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Analysis study of whole stillage, thin stillage and syrup

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Analysis study of whole stillage, thin stillage and syrup

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DEDICATION

This thesis is dedicated to my parents of their endless love, support and patience. Their constant encouragement and persistent confidence in me, has taken a load off my shoulders.

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ABSTRACT

Ethanol is used as a fuel additive has resulted in rapid of growth of ethanol production. Thus the bio-based ethanol production has been one of the fastest growing industries in the U.S. The dry grinding corn ethanol process is more predominant than other ethanol production process in the U.S, due to the low capital costs. In the dry grinding process, the corn is fermented to produce ethanol and distillers dried grains with solubles (DDGS). The generated DDGS are primarily used by farmers to feed livestock. However, drying distillers grains consume energy and costs money. As a result, DDGS are more expensive than other distiller grains (Gorden, 2008). To reduce operational costs through drying process and environment impacts, such as greenhouse emissions, some other distiller grains with relatively high moisture content, including whole stillage, thin stillage, and syrup could be considered as alternative animal feed ingredients.

The physical and biological properties tests provided the information background information about operational processes, and valuable components change over time. The objectives of the first study were to determine various physical and biological properties of whole stillage, thin stillage, and syrup, and the allowable shelf life under different storage temperature levels.

The thin stillage and whole stillage had high initial average moisture contents of 92% (w.b.) and 87% (w.b.) respectively, and initial water activity of 0.99; the high water content marked samples easily susceptible to rapid spoilage. Time had a significant effect ($P < 0.05$) on properties of co-products. Both thin stillage and whole stillage samples got mold growth after 5 days incubation at 32^{o}C. Thin stillage had the greatest separation rate in settling experiment. However, syrup had relative low initial average moisture content

of 62% and initial water activity of 0.92. No mold growth and settling separation happened in syrup samples. There were no evidence showing a linear relationship exists between Hunters L*, a* and b*, and mold growth. The Solvita® test showed that high-temperature treatment caused high CO_2 production in all samples. The exponential models described the relationship between storage time (from 0 to 5 days at 25^oC and 35^oC) and CO_2 concentration for three co-products.

The physical and biological properties study is just the first step to explore opportunities for utilizing these co-products. Follow-up study should work should work on separation process to concentrate the valuable components from ethanol co-products.

Evaporation is the typical method used to concentrate solids in these co-products, but it requires a large amount of water and energy consumption. In order to overcome the problems that associated with the evaporator, membrane filtration could be applied that may provide a cheaper and efficient way to improve value for whole stillage, thin stillage, and syrup. Fractionation of these wet co-products by using ultrafiltration was conducted to evaluate membranes as an alternative to evaporators in ethanol production. A study has been showed that ultrafiltration required less energy than evaporation (Rausch and Belyea 2006). This study indicates that ultrafiltration could be a better choice that can be applied in biotechnology industries to concentrate valuable components. However, an important problem associated with membrane technologies is flux decline and membrane fouling. An understanding of causes of flux decline is necessary to minimize or avoid fouling and to make membrane application economic.

The membrane size, stirring speed and volume capacity had significant effects ($P < 0.05$) on flux during the ultrafiltration for whole stillage and thin stillage. The flux

increased by 30% maximum as siring speed increased from 160 to 320 rpm for YM 10 membrane in these two samples. The effect of membrane size on solid recovery was significant ($P < 0.05$). The solid recovery for YM 100 membrane in whole stillage ranged from 75% to 83%, and 74% to 84% for thin stillage, however, the YM 10 kDa was ranging from 80% to 90% in whole stillage, and 84% to 90%. Retentate products from ultrafiltration could be further used as an ingredient to feed animals, and the permeate stream could be recycled in dry grind plants to help in reducing process water requirement.

CHAPTER 1: INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

The need for energy helps the U.S. rely less on foreign supplies and the hope of becoming greener society has encouraged the growth and development of the ethanol industry. The ethanol is used as an alternative to and additive for fossil fuels has increased steadily in the U.S. since the 1980s. Corn is the major raw material that the ethanol industry utilizes to produce ethanol in the U.S (Graboski, 2002). Other potential feedstocks, including sorghum, barley, wheat, rye and cereals, are also used to produce fuel ethanol (Singh, 2010).

There are two major type processes for the conversion of corn to fuel ethanol, wet milling, and dry grinding. The goal of wet milling process is to produce fuel ethanol as well as some other co-products, including corn oil, corn gluten feed, and corn gluten meal. However, the goal of dry grind process is to ferment as much corn as possible. A majority of the ethanol production (80%) in the U.S is made using the dry grind technology due to the low capital costs and relative high ethanol production (Singh, 2005).

At the end of distillation process of dry grinding method, ethanol/water is separated from distillers grains. The ethanol and water mixture is sent to the molecular sieve to remove water, and distiller grains are sent to some other processes to produce distiller dried grain with solubles (DDGS) which is an essential ingredient in animal feeding (Makkar 2012). However, drying distillers grains requires energy, time and money; as a result, DDGS are more expensive than other distillers grains. To reduce the operation costs, some other distillers grains with relatively high moisture content,

including whole stillage, thin stillage, and syrup could be considered to use as feed ingredients.

The recent growth of fuel ethanol production brings much attention to DDGS, but little study has been done on the upstream components that make up DDGS. Increasing using of upstream components (whole stillage, thin stillage and syrup), the environmental impacts, such as greenhouse gas emission, as well as the overall energy consumption throughout the drying process, could be significantly reduced. Also, a large volume of upstream components is produced and sold, making them essential towards ethanol profitability. However, utilizing upstream components in the marketplace have many limitations. The shelf-life is short due to high moisture content, and the nutritional profile is not favorable for many animals, and ultimately, those products cannot be sold for high values.

Studies work on upstream components (whole stillage, thin stillage, and syrup) is an essential way to keep the ethanol industry profitable. In order to fully understand the potential value of these upstream components, this study took an initial look at the physical and biological analyses of whole stillage, thin stillage, and syrup gives insight about handling, storage, and processing operations. Those properties are necessary for developing value separation methods. Separation of an organic stream into a concentrated product would give farmers control of nutrient loading on animal feeding. Greater separation of the nutrients added value to the product and justified on this stream. This study supplements the research on separation solids from whole stillage and thin stillage by using ultrafiltration process.

1.2 U.S. Ethanol Industry

Fuel ethanol production is one of the fastest growing industries in the United States. The development of the U.S. corn ethanol has shaped the U.S. energy policies over the past four decades. The policies that promote the rise of ethanol production help solve economic, environmental, and national security concerns. The development of corn ethanol production has created more job opportunities, lowered greenhouse gas emission, and reduced reliance on foreign oil supplies. This review demonstrates the development of ethanol production in the United States and the benefits of ethanol as part of the United States energy portfolio.

Development of ethanol production

The development of ethanol production is motivated by fossil fuel exhaustion, less harmful environmental emission production, easy accessibility, and renewable nature. Figure 1.1 summarizes the annual U.S. ethanol production over time (RFA. 2014). The ethanol production consistently increased since 1980, and the production hit record of nearly 14 billion gallons in 2011 (Figure 1.1). The ethanol industry grew by 200 plants and 13.3 billion gallons of ethanol in 2013 (RFA, 2014).

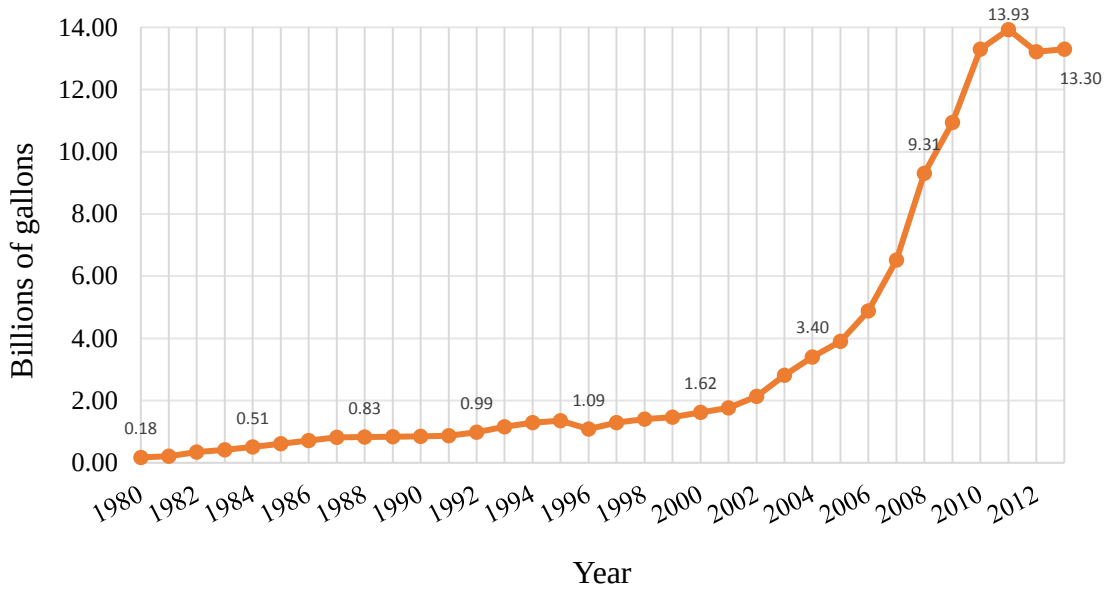

Figure1.1 Annual U.S. ethanol production based on data from Renewable Fuel Association (RFA), 2014

Benefits of ethanol production

The increasing of ethanol production in the United States brought economic, environmental and national security benefits. In 2013, the production of 13.3 billion gallons of ethanol supported 85,504 direct jobs in renewable fuel production and agriculture field, as well as 300,277 indirect and induced jobs in other areas, and an added $30.7 billion household income and $44 billion to the nation's Gross Domestic Product (RFA, 2014). With the expansion of ethanol industry, a greater availability of distillers grains could be used in the animal feed market (RFA 2015 a).

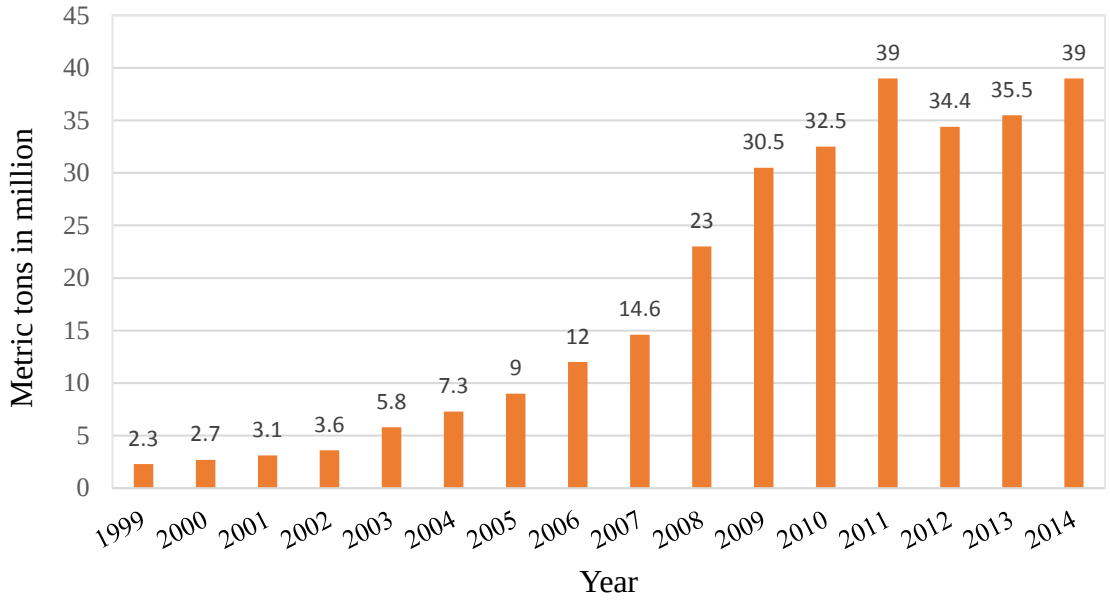

Figure 1.2 Annual distillers grain production based on the data provided by RFA by RFA, 2014

At the end of ear 2014, 14.3 billion gallons of ethanol and 39 million metric tons of distillers dried grains with solubles (DDGS) were produced (RFA 2015 a and 2015 b). The ethanol sector has quickly and quietly become one of the largest contributors to the U.S. feed supply. Only the grain's starch is consumed by ethanol production process and the remaining protein, minerals, fat and fiber pass through the process and are concentrated into highly valued and nutritious co-products that can be fed to livestock. One-third of a 56-pound bushel of grain that passes through ethanol process and enters to the animal feed market (RFA 2015 a). These co-products are primarily used to feed beef cattle, dairy cows, swine, poultry, and fish in nations and around the world. Since the co-products makes an enormous contribution to the global animal feed supply, its marketability is important to the viability of ethanol production. Regarding national

security, U.S. dependence on imported crude oil and petroleum products has not significantly increased since the early 1990s (RFA, 2014). After reaching a peak at 60% in 2005, import dependence has fallen steadily and settled at an estimated 35% in 2013 (Figure1.3.) For the past five years, ethanol has contributed to 10% of U.S. nation's gasoline supply, and the ethanol produced in 2013 saved an amount of gasoline refined from 462 million barrels of crude oil (RFA, 2014). Reducing the importing oil from other countries could help to avoid political and economic consequences in the U.S. caused by the interruption in oil supply countries.

1.2 Ethanol Production Process Overview

Corn is the major raw material that the ethanol industries utilize to produce fuel ethanol (Graboski, 2002). There are two major type processes for the conversion of corn to ethanol, including wet milling and dry grinding. Wet milling process could produce ethanol as well as a diversity of co-products that can be used in animal feeding. The yield of ethanol from wet milling process is more than 100 million gallons per year (Graboski, 2002). However, the majority of the fuel ethanol production in the U.S. is made using the dry grind technology, due to the low capital and operational costs (Singh, 2005).

Dry-grind corn ethanol process

Currently in the United States, the dry-grind corn ethanol process is most prevalent. Average production levels include 2.8 gallons of ethanol and 18 pounds of distiller's grains and 18 pounds of CO_2 from every 56-pound bushel of corn (Koza, 2012). Two third of maize is carbohydrates, which are converted to fermentable sugars and ultimately to ethanol and CO_2. The residual corn is processed into distiller grains; it contains proteins, fibers, oil, and remaining yeast cells. Figure 1.3 demonstrates the general process of dry

grinding, and the process starts with the whole corn grinding into flour, which is referred to as “meal”. Enzymes are added to the mash to convert the starch to simple sugars. Ammonia is added for PH control and a nutrient provider. The product is cooked in a high-temperature to reduce bacteria levels before entering fermentation tank. The product is then cooled and transferred to a fermentation tank where the yeast is added, and simple sugars are converted into ethanol and carbon dioxide. Usually, the fermentation process needs about 40 to 50 hours. After fermentation, the resulting material which is called beer that consists of 10% alcohol, water, and some other solids that are not fermented, and then is transferred into distillation tank where ethanol is separated from other distiller grain which is called whole stillage. Whole stillage is sent to the centrifuge to remove excess liquid to form thin stillage, and then thin stillage is then condensed by evaporation process. The condensed liquid is called syrup or condensed distillers soluble (CDS) and the remaining solids are referred as wet distillers’ grains (WDG). The WDG and CDS mixture is called wet distillers’ grains with soluble (WDGS), which is an important ingredient in animal feeding (Makkar, 2012). However, the residual coarse grain solids and CDS are mixed and dried to produce distiller dried grain with solubles (DDGS) (Makkar, 2012).

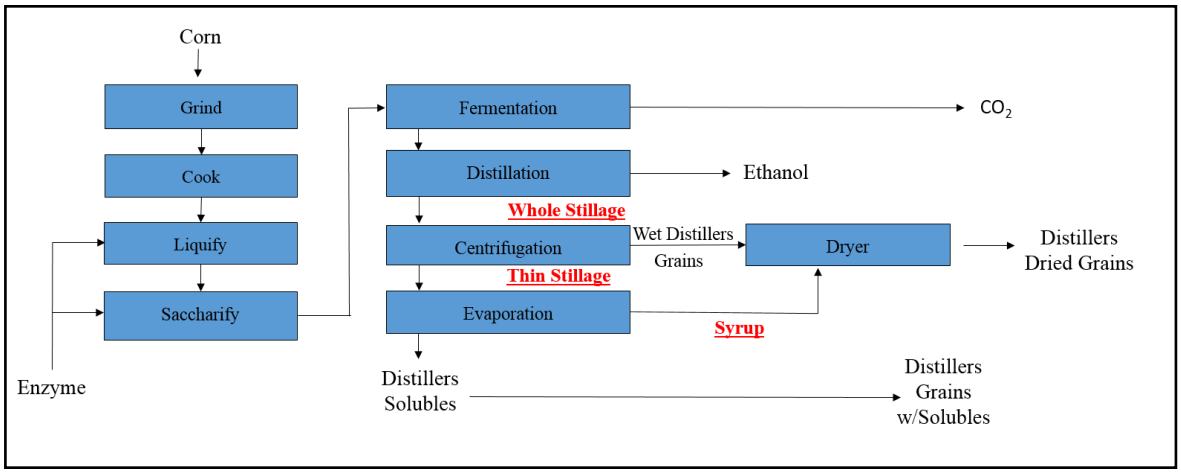

Figure 1.3 The overview of dry-grind corn ethanol production process

Distillers grains

Whole stillage (WS) is centrifuged to form a liquid fraction which is called thin stillage (TS), and the solid fraction that is wet distiller grains (WDG). The thin stillage is further condensed by removing water through an evaporator to form syrup which is known as condensed distillers solubles (CDS). The WDG can be mixed with CDS and dried to produce DDGS. The compositions of DDGS include approximately 25% to 35% protein, 3% to 14% fat, 7% to 10% fiber, and approximately 7% to 14% moisture (Bhadra et al., 2009; Ganesan et al., 2008; Kim et al., 2007; Rosentrater and Muthukumarappan, 2006; Shurson and Alhamdi, 2008; Srinivasan et al., 2005; Srinivasan et al., 2009; Weigel et al., 1997).

