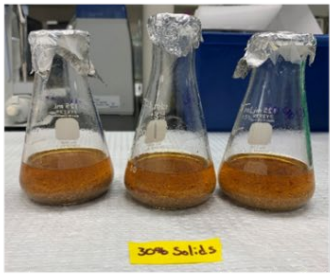
After drying in vacuum oven

De-fatted DDGS Residue

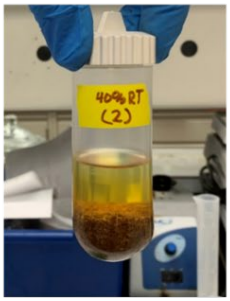
Figure A1. Oil separation steps using enzymes

DDGS mixed at different solid loadings (Left: 40%, Right: 30%)

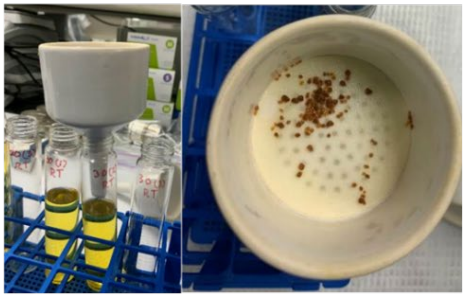
Experimental setup



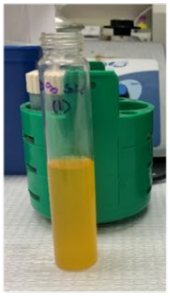
Sample after 2hr incubation



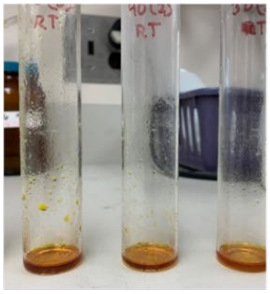
After centrifuging



Transfer using Buchner Funnel

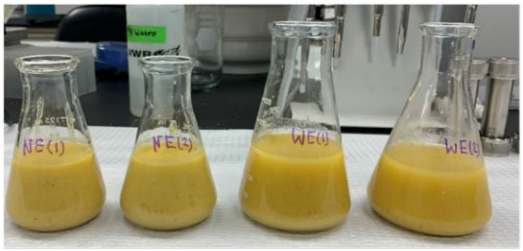


Sample after transfer

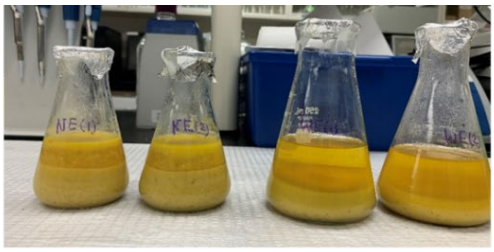


Oil after evaporation

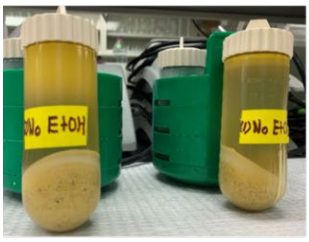
Figure A2. Oil separation steps using ethanol

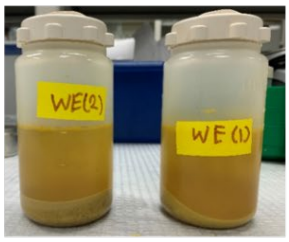


Raw sample; WE: ethanol mixed and NE: no ethanol added

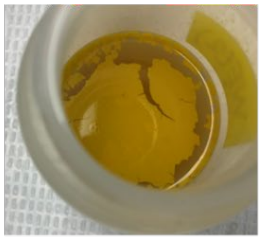


Sample after 2hr incubation (130 rpm)





Sample after centrifugation at 3000g for 20 mins.



Liquid fraction sample

Solid fraction sample

Figure A3. Oil partitioning in whole stillage