DDGS can be shipped to and from any farm in the country and can be stored under reasonable conditions, due to its long shelf life and low moisture content. However, drying distiller grains requires energy, time and costs money; as a result, DDGS are more expensive than other distiller grains (Gorden, 2008). In order to lower operational costs,

WDG is considered as an alternative animal feed ingredient, which has 30%-35% moisture content, but is less efficient to ship due to its short shelf life (Dooley et al. 2008). As a consequence, WDGS is typically utilized by the farms that are next to or close to an ethanol production plant and with the ability to be delivered rapidly. However, the recent growth of fuel ethanol production resulted in an increased availability of ethanol co-products for the ingredients of the livestock food. Thus the interest in using co-products which contain relative high moisture content has also increased. Moreover, increasing using the intermediate products, including the whole stillage, thin stillage, and syrup in the marketplace, the environmental impacts, such as greenhouse gas emissions, as well as the overall energy consumption, throughout the drying process could be significantly reduced. Thus, the storability of whole stillage, thin stillage, and syrup play a significant role in economic and energetic balances in animal feeding market.

1.3 Solid-Liquid Separation

The application of solid-liquid separation started with small-scale water purification and, later, the preindustrial production of foods, dyes, beers and wines (Sparks, 2012). The development of filtration technology has been both driven by the demand and availability of technologies.

Liquid- solid separation involves the separation of two phases, solid and liquid, from a suspension. It is used in many processes for four main reasons, including recovery of the valuable solid component, liquid recovery, recovery of both solid and liquid, or recovery of neither phase. Separation of solids from liquids slurry is a challenging task in many industrial processes.

1.4 Membrane Filtration

Membrane technology is an alternative to the evaporator is ethanol production for dewatering corn processing streams (Arora et al, 2010). A membrane is a barrier which separates two streams and restricts the transport of various chemical species in a rather specific manner. Separations in membrane processes are the result of differences in seperation rates of chemical species through the membrane barrier. Transport rate is determined by the driving force or forces acting on individual components, their permeability and concentration which determine how large a flux is produced by a given driving force. Permeability is determined primarily by the solute's molecular size, charge and physical structure if the membrane material, and the concentration of the solute is the primarily determined by chemical compatibility of the solute and the membrane material (Zeman and Zydney 1996). Membrane filtration has been applied to corn process streams separation and has several advantages (Templin et al., 2005). Filtration could reduce the energy needed to remove water from the co-product stream due to the process does not involve phase change of water. Because filtration does not add heat to the co-product stream, quality of protein in the co-products should not be compromised (Templin et al, 2005).

1.5 Membrane Classification

The membrane is the most important part of the separation process. Membrane separation involves separating components from liquid fluid or gaseous streams by forcing the stream to pass through the surface of a membrane. The working principle of membrane technology is that the membrane only allows the components smaller than the membrane pore size to pass through, while larger components are retained. Beside the

pore size differences, other conditions could influence the membrane performance, including temperature, transmembrane pressure, flow rate and PH (Shgort 1998, Tanny et al. 1982, Saksena and Zydney 1994, Ghosh and Cui 1998). The membrane technology is a family of processes that include microfiltration, ulfiltration, nanofiltration and reverse osmosis. Membrane processes separate molecules bases on the size and molecular weight of components. Microfiltration (MF) is the most open membrane that can separate macro materials and suspended solids with the size range of 0.05 to 2.0 microns. The operation pressure of MF is approximately15 to 60 psi. Typical materials removed by MF separation process are starch, bacteria, fat, molds, yeast and emulsified oils. Ultrafiltration (UF) is a low-pressure fractionation process that can remove all microbiological species and some viruses. UF separates dissolved solutes of 0.005 to 0.1 microns, which corresponds to a molecular with the weight cut off (MWCO) of about 1,000 to 300, 000 Daltons, and an operating pressure of approximately 30 to 100 psi. Based on the molecular weight cut off selected, the membrane will concentrate high molecular weight species while allowing dissolved lower molecular weight materials to pass. UF membranes are used in numerous industries for concentration and clarification. Nanofiltration (NF) membranes have a nominal pore size of approximately 0.001 microns and an MWCO of 1,000 to 100,000 Daltons. Pushing fluid through these smaller membrane pores requires a higher operation pressure than either MF or UF. Operational pressure ranges from 90 to 150 psi. NF membrane can remove virtually all cysts, bacteria, and viruses. Reverse osmosis (RO) is the most efficient separation process, which can remove nearly all inorganic contaminants from water. RO can also effectively separate radium, natural organic substances, pesticides, cysts, bacteria, and viruses. The

pore size of RO membrane is about 0.0001 microns, and the range of operation pressure is from 300 to 1200 psi. RO has been widely applying for desalinating sea water and reclaiming brackish water.

1.6 Membrane Filtration in Dry Grinding Industry

Wu et al. (1983) used an RO membrane unit to separate water from thin stillage solubles. The thin stillage was filtered through cheesecloth under vacuum. Thin stillage was centrifuged at 45,200 x g with a continuous centrifuge to separate suspended solids and soluble fractions of thin stillage. The centrifuged stillage solubles was used for the experiment. A 200 molecular weight cut off (MWCO) RO membrane with 5 A pore size was applied at the transmembrane pressure of 200 psi. The total volume of the stillage solubles was 9900 mL contains 1.13% total solids, and 0.265 mg/mL total nitrogen content was provide to the experiment. Approximately 76% of the original stillage volume, 4.6% of total solids and 3.2% of total nitrogen were recovered as permeate. In the second experiment, a pressure leakage problem came out due to the excessive pressure build up in the membrane system. To overcome this problem, UF membrane was introduced as a pretreatment to RO. The total UF permeate volume of 5820 mL was used as an input stream for RO. The results are presented in Tables 1.1 and 1.2.

Table 1.1 Reverse osmosis of recycled stillage solubles (Wu et al., 1983)

	Volume (mL)	N (mg/mL)	Solid (%)
Stillage solubles	8360	1.04	4.36
Permeate	4670	0.059	0.76
Concentrate	2370	1.88	8.31

Table 1.2 Ultrafiltration and reverse osmosis of recycled stillage solubles (Wu et al., 1983).

	Volume (mL)	N (mg/mL)	Solid (%)
Stillage solubles	7,000	1.54	6.63
Permeate (UF)	5920	1.06	4.79
Concentrate (UF)	140	4.52	14.83
Permeate (RO)	4120	0.27	2.03
Concentrate (RO)	980	1.58	7.54

This study demonstrated that combination of two types of pore size membranes had better performances in recovering solids and protein compared to a single membrane. Further processing of permeate from recycled stillage solubles is needed to reduce the conductivity of permeate to the level of tap water.

1.7 Conclusion

Distillers grains are the commonly used as an animal feed ingredient; however, drying distillers grains requires energy, time and costs money, which makes DDGS are more expensive than other distiller grains. As the ethanol industry continues to expand production of distillers grains, it is possible that an increased using the upstream of DDGS, for instance, the whole stillage, thin stillage, and syrup, could reduce the environmental impacts, such as greenhouse gas emission.

However, little research has been done on whole stillage, thin stillage, and syrup. This study took an initial look at the physical and biological properties of these upstream components (whole stillage, thin stillage, and syrup) to provide background for future studies. The physical properties provided the information necessary design in handling

and processing operations, while the biological analysis provided information about potential valuable components change during the storage process.

In addition to looking at the properties of whole stillage, thin stillage, and syrup this study looked at the ultrafiltration process of whole stillage and thin stillage. Two regenerated cellulose membranes, YM 10 and YM 100 with pore sizes of 10 and 100 kDa, membranes were applied to evaluate ultrafiltration characteristics.

1.8 Thesis Organization

The thesis is organized into three parts, an introduction and literature review and two research studies. The introduction and literature review are in Chapter 1 that outlines the motivation behind the start of the ethanol industry, the current state of the ethanol industry, production process, co-products and separation technologies.

Chapter 3 investigates the physical and biological properties of whole stillage, thin stillage, and syrup. Chapter 4 identifies ultrafiltration process of whole stillage and thin stillage. Chapter 5 is the overall conclusions for this thesis. Chapter 6 presents recommendations for future study.

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CHAPTER 2: OBJECTIVES AND HYPOTHESIS

2.1 Objective and Hypotheses 1

2.1.1 Objective

The objective of the study presented within Chapter 3 was to test physical, and biological properties of whole stillage, thin stillage and syrup.

2.1.2 Hypotheses

It was hypothesized that whole stillage, thin stillage, and syrup have different characteristics.

2.2 Objective and Hypotheses 2

2.2.1 Objective

The objectives of this study presented within Chapter 4 were to evaluate operating parameters (stirring speed and volume capacity) on flux decline during batch ultrafiltration of thin stillage and whole stillage, and compare their ultrafiltration characteristics, including flux and solid recovery.

2.2.2 Hypothesis

It was hypothesized that: 1) the flux rate decrease over time in whole stillage and thin stillage samples, 2) membrane pore size of YM 10 has greater solids recovery for both whole stillage and thin stillage samples, and 3) membrane pore size of YM 100 has greater permeate flux rate in both whole stillage and thin stillage samples.

CHAPTER 3: PHYSICAL AND BIOLOGICAL PROPERTIES OF WHOLE STILLAGE, THIN STILLAGE AND SYRUP

3.1 Abstract

The production of bio-based ethanol has been one of the fastest growing industries in the U.S. during the last decade. Thus, wider exploration of ethanol co-product uses is necessary in ethanol plant. Currently, process steams such as whole stillage, thin stillage and syrup are processed into distillers dried grains with solubles and fed to livestock. The storability of whole stillage, thin stillage and syrup influences the economic and energetic balances of fuel ethanol production. But there are few investigations of the shelf life for those products, or how to measure these quantities.

The objectives of this research were to test physical and biological properties of whole stillage, thin stillage, and syrup, and determine storability and allowable shelf life for these materials as influenced by storage temperature levels. Using standard laboratory methods, several properties were determined, including moisture content, water activity, thermal properties (conductivity, resistivity, volumetric heat capacity, and diffusivity), color, mold development and CO_2 production. Also, the separation processes due to settling were observed over 72 hours. The thin stillage and whole stillage had relative high average moisture contents of 92% (w.b.) and 87% (w.b.) respectively, and mean water activity of 0.99; the high water content marked samples easily susceptible to rapid spoilage. Time had a significant effect ($P < 0.05$) on properties of co-products. Both thin stillage and whole stillage samples got mold growth after 5 days incubation at 32^{o}C. Thin stillage had the greatest separation rate in settling experiment. However, syrup had relative low average moisture content of 62% and average water activity of 0.92. No

mold growth and settling separation happened in syrup samples. There were no evidence showing a linear relationship exists between Hunters L*, a* and b*, and mold growth. The Solvita® test showed that high-temperature treatment caused high CO2 production in all samples. The exponential models described the relationship between storage time (from 0 to 5 days at 25ºC and 35ºC) and CO_2 concentration for three co-products.

This study is just the first step explore opportunities for utilizing valuable components from these co-products. Follow –up study should work on separation process to concentrate the valuable components of these co-products. Exploring the potential value of ethanol co-products could maintain and improve the profitability of ethanol industry.

3.2 Introduction

The corn ethanol industry has evolved into an invaluable economic engine for communities across the nation. In 2014, fuel ethanol production in the U.S. was 54,131 m^3 that supported 83,949 direct jobs in the agricultural sector and 295,265 indirect jobs across all sectors of the economy (RFA, 2015). Also, the ethanol industry makes an enormous and overlooked contribution to the production animal feed supply. In the ethanol process, one-third of every bushel of grain that enters the ethanol process is enhanced and sent to the animal feed market. Distiller grains, corn gluten feed, and corn gluten meal are the major co-products which are used to feed livestock. Only the starch portion of the grain is to produce ethanol; the remaining protein, fat, and fiber pass through the process. These nutrient-dense co-products are primarily fed to livestock, including beef cattle, dairy cows, swine, poultry, and fish in nations around the world. In 2014, the ethanol industry produced as estimated 39 billion kilograms of feed, making the

renewable fuels sector became the largest animal feed processing segments in the United States (RFA, 2015).

Ethanol production from corn is mainly classified into two types, namely: wet milling, and dry grinding. In the U.S., dry grinding is more predominant in ethanol production plants. In dry grinding ethanol processing, corn is ground into a fine powder which is then cooked to form starch. The enzymes, alpha-amylase, and glucoamylase are added to liquefy and hydrolyze the starch slurry into simple sugars for fermentation. Yeast is to fermentate the simple sugar to alcohol. The resulting mash called beer is distilled to separate ethanol with whole stillage. The whole stillage is then centrifuged to concentrate the solids, wet distiller grains (WDG) from the supernatant. Thin stillage from supernatant which contains the unfermented components (oil, fiber, protein, and minerals) and fermentation byproducts, then sent to an evaporator to make condensed distiller solubles (CDS). WDG and CDS are blended and dried in a large rotary drum dryer resulting in the DDGS. The properties of DDGS have been drawn great interest to researchers in the area of ethanol production and especially to people in the feed industry. The DDGS is sold as a feed ingredient for livestock due to its high proportion of nutritional components (Table 3.1). From Table 3.1, the relatively high content of protein affects the market value of DDGS. In addition, low moisture content (10 to 13%) in DDGS helps to prevent microbial degradation and to maintain product stability (Bhadra et al., 2009; Ganesan et al., 2008; Kim et al., 2007; Rosentrater and Muthukumarappan, 2006; Shurson and Alhamdi, 2008; Srinivasan et al., 2005; Srinivasan et al., 2009; Weigel et al., 1997).

Table 3.1 Average composition of nutrients (dry matter basis) in corn DDGS (Bhadra et al., 2009; Ganesan et al., 2008; Kim et al., 2007; Rosentrater and Muthukumarappan, 2006; Shurson and Alhamdi, 2008; Srinivasan et al., 2005; Srinivasan et al., 2009; Weigel et al., 1997).

Nutrient	Average
Crude protein, %	30.9
Crude fat, %	10.7
Crude fiber, %	7.2
Ash, %	6

However, drying distillers grains requires energy, time, and costs money; as a result, DDGS are more expensive than other distiller grains (Gorden, 2008). To lower operational costs, some other distiller grains could be considered as an alternative animal feed ingredient. Whole stillage is the product from distiller tank and then sent to centrifuge to produce liquid fraction (thin stillage) and solids fraction (wet distillers' grains). The thin stillage is then sent to the evaporator to make condensed distiller solubles (CDS). The mixture of wet distillers grains (WDG) and CDS is called wet distillers grains with soluble (WDGS), which can be used as an ingredient in animal feed. However, WDGS has 30%-35% moisture content and are less efficient to transport due to its short shelf life (Dooley et al. 2008). As a consequence, WDGS need to be delivered rapidly and utilized by animal feeding operation nearby an ethanol plant.

The recent growth of fuel ethanol production brought up an increased availability of ethanol co-products for animal food, thus the interest in using the co-products which are produced upstream of DDGS process also increased. Increasing using the upstream products in the marketplace, for instance, the whole stillage, thin stillage, and syrup, the environmental impacts, such as greenhouse gas emission, as well as the overall energy

consumption throughout the drying process and fuel ethanol production, could be significantly reduced. Thus, the storability of whole stillage, thin stillage, and syrup play a significant role in economic and energetic balances in animal feeding market.

While many studies have focused on properties of DDGS, little has been done to examine the properties of upstream components (whole stillage, thin stillage, and syrup). In order to fully understand the potential value of these upstream co-products, this study worked on physical and biological properties of whole stillage, thin stillage, and syrup to provide background for future research. The physical properties provide the information necessary to design and utilize unit operations, including handling, storage, and processing, while the biological analyses provide information about potential valuable components change over time. Thus, the objectives of this study were to: (1) determine various physical and biological properties of whole stillage, thin stillage, and syrup, (2) allowable shelf life for these three co-products as influenced by different storage temperature levels.

3.3 Materials and Methods

3.3.1 Initial Characterization

3.3.1.1 Experimental Design and Sampling

Fresh whole stillage, thin stillage, and syrup were collected from Lincolnway Energy plant in Nevada, Iowa. The samples were stored in sealed plastic buckets with screwed lid in a refrigerator at 4°C until needed.

Initial properties of whole stillage, thin stillage, and syrup were characterized at the outset of the experiment (t=0 day). The initial properties

(Figure 3.1) included moisture content, water activity, thermal conductivity, thermal resistivity, thermal diffusivity, volumetric specific heat, separation due to settling, and color analysis. The settling test was evaluated using two replications, and the rest tests were determined using three replications

3.3.1.2. Moisture Content and Water Activity

Moisture content was determined following the standard method S352.2 (ASAE 2004), using a forced-convection laboratory oven (Heratherm, Thermo Scientific Inc., Odessa, TX) at 135^{o}C for 2 hours. Water activity was measured by a calibrated water activity meter (Aqualab, Decagon Devices Inc., Pullman, WA).

3.3.1.3 Thermal Properties

Thermal conductivity, resistivity, diffusivity and volumetric specific heat were determined with a thermal properties meter (KD2, Decagon Devices, Pullman, Wash).

3.3.1.4 Separation due to Settling

For the settling test, products were homogenized then placed into conical settling columns. Two conical settling columns were assigned for each product. The Six conical settling columns were placed on the horizontal lab table at room temperature (25^{o}C). The volume of the top layer and bottom layer of each column was recorded hourly in the first 7-hour period, and then results were only reported at the 24th and 72nd hour.

3.3.1.5 Color Test

Color was measured using a spectrocolorimeter (CR-400 Chroma Meter, Konica Minolta, Ramsey, NJ) using the *L**-*a**-*b** opposable color scales (Hunter Associates Laboratory, 2002). Where *L**-value quantified the brightness/darkness; *a**-value denoted the redness/greenness, and *b**-value depicted the yellowness/blueness.

3.3.2. Physical and Biological Characterization of Samples over Time

3.3.2.1 Experimental Design and Sampling

Homogenized, fresh whole stillage, thin stillage, and syrup were evenly distributed into 180 sterile, plastic (100x 15 mm) petri dishes at room temperature. Each dish contained the same depth (10 mm). Sixty petri dishes of each co-product were divided into three groups. Each group (20 petri dishes) with lids containing samples were assigned to one of three incubation temperatures: 12^{o}C, 25^{o}C, and 32^{o}C. Three different temperatures were used to understand how these co-products behave as the temperatures changes during storage or processing. 25^{o}C was to set at average room temperature; 12^{o}C as chosen to predict behavior as products are processed in cooling operations or during storage at winter conditions; 35^{o}C was chosen to predict behavior as products are processed in heating operations or during storage at late summer conditions. The observation period was ten days.

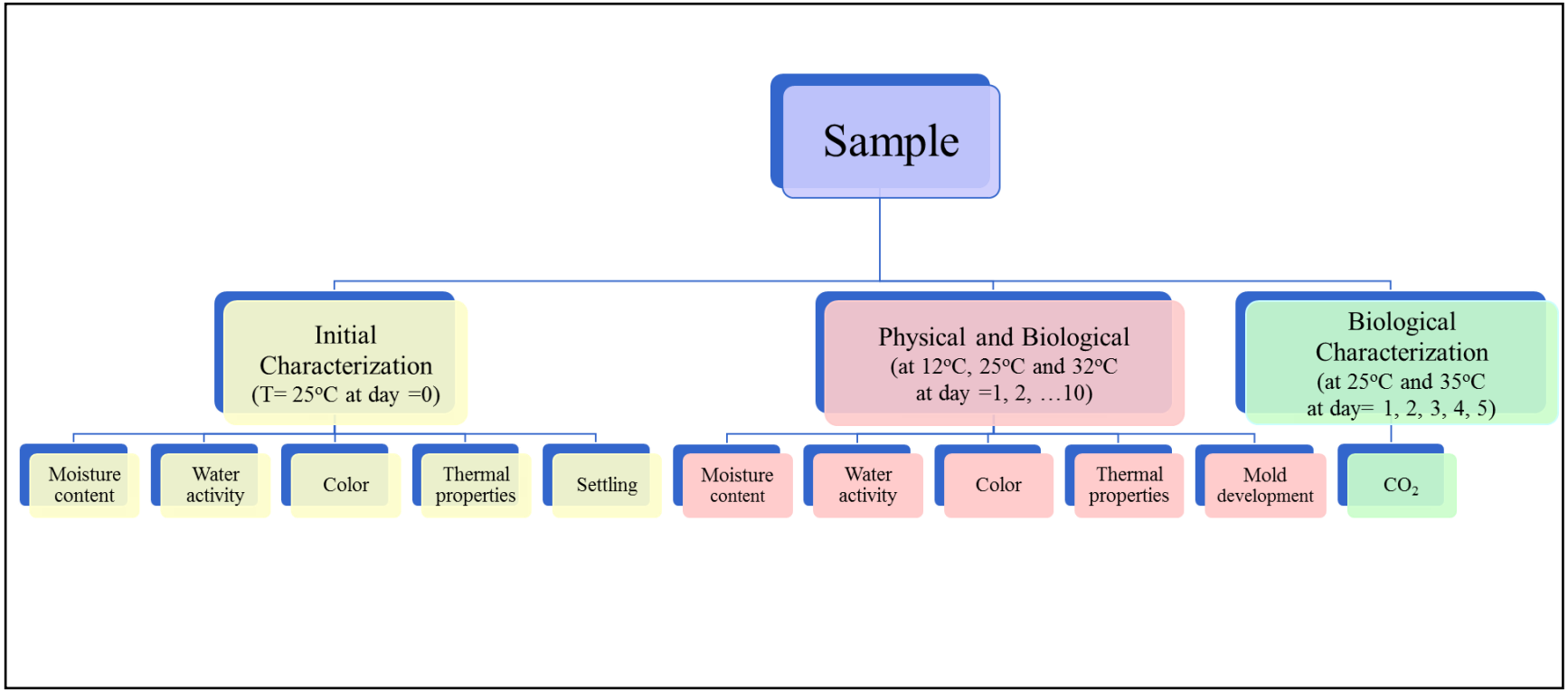

Figure 3.1 Experimental approach

At 1-day intervals, two randomly selected dishes containing each co-product at each temperature treatment were analyzed for moisture content, water activity, thermal properties, color, and the presence of mold, as shown in Figure 3.1. Two replications were applied for each test. Changes in the response variables (moisture content, water activity, thermal properties, color, and visual changes in mold) were plotted with time during the experiments.

3.3.2.2 Moisture Content and Water Activity

Moisture content was determined following the standard method S352.2 (ASAE 2004), using a forced-convection laboratory oven (Heratherm, Thermo Scientific Inc., Odessa, TX) at 103^{o}C for 2 hours. Twenty to thirty grams of each sample were transferred into aluminum moisture containers of known mass from petri dish. They were dried at 103^{o}C for 2 hours, placed on the working bench to cool to room temperature, and then weighed.

Water activity was measured by a calibrated water activity meter (Aqualab, Decagon Devices Inc., Pullman, WA), and tested at room temperature (24^{o}C $\pm$ 1^{o}C). Samples were placed in plastic dishes, and each dish had the same depth (1 mm) sample.

3.3.2.3 Thermal Properties

Thermal conductivity, resistivity, diffusivity and volumetric specific heat were determined with a thermal properties meter (KD2,

Decagon Devices, Inc., Pullman, WA) that utilized the line heat source probe technique. Two randomly selected samples from each temperature condition were poured into 50mL glass beakers. The sample in each beaker had the same depth (40mL).

3.3.2.4 Color Test

Samples were transferred to metal dishes. The color was measured using a spectrocolorimeter (CR-400 Chroma Meter, Konica Minolta, Ramsey, NJ) using the *L*-a*-b** opposable color scales (Hunter Associates Laboratory, 2002) according to manufacturer's guidelines. To measure color, each metal dish containing a sample was placed under the machine's sample observation port, and the three reflectance spectra measurements were collected.

3.3.2.5 Mold Observation

For the randomly selected samples on each day, the presence of visible mold in the petri dish was assessed by inspection using the following progressive rating system: 0, no visible mold; 1, any visible mold (< 50% of surface colonized); and 2, extensive colonized (> 50% of surface colonized).

3.3.3 Biological Characterization of Samples over Time

3.3.3.1 Solvita® Technology

Woods End Labs developed and markets the Solvita® that is a patented measurement system with the application for soil, compost,

manure and grain (Woods End Research, 2002). One type measures ammonia (NH_3) and the other is for carbon dioxide (CO_2) in a low and high range. Solvita® technology assesses a component of sample health by measuring carbon dioxide emissions which are primarily due to microbial respiration. Each Solvita® test kit consists of a 273-ml glass jar, a color scale, and a packet containing a polystyrene paddle that has a CO_2 sensitive gel surface on one side (Figure 3.2). The gel changes color according to the CO_2 concentration in the jar due to the PH buffer that is on the gel surface. The CO_2 produced by microbes inside the jar will diffuse into the gel, neutralizes the buffer and will cause a PH drop which will lead to color change on the gel surface. The color number is determined by comparing the color of the gel with the colors on the color card (Figure 3.2). The numbers on the color card are assigned to a series of colors that associate with the processing of color changes as the CO_2 concentration increasing in the jar. The relationship between the color number and CO_2 concentration in the test kit jars will be introduced in section 3.4.4. According to Woods End Laboratory, CO_2 concentrations greater than 3.02% (color 5) will not be measurable.

Brewer and Sullivan (2003) used Solvita® test kit to measure the rate of CO_2 release from compost. Doran et al., (1997) applied this test kit to test soil quality by measuring the respiration rate. Chitrakar et al. (2006) tested this kit for shelled corn and concluded that the equipment was able to quantify the storage condition of shelled corn. They quantified effects

of moisture content and incubation time on fungal growth in shelled corn. Moog et al. (2008) used Solvita® kits to measure the fungal susceptibility of shelled corn that has been rewetted to 21% moisture content and incubated at 24°C. Due to the successful use of Solvita® test kit in measuring CO_2 production from variety sources, this method was well-suited applicable for analysis of distiller grains from ethanol production, such as whole stillage, thin stillage, and syrup.

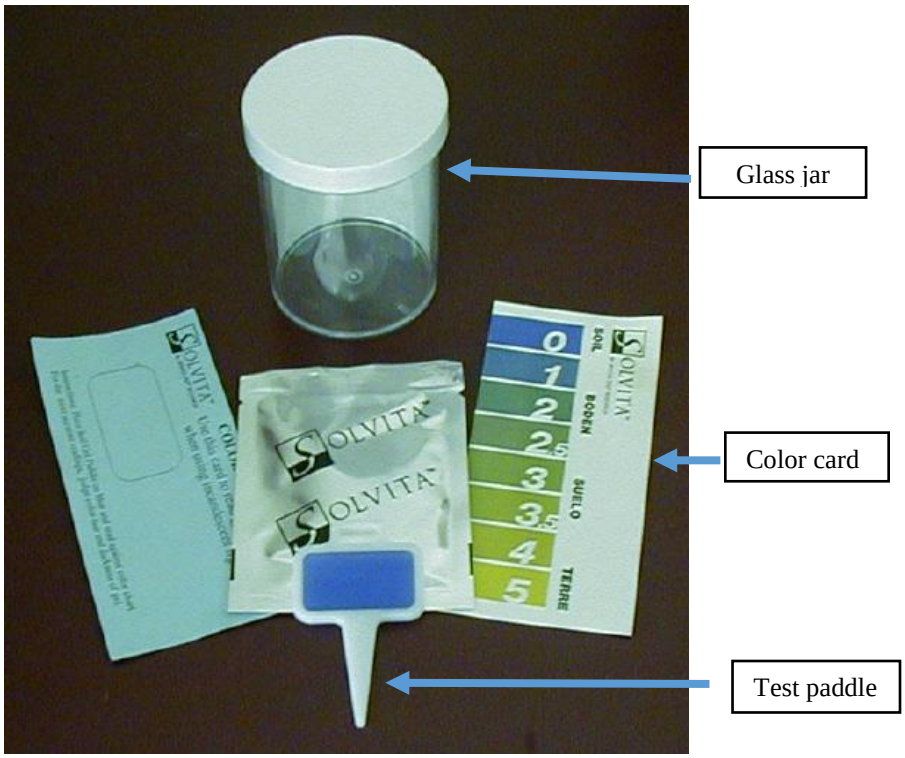

Figure 3.2. Solvita® test kit

3.3.3.2 Experimental Design and Sampling

Five mL of co-products were poured into a Solvita® glass jar with a Solvita® paddle, and the jar was sealed. For each co-product, two glass

jars were assigned to one of two incubation temperatures: 25^{o}C and 35^{o}C. Two different temperatures were used to understand how these products behave as their temperatures as adjusted during storage or processing: 25^{o}C was to set at average room temperature and 35^{o}C was chosen to predict behavior as products are processed in heat operations or during storage at late summer conditions.

CO_2 production (carbon mineralization or respiration) was used to estimate aerobic stability. CO_2 production was quantified by observing the color of paddle gel surface in sealed glass jar containing the sample at every 24-hour during the 5-day experiment (Figure 3.1). To minimize air exchange between the outside atmosphere and the air in the jars, the paddles were not removed, and the surface colors were observed through the jars. Two measurements of a sample were collected. A regression analysis was used to assess the correlation within 5-day respiration from Solvita® gel.

3.4 Data Analysis

Formal statistical analyses on all collected data were performed by Microsoft Excel v. 2013 (Microsoft Corporation, Redmond, WA), and JMP Pro v. 12 (JMP Campus Dr, Cary, NC) software, using a Type I error rate (α) of 0.05, and included summary statistics, which are given as means $\pm$ 1SD; ANOVA and General Linear Models (to test for differences over time); and nonlinear regression.

Table 3.2. Initial properties of whole stillage, thin stillage, and syrup at t = 0 [a]

Property	Replications	Min	Max	Mean	Standard deviation	Coefficient of variation
Moisture content (w.b.)	3	87%	87%	87%	0	0
Water activity	3	0.988	0.99	0.989	0.001	0.001
Thermal Conductivity (W/m °C)	3	0.433	0.84	0.67	0.21	0.31
Resistivity (m °C/W)	3	138.4	1191	520.1	582.85	1.12
Diffusivity (mm^2/s)	3	0.095	0.169	0.13	0.040	0.31
Color						
Hunter L	3	65.92	65.94	65.93	0.0100	0.0002
Hunter a	3	1.36	1.37	1.37	0.0100	0.0073
Hunter b	3	41.79	41.82	41.81	0.0200	0.0005

Property	Replications	Min	Max	Mean	Standard deviation	Coefficient of variation
Moisture content (w.b.)	3	92%	92%	92%	0	0
Water activity	3	0.985	0.987	0.986	0.0012	0.001
Thermal Conductivity (W/m °C)	3	0.439	0.723	0.607	0.149	0.25
Resistivity (m °C/W)	3	138.4	227.7	172.73	48.09	0.28
Diffusivity (mm^2/s)	3	0.119	0.154	0.14	0.0176	0.13
Color						
Hunter L	3	64.32	64.36	64.35	0.0231	0.00
Hunter a	3	-0.7	-0.69	-0.697	0.00577	-0.01
Hunter b	3	42.61	42.64	42.63	0.0173	0.00

Property	Replications	Min	Max	Mean	Standard deviation	Coefficient of variation
Moisture content (w.b.)	3	60%	63%	61.89%	0.02	0.003
Water activity	3	0.923	0.924	0.923	0.000577	0.001
Thermal Conductivity (W/m °C)	3	0.415	0.588	0.511	0.0882	0.17
Resistivity (m °C/W)	3	170.2	240.8	199.77	36.67	0.18
Diffusivity (mm^2/s)	3	0.13	0.149	0.14	0.0095	0.07
Color						
Hunter L	3	59.43	59.45	59.44	0.0115	0.00019
Hunter a	3	5.64	5.65	5.647	0.00577	0.00102
Hunter b	3	43.92	43.99	43.96	0.0289	0.00069

[a] n= 3 for all properties studied.

3.5 Results and Discussion

3.5.1 Initial Characterization

Initial properties of whole stillage, thin stillage, and syrup results are summarized in Table 3.2 which provides replications, minimum, maximum, and mean values, as well as the standard deviation for each parameter studied. Many of the properties generally have relatively low variability, with the coefficient of variation values ranging from 0 for moisture content in whole stillage and thin stillage samples to just 0.3 for conductivity in whole stillage sample. Resistivity appeared to exhibit some variation. This could be due to the nature of insufficiently mixed solid.

3.5.1.1 Moisture Content and Water Activity

Moisture content and water activity relate to product's shelf life. Moisture content is the amount of water contained in a material, including "bound" and "free" water. However, water activity in food which is the "free" water that can support the growth of microorganisms.

The samples in this study have initial average moisture contents of 87% (w.b.) in whole stillage, 92% (w.b.) in thin stillage and 62% (w.b.) in syrup (Table 3.2). Since thin stillage is the liquid fraction of whole stillage, it was expected to have the highest initial moisture content. The high moisture content of both whole stillage and thin stillage make them highly susceptible to rapid spoilage and microbial growth. In order to keep the products from spoiling and to reduce the cost associated with transpiration, the moisture content would need to be reduced to

approximately 6% (w.b.) to whole stillage and 11% (w.b.) to thin stillage, which is the recommended moisture content foe feed products as it allows them to be microbiologically safe and reduces the cost of transport (Beauchat, 1981; Wang et al., 1997). When comparing with whole stillage and thin stillage, syrup had relative low moisture content (62% w.b.), but it is still at high risk of spoilage. The shelf life for these co-products stream with high moisture content usually ranged from 4 to 7 days depending on storage conditions (Tjardes and Wright 2002).

Correspondingly, the mean water activity in these three materials were 0.99 in both whole stillage and thin stillage, and 0.92 in syrup (Table 3.2). The parameter of water activity quantities the amount of "free" water available for use by microorganisms and chemical agents, and therefore a measure of a material's susceptibility to spoilage and deterioration. Usually, products with no free water ($a_w = 0.0$) are not at risk for spoilage. However, materials with free surface water ($a_w = 1.0$) are at high risk for rapid degradation. Not surprisingly whole stillage thin stillage and syrup all had very high values of water activity (greater than 0.9), that indicate that these three materials appear to be at high risk of spoilage by microorganism growth.

The moisture content and water activity data collected from whole stillage and thin stillage in this study were similar to values published from others (Wood, 2013 and Byun, 2008). However, the data of syrup was slightly less than a previous study (Wood, 2013), the difference could

come from the different experimental and sampling procedures and various locations of sample generating.

3.5.1.2 Thermal Properties

Understanding how the products thermal properties behave is essential in design and utilization of unit operations, like how heating or cooling the product may need during the process.

The measurement of thermal conduction indicates the ability of a substance to transfer heat via conduction and serves as a way of predicting the rate of energy loss by the material (Stroshine, 2001). The mean thermal conductivity of whole stillage was determined to be 0.67 W/ (m ^{o}C), while it was approximately 0.607 W/ (m ^{o}C) in thin stillage and 0.511 W/ (m ^{o}C) in syrup. The thermal conductivity of distillers dried grains with solubles (DDGS) ranged from 0.06 to 0.08 W/ (m ^{o}C) (Rosentrater, 2006). The thermal conductivity value of DDGS is less than the experimental data collected from whole stillage, thin stillage, and syrup which was due to the relative high moisture content of these three co-products. It can be concluded that whole stillage, thin stillage and syrup have a higher thermal conductivity than DDGS which means that these co-products have higher heat transfer rate via conduction process.

Thermal diffusivity measures a substance's ability to conduct heat relative to its ability to store heat (Stroshine, 2001). The average thermal diffusivity for both thin stillage and syrup were 0.14 mm^2/s, while that for whole stillage was 0.13 mm^2/s (Table 3.2).

The thermal diffusivity of distillers dried grains with solubles (DDGS) ranged from 0.13 to 0.15 mm^2/s (Rosentrater, 2006). The diffusivity value of DDGS was very close to the experimental data of whole stillage, thin stillage, and syrup, because these three co-products are the upstream components of DDGS.

3.5.1.3 Color Test

The initial color of three co-products can be found in Table 3.2. At the outset of the experiment (t=0), color does show some variation between three co-products. *L** value (brightness - darkness) varied from 59.44 to 65.93; *a** value (redness - greenness) was -0.7 to 5.65; and *b** value (yellowness to blueness) varied from 41.81 to 42.63. When comparing the average color scale data for three co-products, whole stillage had largest positive L* value, which indicated that it was brightest. Thin stillage had lowest negative a* value, which showed that it was initially greenest. And largest positive b* value demonstrated that syrup was the most yellow. According to the DDGS values from a published study (Rosentrater, 2006), L* ranged from 43.48 to 48.8, a* was from 8.3 to 9.7, and b* ranged from 19.4 to 23.0. These results indicated that whole stillage, thin stillage, and syrup in this study were initially bright, greener and more yellow compare to the DDGS from the previous study.

3.5.1.4 Separation due to Settling

Settling was used to separate the particle from the liquid. As time goes on, the interface between the liquid and solid changes from non-existent at beginning, to having very distinct edges at the end.

The results were plotted with time to the ratio of top volume and bottom volume (Figures 3.3, 3.4, and 3.5). Two replications were combined into a single regression line for each co-product. A logarithmic model was fitted to each set of date, except for syrup. No separation happened in syrup (Figure.3.5). R^2 values of thin stillage and whole stillage were 0.98 and 0.89 respectively. Based on the R^2 value, the regression equation was able to determine the ratio of the volume change during storage. Since the data was collected every hour during the first 7 hours, and then, results were only reported at time points 24 hours and 72 hours, which is why the data are more intensive in the first 7 hours. For thin stillage and whole stillage (Figures 3.3 and 3.4), the volume ratio of the top layer and bottom layer increased significantly in the first 7-hour and then slowly increased. However, the ratio increased more dramatically in thin stillage, which means the particle moved faster in thin stillage (Figure 3.3).

The effect of settling is a necessary to design material mixing, handling and storage processes. Typically, the slurry co-product has always needed to be re-homogenized after a period of time in storage, due to the settling of the abrasive content. In this study, sedimentation

happened in whole stillage and thin stillage after 1-hour storage, which indicated that these co-products need be re-homogenized after 1 hour. To fully characterize the behavior of a co-product that contains solid and liquid over time, detailed shelf life and aging studied are needed to evaluate the mixing requirements needed to re-homogenize the co-product at different ages to keep it fresh and maximize shelf life.

3.5.2 Physical and Biological Characterization of Samples over Time

3.5.2.1 Moisture Content

Moisture content indicates the amount of water in the sample. Moisture content is related to the freshness and stability for the storage of the material. Moisture contents (w.b) of whole stillage, thin stillage and syrup are summarized in Figure 3.6. The whole stillage was found to have moisture content about 4% less than that of thin stillage at about 93% (w.b) after the first day incubation at three temperature treatments. Since thin stillage is the liquid fraction of whole stillage, it was expected to have the highest overall moisture content (97% in wet basis). Syrup is the product from evaporation where much of liquid is removed. Thus, the overall moisture content of syrup was about 65% (w.b).

In this study, temperature and time treatments had significant effects ($p < 0.05$) on moisture contents of these three co-products (Tables 3.3 and 3.4), especially the effect of time treatments ($p < 0.0001$) (Table 3.4). As expected, the samples in the 32^{o}C incubation had the lowest moisture at the end of day 10 (Figure 3.6), due to the highest temperature causing the greatest evaporation rate.

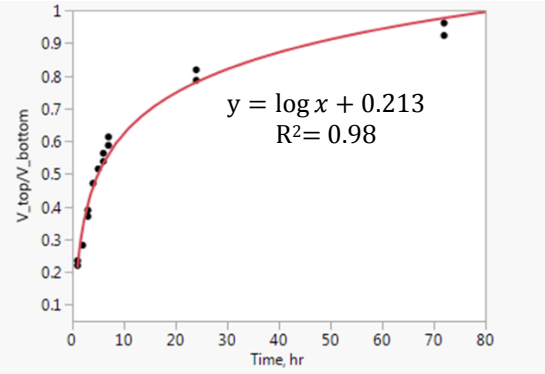

Figure 3.3. Settling result of V_{top} / V_{bottom} in thin stillage

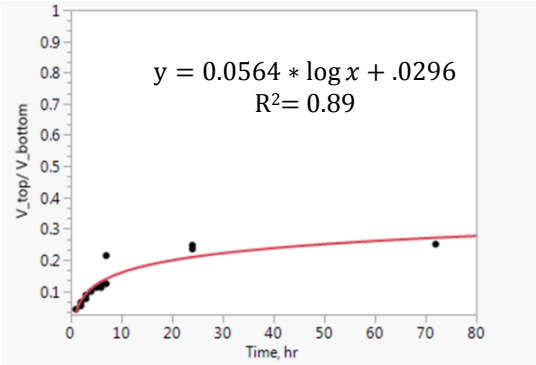

Figure 3.4. Settling result of V_{top} / V_{bottom} in whole stillage

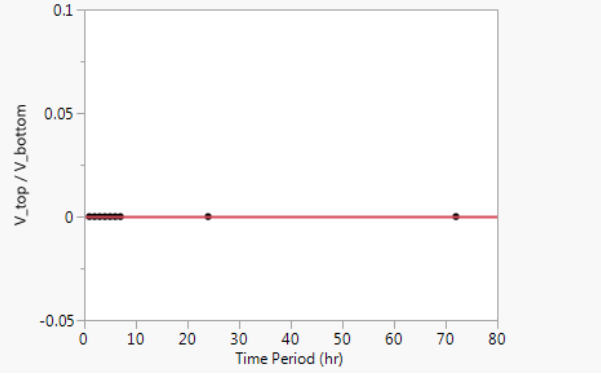

Figure 3.5. Settling result of V_{top} / V_{bottom} in syrup sample

Table 3.3 Moistures contents of three co-products effects by temperatures [a]

Treatments	Whole stillage		Thin stillage		Syrup	
	Mean (%)	SD[b]	Mean (%)	SD[b]	Mean (%)	SD[b]
12°C	86 A	0.02	92 A	0.01	66 A	0.01
25°C	85 AB	0.02	92 A	0.01	64 B	0.01
32°C	84 B	0.03	91 B	0.01	64 B	0.02

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of temperature was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

Table 3.4 Moistures content of three co-products effects over time [a]

Time	Whole stillage		Thin stillage		Syrup	
	Mean (%)	SD[d]	Mean (%)	SD[d]	Mean (%)	SD[d]
1	89 A	0.01	93 A	0.007	92 A	0.002
2	88 AB	0.01	92 AB	0.02	92 AB	0.0008
3	87 BC	0.01	92 AB	0.005	92 ABC	0.002
4	87 BCD	0.01	92 AB	0.005	92 BCD	0.002
5	86 CD	0.01	92 ABC	0.01	92 CDE	0.002
6	85 DE	0.01	91 BC	0.02	91 CDE	0.005
7	84 EF	0.01	91 BC	0.02	91 DE	0.006
8	84 EF	0.01	91 BC	0.007	91 EF	0.005
9	83 F	0.01	91 C	0.01	91 FG	0.006
10	81 G	0.01	89 D	0.01	91 G	0.008

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of time was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

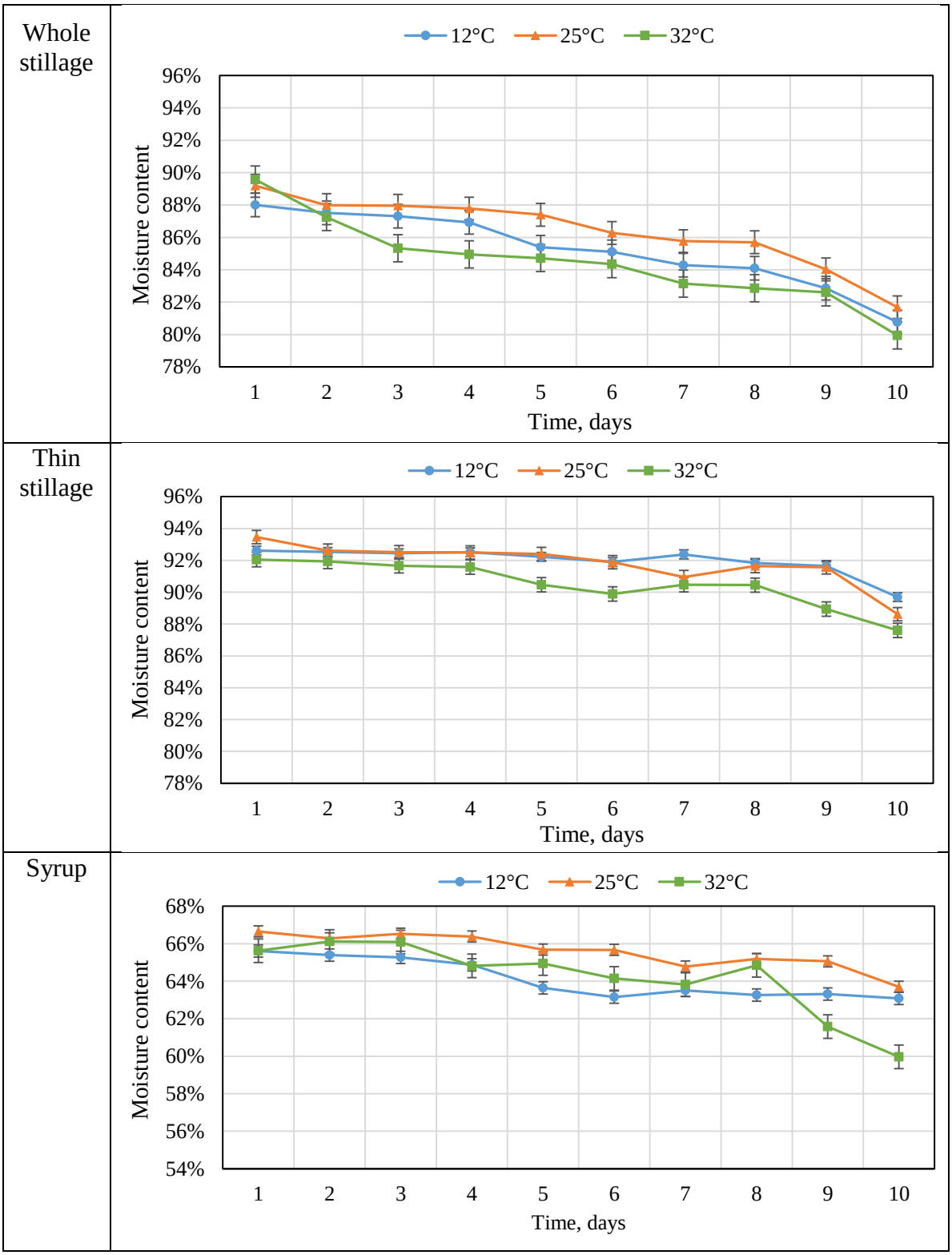

Figure 3.6. Average moisture content changes over time. Error bars are 1 standard deviation.

3.5.2.2 Water Activity

Water activity measures the amount of "free" water available for use by microorganisms and chemical agents, and is therefore a measure of a material's susceptibility to spoilage. Thus, products with no free water (aw=0.0) are not at risk for degeneration, while materials with free water (aw=1.0) are at high risk for rapid spoilage (Rosentrater and Lehman, 2008). Figure 3.7 describes the mean values of water activity of the three co-products change over time. In general, the values of water activity in three materials decreased over the 10-day experiment, but the value were still high. The "free" water in whole stillage and thin stillage ranged from 0.96 to 0.98 at the end of the experiment, While the value of water activity in syrup changed from 0.92 to around 0.9 (Figure 3.7). The results indicated that all three co-products were easy for rapid degradation. The samples incubated at 12^{o}C and 25^{o}C had a lower water activity value, compared with the samples in 32^{o}C incubator, due to high temperature decreased the water content.

Tables 3.5 and 3.6 show that temperature and time treatments had effects on water activity of the co-products, especially the effect of time treatments were significant ($p < 0.0001$) (Table 3.6).

Table 3.5 Water activity of three co-products effects by temperatures [a]

Treatments	Whole stillage		Thin stillage		Syrup	
	Mean	SD[b]	Mean	SD[b]	Mean	SD[b]
12°C	0.98 A	0.007	0.98 A	0.009	0.92 A	0.004
25°C	0.98 AB	0.005	0.98 A	0.001	0.92 A	0.004
32°C	0.97 B	0.009	0.98 A	0.001	0.91 B	0.009

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of temperature was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

Table 3.6 Water activity of three co-products effects over time [a]

Time	Whole stillage		Thin stillage		Syrup	
	Mean	SD[d]	Mean	SD[d]	Mean	SD[d]
1	0.99 A	0.001	0.99 A	0	0.92 A	0.002
2	0.98 B	0.003	0.98 AB	0.0006	0.92 AB	0.0008
3	0.98 B	0.004	0.98 BC	0.003	0.92 ABC	0.002
4	0.98 C	0.002	0.98 BC	0.002	0.92 BCD	0.002
5	0.98 CD	0.001	0.98 C	0.0006	0.92 CDE	0.002
6	0.98 DE	0.002	0.98 D	0	0.91 CDE	0.005
7	0.97 E	0.003	0.98 DE	0	0.91 DE	0.006
8	0.97 EF	0.004	0.98 E	0.001	0.91 EF	0.005
9	0.97 F	0.006	0.98 F	0.001	0.91 FG	0.006
10	0.97 F	0.006	0.97 G	0.0006	0.91 G	0.008

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of time was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

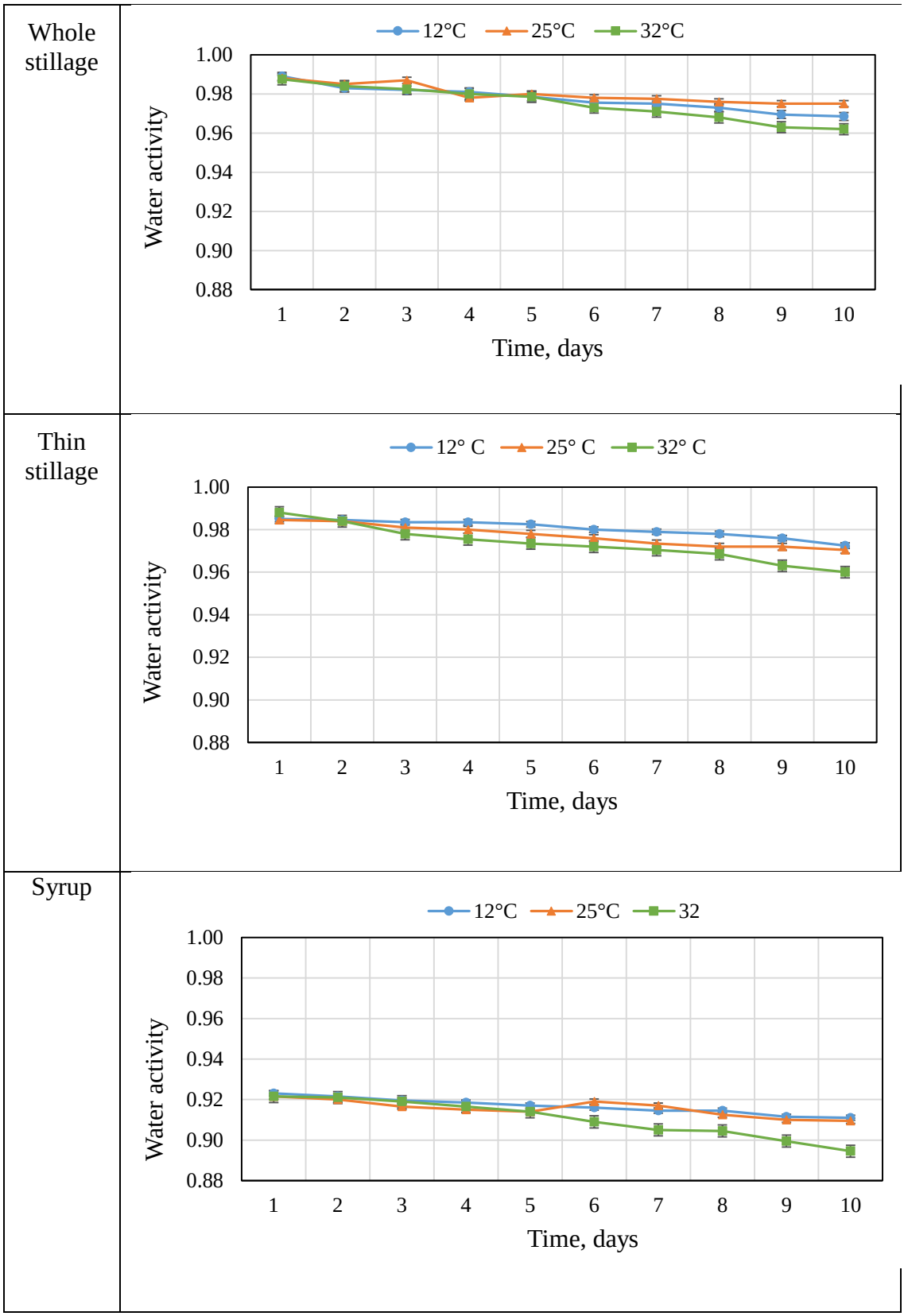

Figure 3.7 Mean water activity changing over time. Error bars are 1 standard deviation

3.5.2.3 Mold Observation

Visible mold is an indicator of progressing spoilage that is easily assessed and therefore the development of molds on whole stillage, thin stillage and syrup were measured by visual inspection and assignment of the numerical rating. Tables 3.7 and 3.8 demonstrate that temperature and time treatments had significant effects ($P < 0.05$) on mold growth on whole stillage and thin stillage. Figures 3.8 and 3.9 show the mold development over time on whole stillage and thin stillage. When samples were held at 32^{o}C incubator, mold first appeared at day 5 on the surface of both whole stillage and thin stillage samples. For 25^{o}C treatment, whole stillage grew mold at 5th day, and the first mold was observed on thin stillage at day 6. Compared with the 32^{o}C and 25^{o}C treatments, samples had a longer shelf life in 12^{o}C treatment. The appearance of mold in whole stillage and thin stillage was between 8 and 9 days. Surprisingly, no mold was found on surface of syrup at three temperature conditions during the 10-day experiment.

Table 3.7 Mold developments of whole stillage and thin stillage effect by temperatures [a]

Treatments	Whole stillage		Thin stillage	
	Mean	SD[b]	Mean	SD[b]
12°C	0.25 B	0.44	0.2 B	0.41
25°C	0.75 A	0.72	0.6 A	0.68
32°C	0.85 A	0.81	0.75 A	0.72

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of temperature was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

Table 3.8 Mold developments of whole stillage and thin stillage over time [a]

Time	Whole stillage		Thin stillage	
	Mean	SD[d]	Mean	SD[d]
1	0 C	0	0 D	0
2	0 C	0	0 D	0
3	0 C	0	0 D	0
4	0 C	0	0 D	0
5	0.67 B	0.52	0.33 CD	0.52
6	0.67 B	0.52	0.67 C	0.52
7	0.67 B	0.52	0.67 C	0.52
8	1 B	0.63	0.67 C	0.52
9	1.67 A	0.55	1.17 B	0.41
10	1.67 A	0.52	1.67 A	0.52

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of time was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

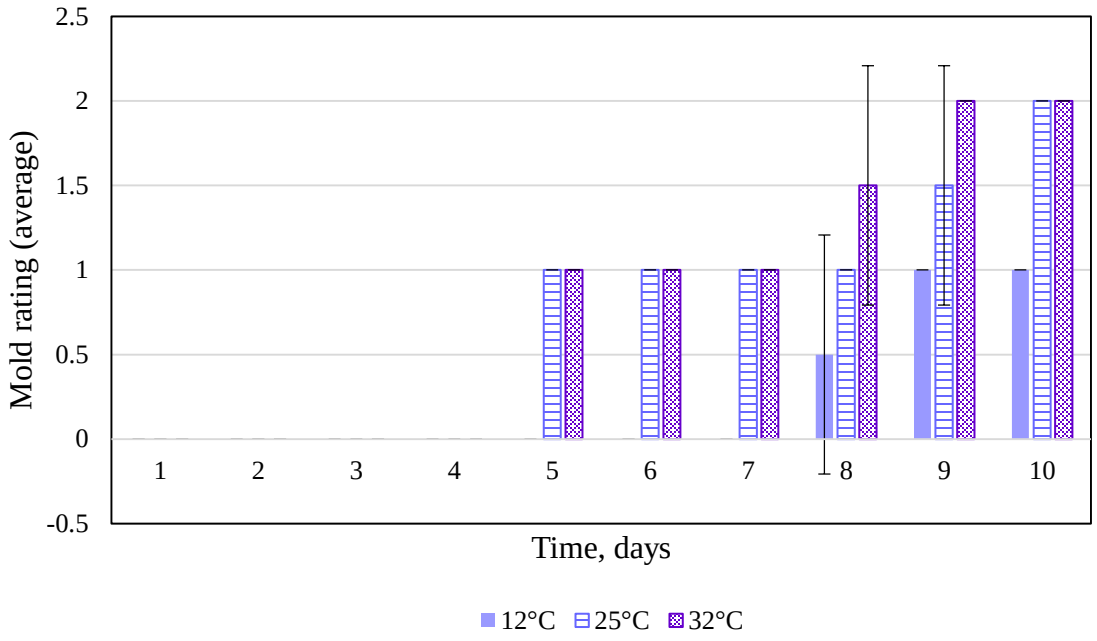

Figure. 3.8. Mold development in whole stillage. Error bars are 1 standard deviation.

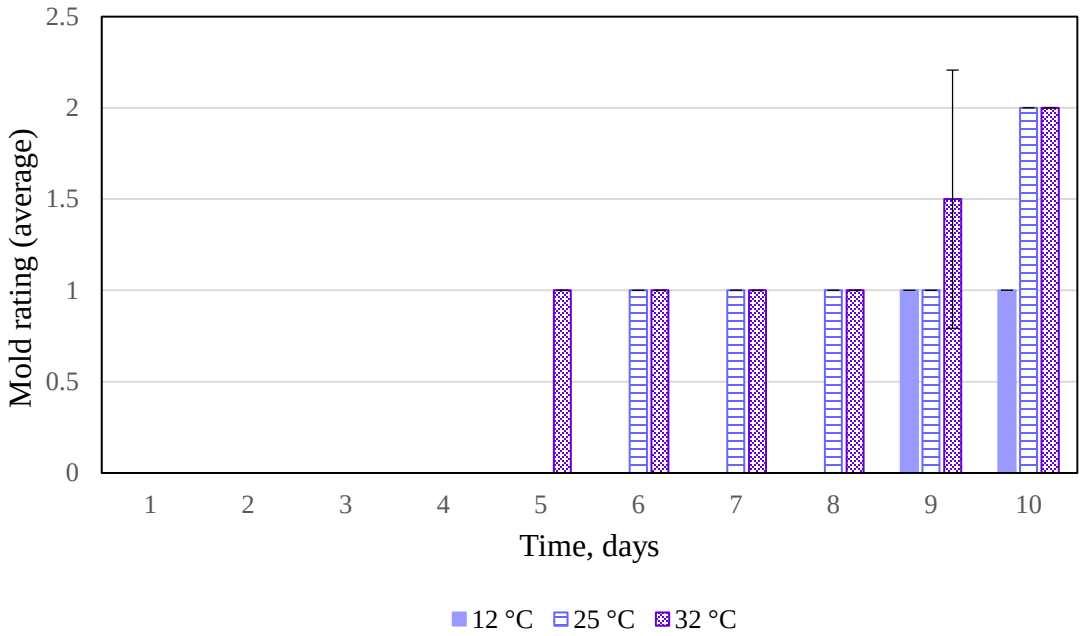

Figure 3.9. Mold development in thin stillage. Error bars are 1 standard deviation.

3.5.2.3 Color Test

The presence of the mold on the surface causes visual color changes in the visual observation of the samples. In a previous study, Hunter *L**, *a**, and *b** color parameters were not as straightforward as microbial growth and activity in distiller's wet grains (Rosentrater and Lehman, 2008). The color values of three co-products are summarized in Figures 3.10, 3.11 and 3.12. In the current study, color (Hunter *L**, *a**, and *b**) appeared to change over time, but these changes did not directly correspond with the mold growth.

Hunter *L** decreased as the retention time increased in all three co-products at any of the three temperature treatments. The day 2's data for thin stillage and syrup seems different, due to equipment error. There was a significant ($P < 0.001$) time effect on Hunter *L** value changed over time on these three co-products (Tables 3.10, 3.12, and 3.14). However, no observable correlation between temperature/time and the *L** value was detected in three co-products. A previous study mentioned the Hunter *L** did not predict any of the microbial stability parameters very well on distiller's wet grains (Rosentrater and Lehman, 2008). Similarly, storage time had a significant effect ($P < 0.05$) on Hunter *a** and *b** values (Table 3.10). However, for the most case, temperature didn't found to be significant on Hunter *L**, *a**, and *b** values (Tables 3.11 and 3.13), except the case of the Hunter *a** value in whole stillage samples (Table 3.9).

The changes of Hunter *a** and *b** values were related to the some of the first appearance of mold on samples. In whole stillage (Figure 3.10), the value of Hunter *a** and *b** suddenly changed at day 8 when the ambient temperature was

12°C (Figure 3.10). Similar situation was fund in thin stillage samples in 12°C incubator. Even though Hunter *a** and *b** values had some noticeable changes when the first appearance of mold on the whole stillage and thin stillage at 12°C temperature treatment (Figure 3.10), there is no evidence showing a linear relationship exist between Hunters *a** and *b**, and mold growth.

Table 3.9 Color of whole stillage effect by temperatures [a]

Treatments	Hunter L		Hunter a		Hunter b	
	Mean	SD[d]	Mean	SD[d]	Mean	SD[d]
12°C	59.16 A	2.25	1.51 A	0.46	37.4 A	1.41
25°C	59.76 A	0.69	1.48 B	0.07	37.33 A	1.09
32°C	58.48 A	1.73	1.06 A	0.31	36.91 A	1.98

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of temperature was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

Table 3.10 Color of whole stillage over time [a]

Time	Hunter L		Hunter a		Hunter b	
	Mean	SD[d]	Mean	SD[d]	Mean	SD[d]
1	62.43 A	1.37	1.08 A	0.1	39.72 A	1.09
2	60.67 B	0.08	1.12 A	0.02	39.25 A	1.16
3	59.82 BC	0.67	1.1 A	0.17	37.43 BC	0.58
4	59.22 CD	0.71	1.3 A	0.17	37.02 BC	0.19
5	59.2 CD	0.54	1.35 A	0.14	36.78 C	1.07
6	58.97 CDE	0.92	1.35 A	0.32	36.7 C	0.17
7	57.49 F	1.49	1.42 A	0.32	36.8 BC	0.1
8	57.83 EF	1.05	1.84 A	0.71	37.7 B	0.7
9	57.51 F	1.31	1.61 A	0.47	35.38 D	1.01
10	58.2 DEF	0.85	1.37 A	0.28	35.41 D	0.67

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of temperature was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

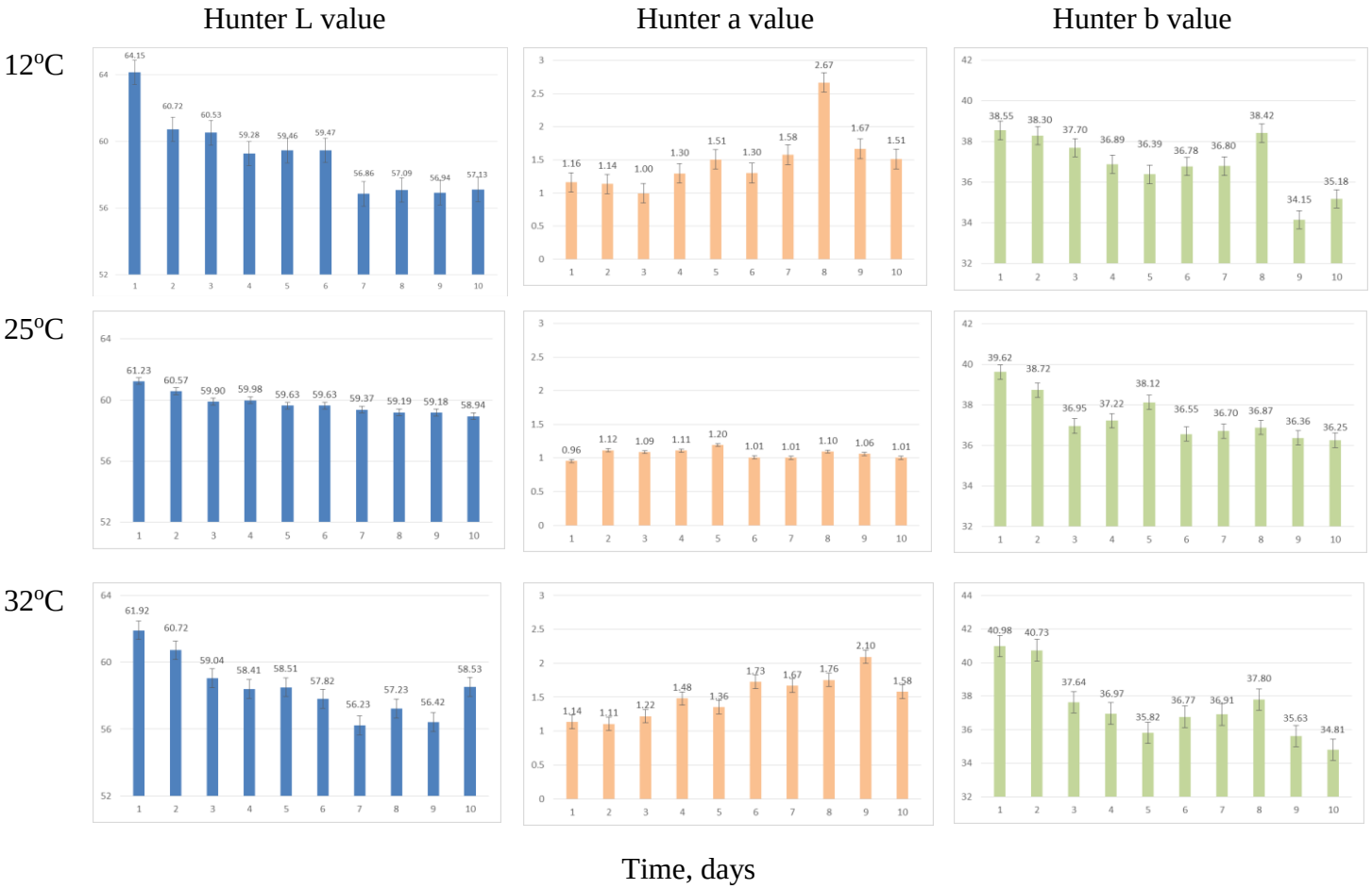

Figure 3.10. Average hunter color values change over time for whole stillage sampling due to incubation temperatures. Error bars are 1 standard deviation.

Table 3.11 Color of thin stillage effect by temperatures [a]

Treatments	Hunter L		Hunter a		Hunter b	
	Mean	SD[d]	Mean	SD[d]	Mean	SD[d]
12°C	59.58 A	9.96	-1.08 A	0.67	40.02 A	1.97
25°C	58.88 A	9.68	-1.32 A	0.6	40.84 A	1.38
32°C	58.59 A	9.74	-0.84 A	0.58	41.26 A	2.28

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of temperature was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

Table 3.12 Color of thin stillage over time [a]

Time	Hunter L		Hunter a		Hunter b	
	Mean	SD[d]	Mean	SD[d]	Mean	SD[d]
1	64.86 A	1.05	-0.66 A	0.05	45 A	1.9
2	30.69 D	0.12	-1.09 ABC	0.02	40.57 CD	0.18
3	61.54 BC	1.22	-1.23 BCD	0.33	40.56 CD	1.06
4	61.44 BC	1.36	-1.50 CDE	0.59	40.43 CD	1.21
5	62.24 B	1.66	-1.83 E	0.44	41.54 BC	2.08
6	62.4 B	0.44	-0.58 A	0.06	41.54 BC	0.77
7	62.38 B	0.21	-0.89 AB	0.27	42.46 B	0.33
8	61.88 B	1.4	-0.53 A	0.16	40.99 BC	2.04
9	60.48 C	1.45	-1.70 DE	1.12	39.08 D	1.37
10	62.28 B	0.47	-0.78 AB	0.59	41.55 BC	0.91

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of temperature was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

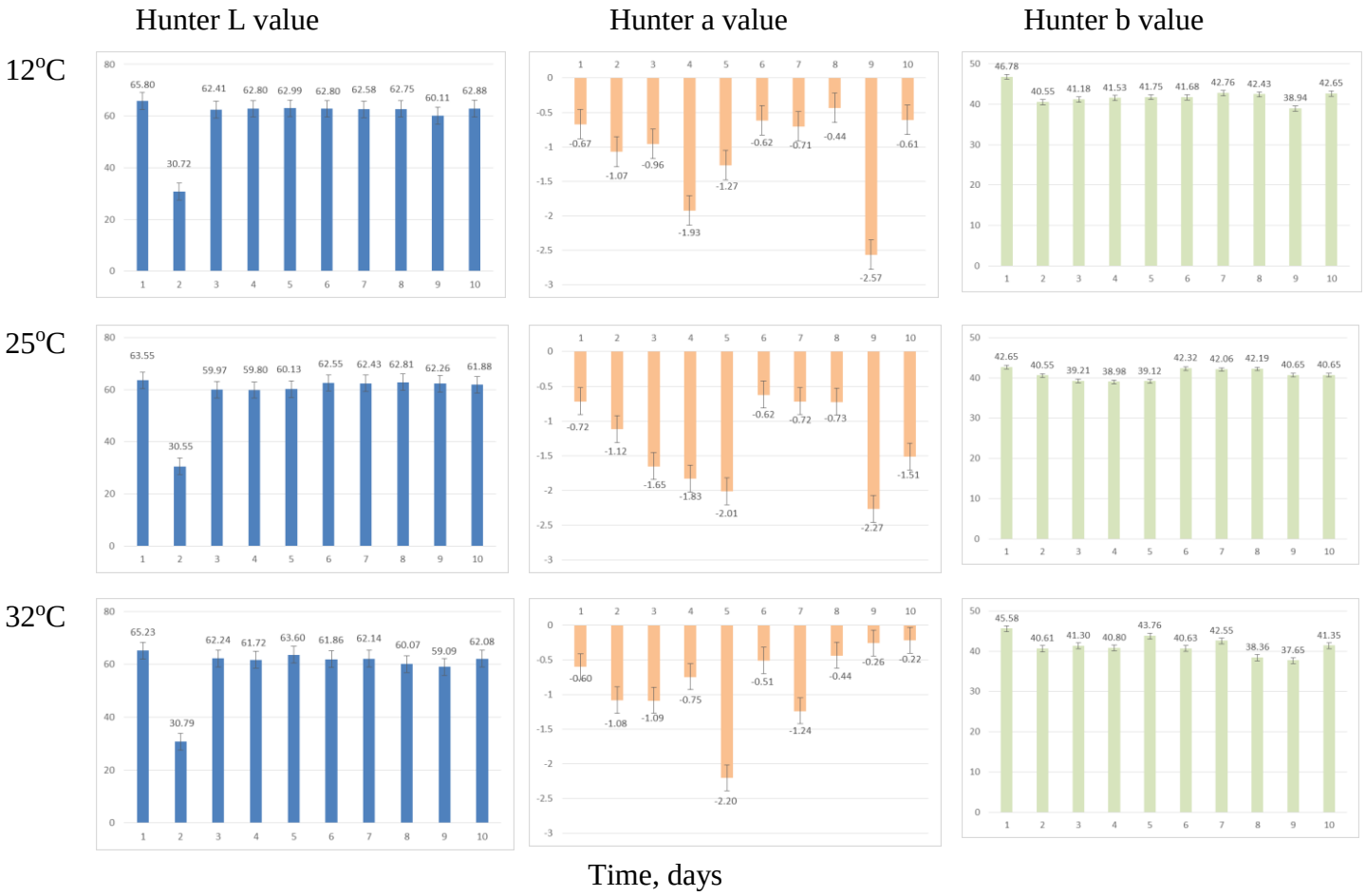

Figure 3.11 Average hunter color values change over time for thin stillage sampling due to incubation temperatures. Error bars are 1 standard deviation.

Table 3.13 Color of syrup effect by temperatures [a]

Treatments	Hunter L		Hunter a		Hunter b	
	Mean	SD[d]	Mean	SD[d]	Mean	SD[d]
12°C	52.09 A	7.35	4.61 A	1.28	36.67 A	1.21
25°C	51.91 A	7.31	4.37 A	1.15	36.40 A	0.91
32°C	49.21 A	6.42	5.13 A	1.44	33.70 A	2.07

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of temperature was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

Table 3.14 Color of syrup over time [a]

Time	Hunter L		Hunter a		Hunter b	
	Mean	SD[d]	Mean	SD[d]	Mean	SD[d]
1	54.95 A	0.63	4.59 C	0.28	37.45 A	0.09
2	30.66 C	0.07	1.1 D	0.008	37.27 A	0.14
3	53.32 AB	1.23	5.33 AB	0.81	34.91 B	1.95
4	53.15 AB	1.78	5.22 AB	0.58	34.99 B	2.07
5	53.41 AB	2.1	5.26 AB	0.71	35.56 AB	2.52
6	53.35 AB	1.58	4.86 BC	0.32	34.57 B	1.05
7	52.5 B	2.07	5.62 A	0.37	34.3 B	1.64
8	52.84 B	1.37	4.99 BC	0.24	35.71 AB	1.72
9	53.52 AB	1.76	4.84 BC	0.17	36.36 AB	2.16
10	53.01 B	2.16	5.24 AB	0.47	34.77 B	2.71

[a] Different letters within a given column indicate that, according to least significant difference (LSD) testing, the effect of temperature was significant at the α=0.05 level for that specific dependent variable;

[b] SD is standard deviation

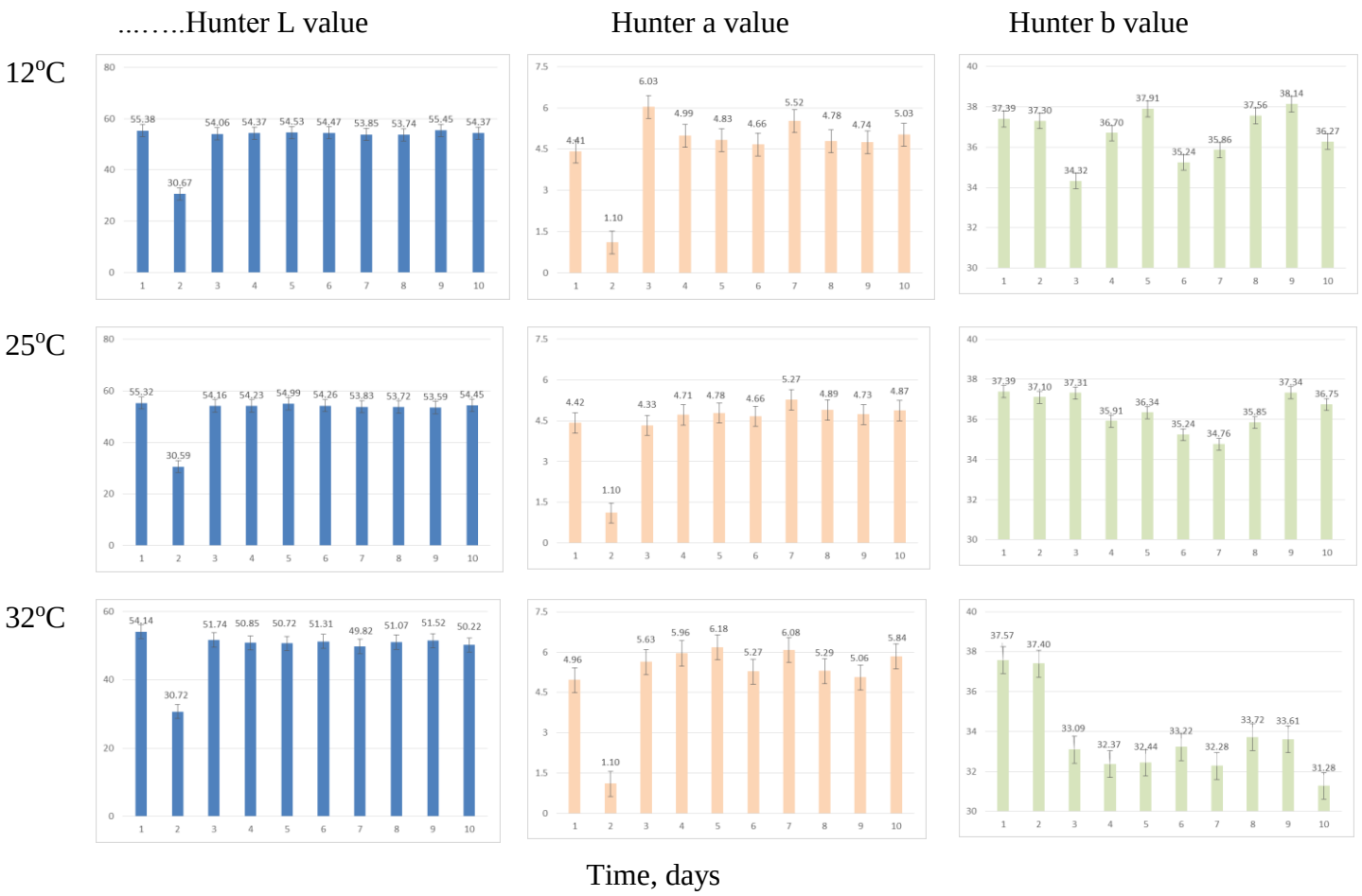

Figure 3.12 Average hunter color values over time for syrup sampling due to incubation temperatures. Error bars are 1 standard deviation.

3.5.3 Biological Characterization of Samples over Time

Before the CO_2 kit results can be used by the grain industry to define degradation levels, the relationship between a sample's kit color readings and the CO_2 concentration in the test kit jar must be understood. The following equation is used to analyze the conversion of color readings (0.5 to 5) to the percent of CO_2 in the test kit jar (Stroshine, 2000):

$$\% CO_2 = \exp(-2.2872 + 0.6784 * (\text{Color reading}))$$

The CO_2 concentration corresponding to color number is showed below:

Table 3.15 CO_2 concentration corresponding to Solvita® color numbers

Solvita® Color Number								
	0	1	2	2.5	3	3.5	4	5
% CO_2	0.1	0.2	0.4	0.6	0.8	1.1	1.5	3.0

It could be difficult to determine paddle color precisely by visual comparison to the color card. Subtle variations in paddle color are hard to distinguish. With experience, it is possible to estimate color number within ± 0.25 color numbers greater than 2.0. For numbers between 0 and 1, the best accuracy that could be achieved in this study was ± 0.5. With experience and care, it was possible to read color numbers to ± 0.25 between 1.0 and 5.0.

CO_2 production is an indicator of microbial activity. In tests on samples of whole stillage, thin stillage and syrup in this study, the results of tests on the co-products shows the color numbers ranged from 1.0 to 5.0 when paddles were inserted during the 5-day experiment. Obviously, a sample with a color number of 5.0 is highly susceptible mold growth, whereas as sample with a color number of 1.0 has a low susceptibility.

Figures 3.13 to 3.18 summarize storage time versus percent CO_2 for whole stillage, thin stillage, and syrup incubated at 25ºC and 35ºC respectively. The effect of temperature and time treatment on CO_2 production was significant ($p < 0.05$). As expected, CO_2 production was greatest at 35ºC, and moderate at 25ºC for three co-products. An exponential model was fitted to each set of data (Figures 3.13- 3.18). R^2 ranged from 0.58 to 0.95, which showed that the most molds were good fit. Because the equipment can't capture the results exceeding the max measurable CO_2 concentration, the CO_2 concentration didn't change once it hit the maximum measurable value.

3.6 Conclusion

DDGS is primarily sold as a feed ingredient for livestock due to its high proportion of nutritional components and low moisture content. However, drying DDGS requires energy, time and costs money; as a result, DDGS is more expensive than other distiller grains. In order to lower operational costs, some other distiller grains with relatively high moisture content, including whole stillage, thin stillage, and syrup could be considered as an alternative material to feed livestock. This study worked on various physical and biological properties, including moisture content, water activity, thermal properties, color, mold growth, and CO_2 production, to provide background for future study.

The initial thin stillage and whole stillage samples had high average moisture contents of 92% (w.b.) and 87% (w.b.) respectively, and average water activity of 0.99; the high water content marked these samples easily susceptible to rapid spoilage. The time treatment had significant effects ($p < 0.0001$) on the values of moisture content and water activity change in these three co-products. Both thin stillage and whole stillage samples showed the first mold growth after five days' incubation at 32^{o}C, due to the high temperature increased microorganisms' activities. Thin stillage had the greatest separation rate in settling experiment. In comparison with the water content in thin stillage and whole stillage, syrup had the average initial moisture content of 62% and water activity of 0.92. No mold growth and settling separation happened in syrup samples. There were no evidence showing a linear relationship exists between Hunters L^*, a^* and b^*, and mold growth. The Solvita® testing showed that high-temperature treatment caused high CO_2 production in all samples. The exponential models described the relationship between storage time (from 0 to 5 days at 25^{o}C and 35^{o}C) and CO_2 concentration for three co-products.

This study worked on physical and biological properties of whole stillage, thin stillage, and syrup to provide information on material handling, processing, and potential valuable components change over time. Thus, the study is just the first step explore opportunities for utilizing valuable components from these co-products. Follow –up study should work on separation process to concentrate the valuable components of these co-products. Exploring the potential value of ethanol co-products could maintain and improve the profitability of ethanol industry.

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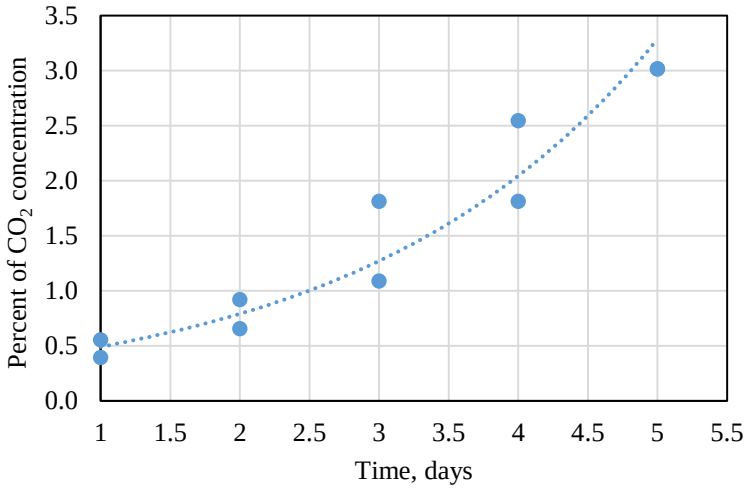

Figure 3.13. % CO_2 in the air in glass jars over time in whole stillage at 25ºC. Each point represents one reading.

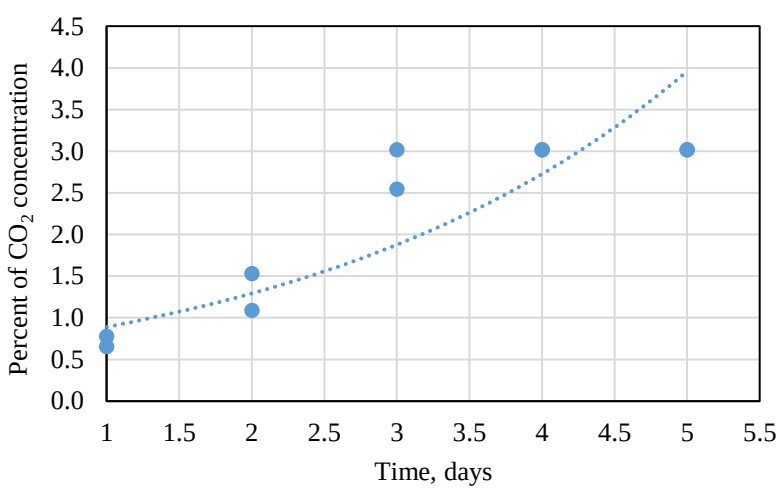

Figure 3.14. % CO_2 in the air in glass jars over time in whole stillage at 35ºC. Each point represents one reading

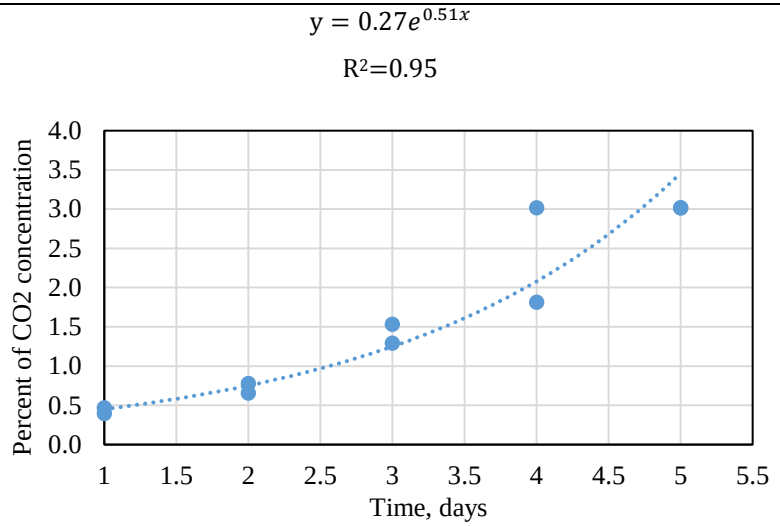

Figure 3.15. % CO_2 in the air in glass jars over time in thin stillage at 25ºC. Each point represents one reading.

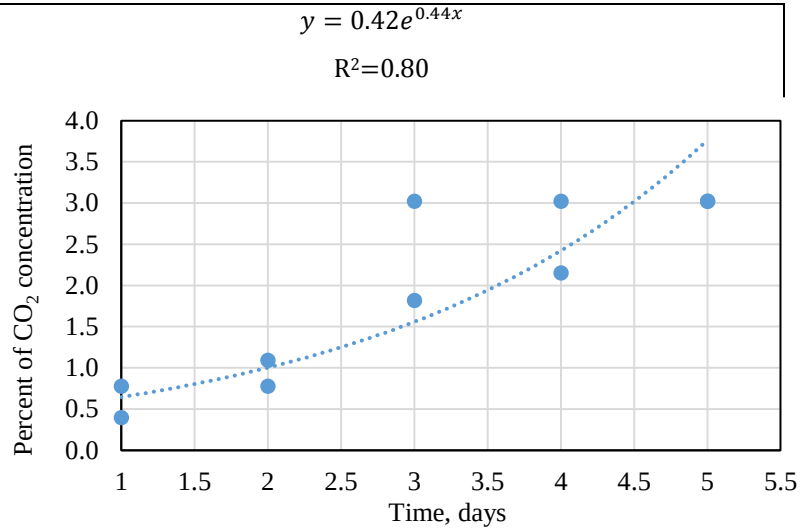

Figure 3.16. % CO_2 in the air in glass jars over time in thin stillage at 35ºC. Each point represents one reading

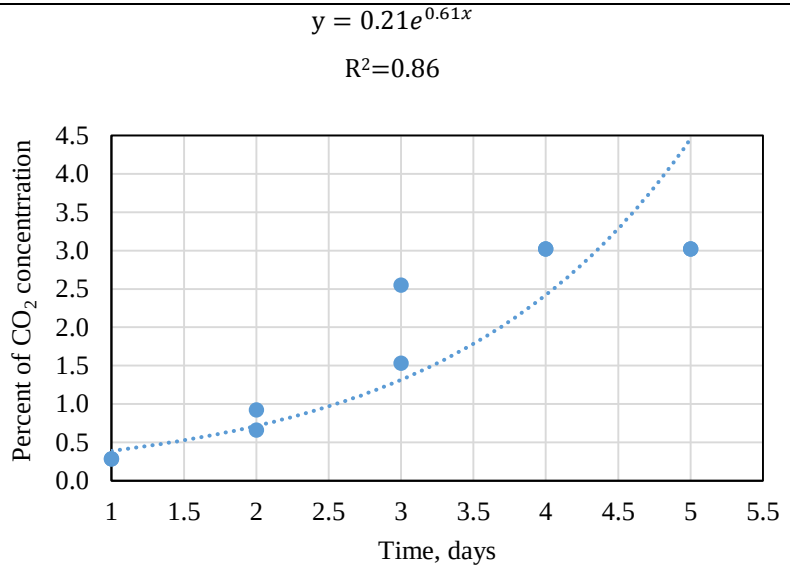

Figure 3.17. % CO_2 in the air in glass jars over time in Syrup at 25ºC. Each point represents one reading.

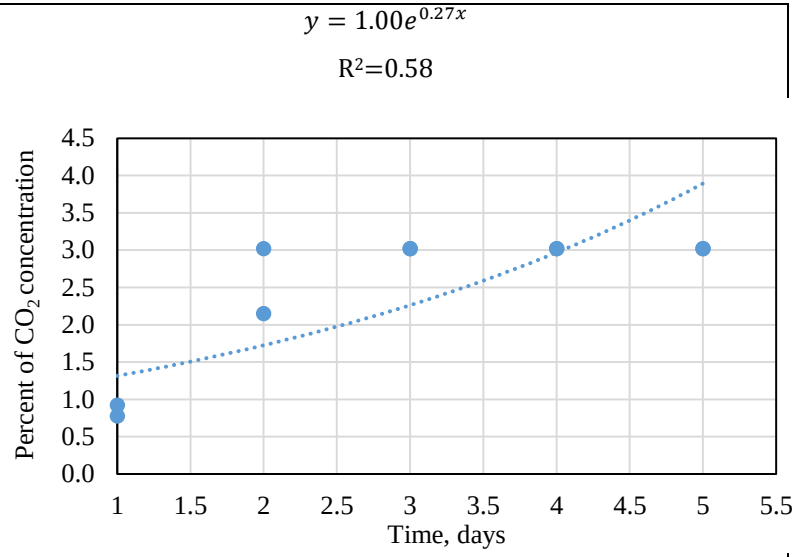

Figure 3.18. % CO_2 in the air in glass jars over time in syrup at 35ºC. Each point represents one reading

CHAPTER 4: ULTRAFILTATION OF WHOLE STILLAGE AND THIN STILLAGE

4.1 Abstract

The corn based dry grind process is the most widely used method in the U.S. for fuel ethanol production. Evaporation is the common method used to concentrate solids in these co-products, but it requires a large amount of water and energy consumption. Fractionation of these wet co-products by using ultrafiltration was conducted to evaluate membranes as an alternative to evaporators in ethanol production. The objectives of this study were to evaluate filtration characteristics of condensed whole stillage and thin stillage for ultrafiltration using a stirred ultrafiltration cell with molecular weight cut offs of 10 and 100kDa regenerated cellulose membranes.

The membrane size, stirring speed and volume capacity had a significant effect ($P < 0.05$) on flux during the ultrafiltration for whole stillage and thin stillage. The flux increased by 30% maximum as siring speed increased from 160 to 320 rpm for YM 10 membrane (10KDa) in these two samples. The effect of membrane size on solid recovery was significant ($P < 0.05$). The solid recovery for YM 100 membrane in whole stillage ranged from 75% to 83%, and 74% to 84% for thin stillage, however, the YM 10 kDa was ranging from 80% to 90% in whole stillage, and 84% to 90%.Retentate products from ultrafiltration could be further used as an ingredient to feed animals, and the permeate stream could be recycled in dry grind plants to help in reducing process water requirement.

4.2 Introduction

From 2001 to 2015, increased demand for ethanol as a fuel additive resulted in rapid growth of U.S. ethanol production. Annual ethanol production increased from 1.8 to 14.8 million gallons (RFA 2014 and RFA 2015). Ethanol is made primarily from corn in the U.S. and is blend with gasoline to reduce air pollution, including emissions of fine particulates and carbon monoxide. Also fuel ethanol has potential to diversify energy resources, reduce dependence on foreign oil, improve trade balances in oil market, and increase energy security (Sovacool and Brown, 2010).

Wet milling and dry grinding are the two major corn-based fuel ethanol production industries. Approximately 80% of fuel ethanol is produced by dry grinding process and the remaining 20% is produced from wet milling. Dry grinding process is preferred over wet milling because it requires lower capital investment but higher ethanol production (Belyea et al. 2004). In the dry grinding process, ethanol is separated from other remaining products which known as whole stillage (15% to 20% solids) in the distillation tank. Then the whole stillage is sent to centrifuge to produce two processing streams; wet cake (30 to 35% solids) and thin stillage (5 to 10% solids) (Zheng, 2013). Thin stillage is then sent to evaporator to remove excess liquid, and the solid that can be used as an additive to distillers grains.

Evaporation is the common method used to concentrate valuable components in ethanol co-products, but it requires a large amount of water and energy consumption. In addition, the evaporators get easily to accumulate deposits on their surfaces that leads to a reduction of heat transfer in a process known as fouling (Arora et al. 2010). Moreover,

evaporation requires large energy input and may result in loss nutritional value of the resulting co-products (Arora et al. 2010).

To overcome the problems associated with evaporator, alternative technologies could be applied that may provide a cheaper and efficient way to concentrate valuable components in co-products that come from dry grinding process. Membrane technology was found to be useful in dewatering processing streams (Arora et al. 2010). The common membrane technologies that are introduced to industrial plants to concentrate valuable chemical and biological components, including microfiltration, nanofiltration, reverse osmosis and ultrafiltration.

Ultrafiltration is not fundamentally different from microfiltration, nanofiltration or gas separation, except in terms of size of the molecules it retains. (Board, 2012). The pore size of ultrafiltration membrane is ranging from 1 to 100 nm (Singh et al. 2007). Typically, ultrafiltration membranes will remove high molecular-weight substances, colloidal materials, and organic and inorganic polymeric molecular-weight organics and ions are not removed (Wang et al. 2010). Ultrafiltration membranes are porous with a typical pore diameter in the range of 1 to 100 nm, corresponding to solute molecular weight in the 1,000 to 1,000,000Da (Peinemann and Nunes, 2011). However, it is more customary to categorize membranes by molecular-weight cut-off (MWCO). Such as a membrane with MWCO of 100 means the membrane can reject 90% of molecular weight with 100. The smaller the MWCO, the tighter the membrane pore size is. For instance, a membrane with molecular weights of 10 kDa would highly retain molecules of that molecular mass or greater, while highly permeating smaller molecules. Nevertheless, molecular-weight cut-off serves as a useful guide in selecting a membrane for a particular

application. Ultrafiltration process is particularly have become increasingly important during past decades due to their high flux at low applied pressure and energetically favorable separation. Ultrafiltration is a less energy required process (9 kJ/kg H_2O) than triple effect evaporation (1300 kJ/kg H_2O) (Rausch and Belyea 2006). This makes ultrafiltration the ideal filtration method in biotechnology industry for nutrient concentration and buffer exchange (Singh et al. 2007).

Despite it several advantages, membrane filtration has not acquired widespread use in corn refinery and corn based dry grind ethanol industries. A major problem associated with membranes is flux decline and membrane fouling. Fouling is a complex phenomenon, depends on the several parameters. An impotent factor was found by Bansal and Chen was the composition of input stream. Accumulation of solute particles over the membrane surface causes formation of gel layer of increasing osmotic pressure at the membrane solution interface with results in permeate flux loss (Berg and Smolders, 1990).

An understanding of causes of flux decline is necessary to minimize fouling and to make membrane application economical. Few studies have been published on concentration of co-products using ultrafiltration process. The objectives of this study were to evaluate operating parameters (stirring speed and volume capacity) on flux decline during batch ultrafiltration of whole stillage and thin stillage, and compare their ultrafiltration characteristics, including flux and solid recovery.

4.3 Materials and Methods

4.3.1 Experimental Materials

Fresh whole stillage and thin stillage were collected from Golden Grain Energy plant in Mason City, Iowa. Samples were stored in sealed plastic buckets with lids at 4^{o}C until needed. For each co-product, 45 mL sample was introduced into centrifuge tube and spun at 3000 rpm for 30 minutes at 25^{o}C room temperature. After centrifuging, upper liquid layer of each co-product was taken. The collected upper layer was analyzed for ultrafiltration process.

4.3.2 Equipment Setting up

A stirred ultrafiltration cell (400mL Amicon, model 8400, Millipore Corporation Bedford, MA) was used for filtering concentrated co-products at room temperature (25^{o}C). Ultrafiltration cell was run in a simulation of continuous operation. Figure 4.1 shows the model of ultrafiltration cell that was used in this study. An air gas was used to apply pressure to the stirred cell. A magnetic stir bas was used to simulate crossflow filtration. Two regenerated cellulose membranes, YM 10 and YM 100 (Millipore Corporation, Bedford, MA) with pore sizes of 10 and 100 kDa, respectively. The membranes are circular flat sheet with a diameter of 76 mm, and effective membrane area of 41.8 cm^2 was used in this study.

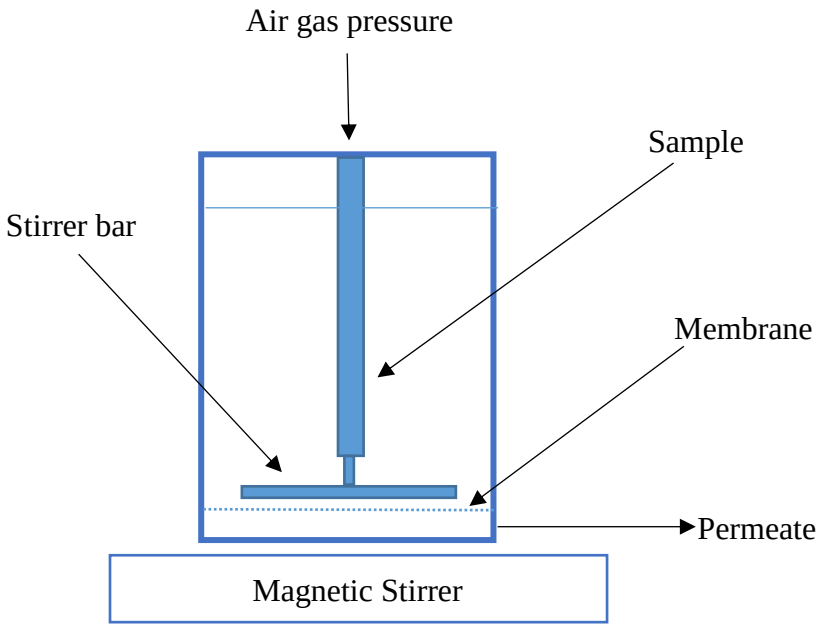

Figure 4.1 Ultrafiltration separation using batch concentration mode

4.3.3 Ultrafiltration Conditions

The air pressure at 20 psi was used to connect to the cell. Flux at higher pressures can be limited due to resistances at the boundary layer and other membrane surface phenomena (Zeman and Zydney 1996). Flux was measured at steady state condition, i.e., 5 minutes after adjusting the pressure. Experiments were performed at room temperature (25^{o}C). Two stirring speeds (160 rpm and 320 rpm) and two capacities (100 mL and 150 mL) were chosen to determine optimum conditions for filtration. The stirring action moves the feed water in a circular pattern around the circular membrane surface. Optimum combination of stirring speed and capacity were used for further filtration studies.

Figure 4.2 presents flow chart of this experiment, the liquid product from centrifugation process were filtered through regenerated cellulosic ultrafiltration membranes YM 10 and YM 100 with 10 and 100 kDa molecular weight cutoff (MWCO), respectively. The experiments were operated in batch concentration mode and experiments were continued until the material was exhausted. Permeate flux was determined volumetrically by measuring the cumulative volume permeated, collected from the bottom of the cell as a function of time using. Both permeate and retentate samples were collected for solid recovery analysis.

4.3.4 Measurement of Membrane Separation Performance

The average permeate flux (J_{av}) was calculated by

$$J_{av} = \frac{V}{At} \quad \text{---------- (Equation 4.1)}$$

Where J_{av} was the average flux (L/ (m^2 hr), LMH), V was the total volume (L) of permeate passed through each UF membrane, A was the effective area of the membrane, which was 0.00418 m^2, and t (hr) was the permeate collection time.

The solid recovery is the rejection of solids by each membrane type, was defined by

$$R\ (\%) = 100^* \left(1 - \frac{Cp}{CR}\right) \text{---------- (Equation 4.2)}$$

Where C_p and C_R were permeate and retentate total solids concentrations, respectively.

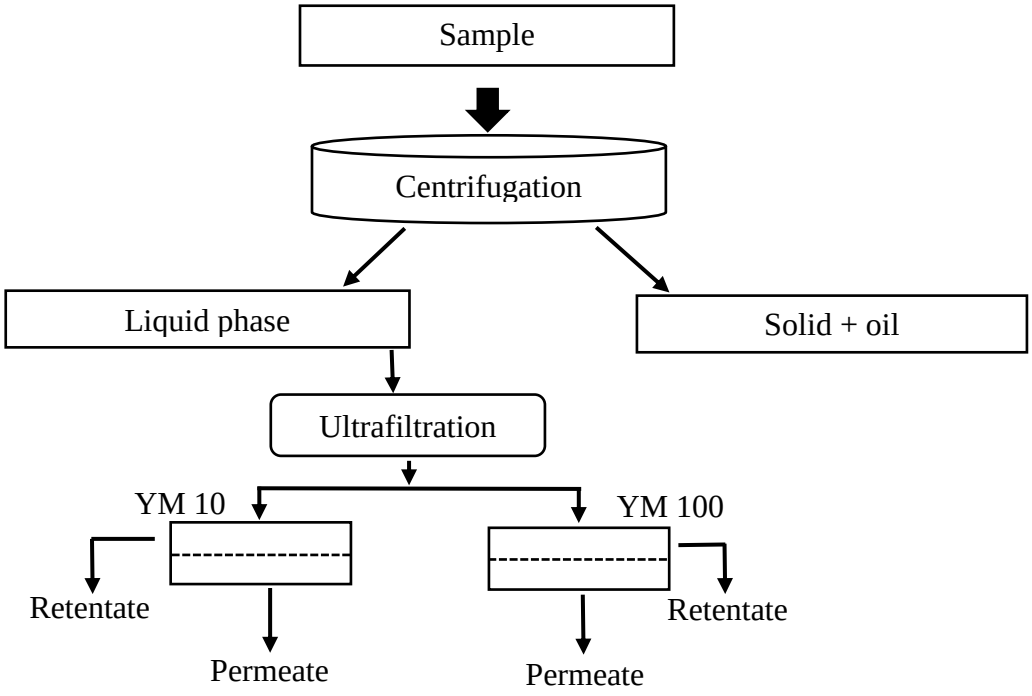

Figure 4.2 Experimental approaching

4.4 Results and Discussion

4.4.1 Thin Stillage

4.4.1.1 Effects of Stirring Speed and Volume Capacity

Effects of stirring speeds (160 rpm and 320 rpm) and volume capacities (100 mL and 150 mL) on permeate flux for two membranes (YM 10 and YM 100) are summarized in Figures 4.3 and 4.4. The stirring speed had a significant effect ($P < 0.0001$) on ultrafiltration flux (Table 4.1). As expected, flux was higher in faster stirring speed process. For YM 10 membrane (Figure 4.3), flux increased 30% as stirring speed increased from 160 to 320 rpm during the first 20-

minute experiment. Higher stirring speed (320 rpm) probably caused an increase in mass transfer rate resulting in higher permeate flux. However, for YM 100 membrane (Figure 4.4), flax increased a little as stirring speed increased from 160 to 320 rpm during 70-minute experiment. The effects due to capacity volume treatment on flux change were significant ($P < 0.05$) (Table 4.1). Figures 4.5 and 4.6 show the flux effects by volume capacity and membrane size at 160 rpm and 320 rpm stirring speed, respectively. At the end of 70-minute experiment, the fluxes with 320 rpm stirring speed for both 100 ml and 150 ml volume capacities were very close, whereas larger differences were noted with 160 rpm stirring speed (Figures 4.5 and 4.6). At lower stirring speed (160 rpm) and small capacity size (100 ml) experimental condition, flux of 100 kDa membrane molecular weights initially 1.5 times larger than the flux of 10 kDa and then decreased dramatically during the processing (Figure 4.5). As the particles started to deposit on the membrane, the rate of flux decreased over time. At small pore size membrane, the deposited layer become more compact and resistance against flow passed through.

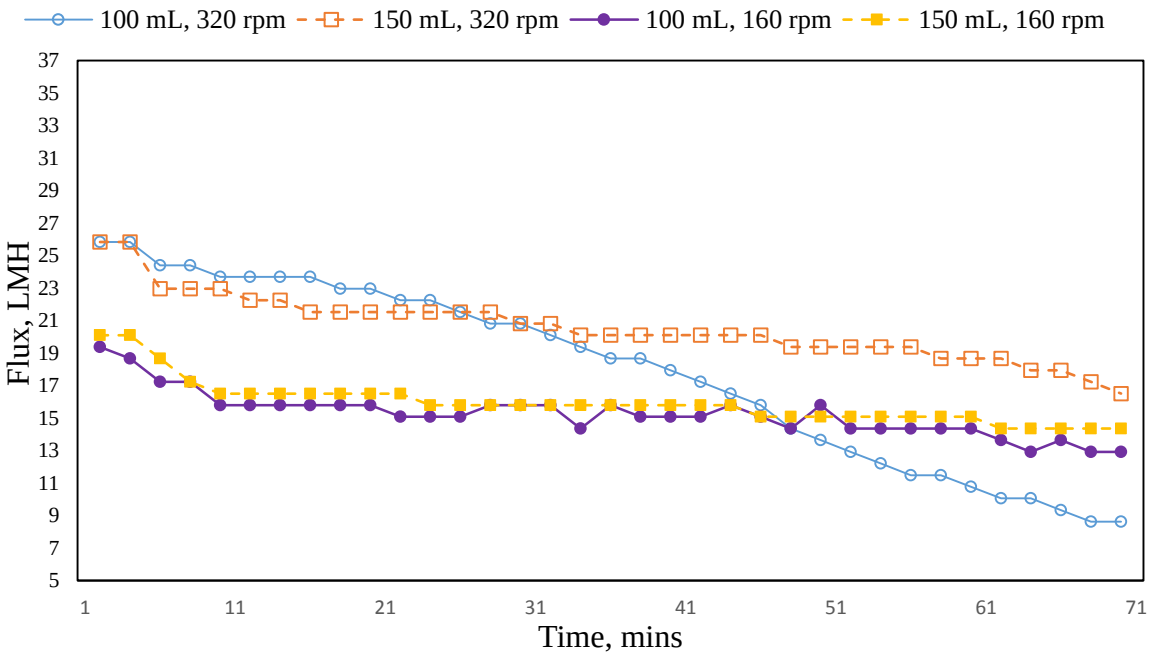

Figure 4.3 YM 10 membrane of flux affected by capacity and stirring speed for thin stillage

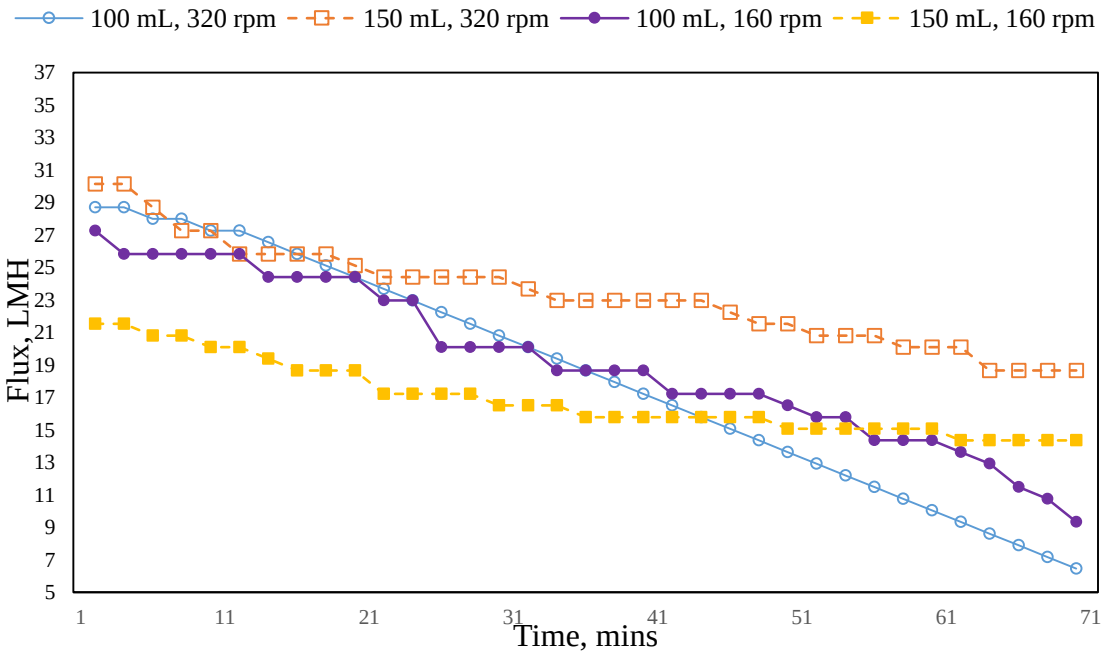

Figure 4.4 YM 100 membrane of flux affected by capacity and stirring speed for thin stillage

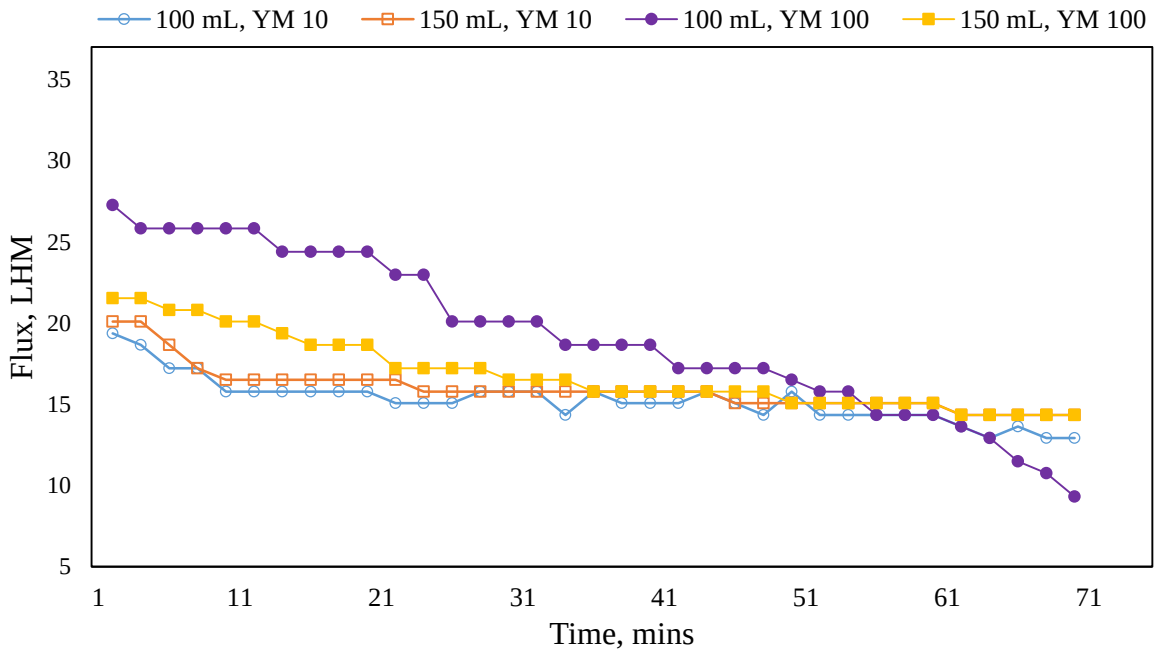

Figure 4.5 Thin Stillage flux as affects by capacity and membrane size at 160 rpm

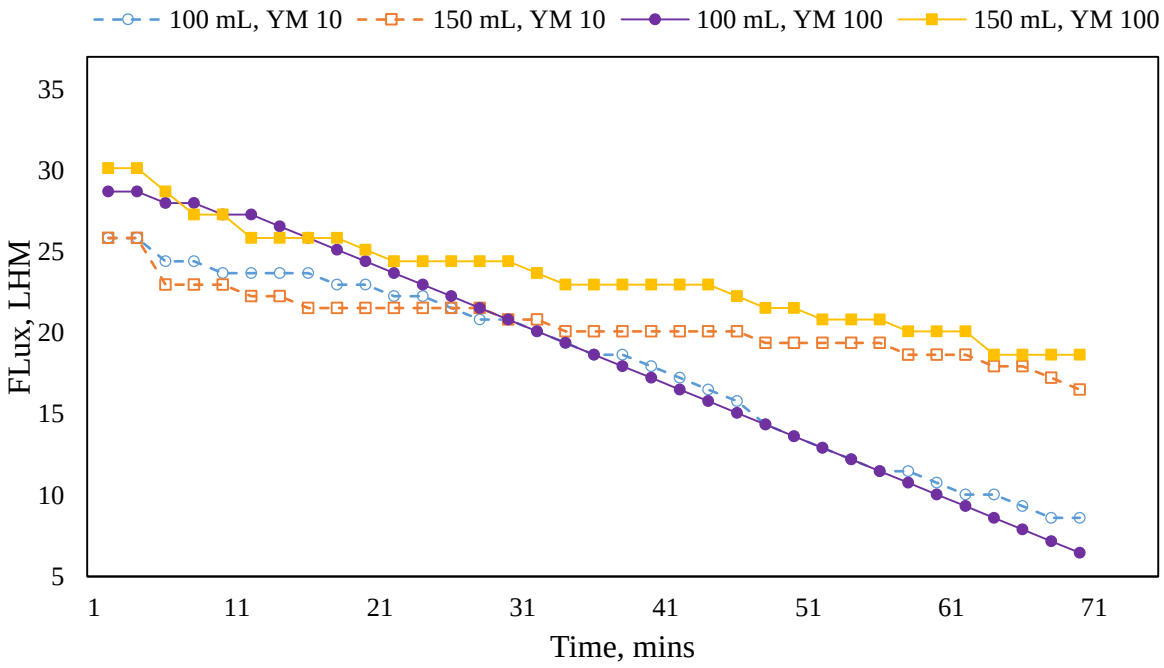

Figure 4.6 Thin Stillage flux as affects by capacity and membrane size at 320 rpm.

4.4.1.2 Effect of Membrane Selection

Selection of a suitable membrane is important to achieve higher permeate flux. The membrane size had a significant effect ($P < 0.005$) on ultrafiltration flux (Table 4.1). Permeate flux of YM 100 declined sharply, while flux of YM 10 decreased relatively constant (Figures 4.3 and 4.4). Since the YM 100 membrane had larger pore size, it was expected to have higher flux than YM 10 membrane at particle stirring speed and capacity size. YM 100 membrane required less stirring speed, thus reducing energy input but more solutes tended to pass through the membrane, reducing rejection of solids.

Table 4.1 Treatment factors effect on flux [a] in thin stillage

Factor	Flux[a]
Membrane size (kDa)	0.0002
Stirring speed (rpm)	< 0.0001
Volume capacity (mL)	0.008

[a] Probability (*P*) of the effects from treatment factor on flux

4.4.1.3 Flux Decline during Ultrafiltration

Flux decline has been attributed to concentration polarization and membrane fouling (Cheryan, 1998). Figure 4.7 summarized the flux changes of thin stillage under different experimental conditions. During 70-minute operation, sharp flux decline on YM 100 was observed (Figure 4.7). The decrease range of flux for YM 100 membrane was from 33% to 66%, and the range for YM 10 membrane was 29% to 67% (Table 4.2). This was attributed to the fouling membrane. As the concentration of solutes in feed increased, the solution viscosity and density increased and diffusivity of a given solute decreased. Since the YM 10 and YM 100 were membranes that can reject nominally 10 and 100 kDa solutes, YM 10 membrane with small pore size that can be blocked easily due to the present of large molecular weight components, thus explaining the generally low flux profiles for YM 10.

Table 4.2 Treatment factors effect on flux change in whole stillage

Treatments			Initial flux	final	Change
Membrane size	Stirring speed (rpm)	Capacity (mL)			
YM 100	160	100	27.27	9.33	66%
		150	21.53	14.35	33%
	320	100	28.71	6.46	78%
		150	30.14	18.66	38%
YM 10	160	100	19.38	12.92	33%
		150	20.10	14.35	29%
	320	100	25.84	8.61	67%
		150	25.84	16.51	36%

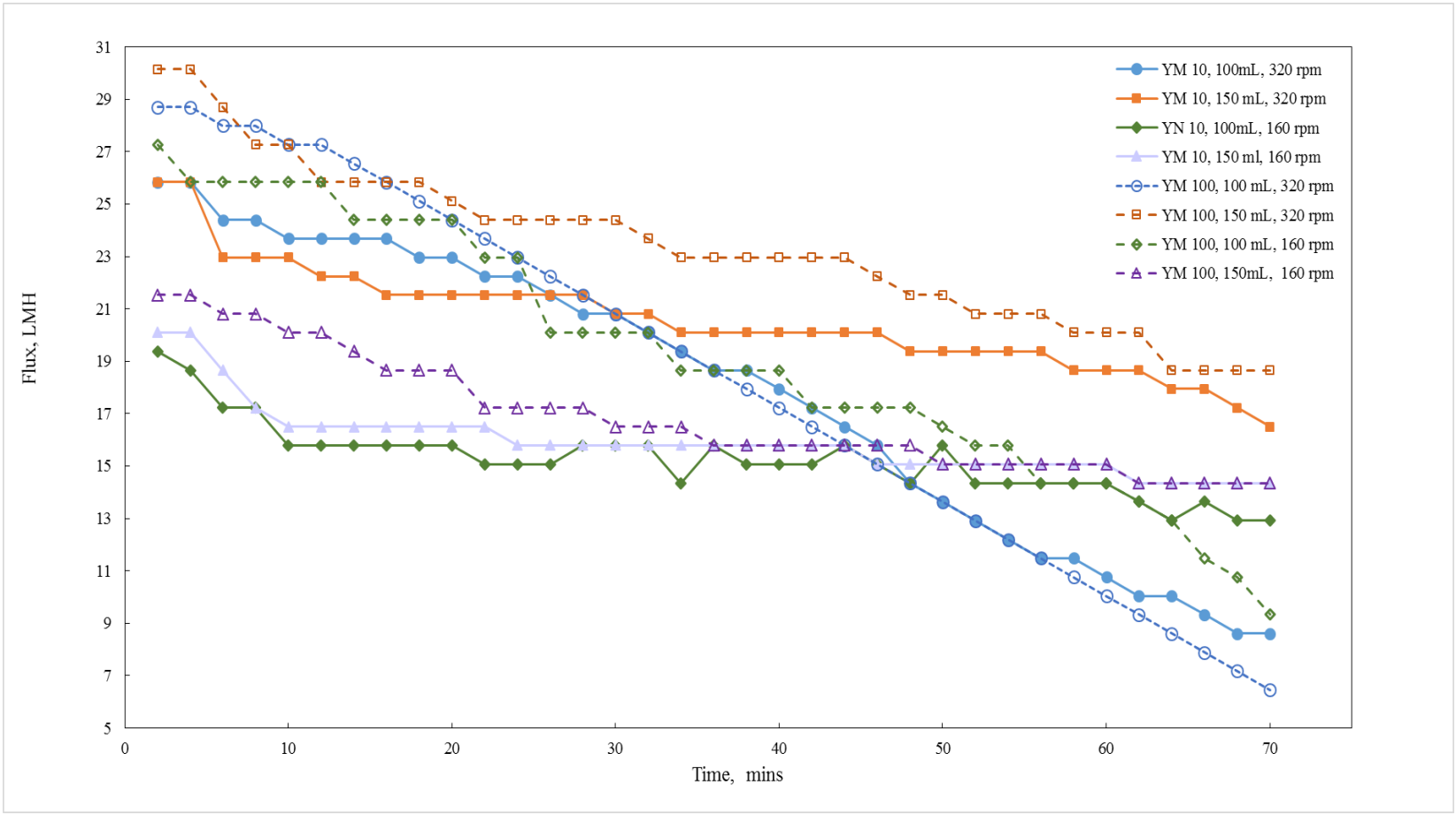

Figure 4.7 The permeate flux profile of thin stillage at 25°C for two membranes; stirring speed; volume capacity for YM 10 and YM 100 membranes were 160 rpm and 320 rpm, 100 mL and 150 mL, respectively.

4.4.1.4 Retentate Recovery

The treatment used in the study included two membrane pore sizes (YM 10 and YM 100), two stirring speeds (160 rpm and 320 rpm), and two volume capacities (100 mL and 150 mL). Membrane size had a significant effect ($P < 0.05$) on solid recovery (Table 4.4). Table 4.3 illustrates the solid recovery following the ultrafiltration for each treatment evaluated. The recovery percent for YM 100 membrane was ranging from 79% to 84%, however, the greatest solid recovery for YM 10 was 90%. The relatively high yield with the YM 10 membrane showed that approximately 84% to 90% of solid were rejected by the YM10 membrane. The solid recovery of thin stillage on YM 10 and YM 100 membranes were overall consistent with similar material analyzed by other literatures (Arora et al, 2010).

Table 4.3 Solid recovery from various experimental treatments in thin stillage

Treatments			Recovery %
Membrane Size	Stirring Speed (rpm)	Capacity (mL)	
YM 100	160	100	84.22
		150	73.53
	320	100	79.17
		150	81.83
YM 10	160	100	86.45
		150	83.72
	320	100	89.9
		150	88.54

Table 4.4 Treatment factors effect on solid recovery [a] in thin stillage

Factor	Solid Recovery [a]
Membrane size (kDa)	0.031
Stirring speed (rpm)	0.485
Volume capacity (mL)	0.462

[a] Probability (*P*) of the effect from treatment factor on solid recovery

4.4.2 Whole Stillage

4.4.2.1 Effects of Stirring Speed and Capacity

The stirring speed had a significant effect ($P < 0.0001$) on ultrafiltration flux (Table 4.5). As expected, flux was higher in faster stirring speed process. Effects of stirring speeds and capacities on permeate flux are summarized in Figures 4.8 and 4.9. The flux increased by 30% and 70% maximum as stirring speed increased from 160 to 320 rpm for YM 10 and YM 100 membranes, respectively. Higher stirring speed probably caused an increase in mass transfer rate resulting in higher permeate flux. However, the flux was highest with 320 rpm stirring speed on YM 100 membrane at the beginning of the experiment, which reached to 34.45 LMH. Permeate flux for large volume capacity (150 mL) attained relatively steady state during 70-minute experiment. Membrane type had a very small effect on flux at 160 rpm stirring speed for both 100 ml and 150 ml capacities, whereas the differences were noted at 320 rpm stirring speed. At high stirring speed (320 rpm) and large capacity size (150 mL), flux of YM 100 membrane initially 1.2 times larger than the flux of YM 10 and then decreased dramatically during the processing (Figure 4.8). In general, both fluxes of YM

100 and YM 10 membrane decreased during the 70-minute experiment. As the particles started to deposit on the membrane, and permeate is getting harder to get through the membrane, so the flux decreased over time. The deposited layer become more compact and resistance against flow passed through on both of two membranes.

4.4.2.2 Effect of Membrane Selection

Selection of a suitable membrane is important to achieve higher permeate flux. The fashions of permeate flux of YM 100 and flux of YM 10 performed similarity with the same stirring speed and volume capacity. At 320 rpm stirring speed, the permeate flux on YM 100 larger than the flux on YM 10 during the first 30-minute of filtration. However, at 160 rpm stirring speed, the permeate flux were similar during the 70 min experiment. Since the YM 100 membrane had larger pore size, it was expected to have higher flux YM 10 membrane at particle stirring speed and capacity size. YM 100 membrane required less stirring speed, thus reducing energy input but more solutes tended to pass through the membrane, reducing rejection of solids.

Table 4.5 Treatment factors effect on flux [a] in whole stillage

Factor	Flux
Membrane size (kDa)	0.04
Stirring speed (rpm)	< 0.0001
Volume capacity (mL)	0.05

[a] Probability (P) of the effects from treatment factor on flux

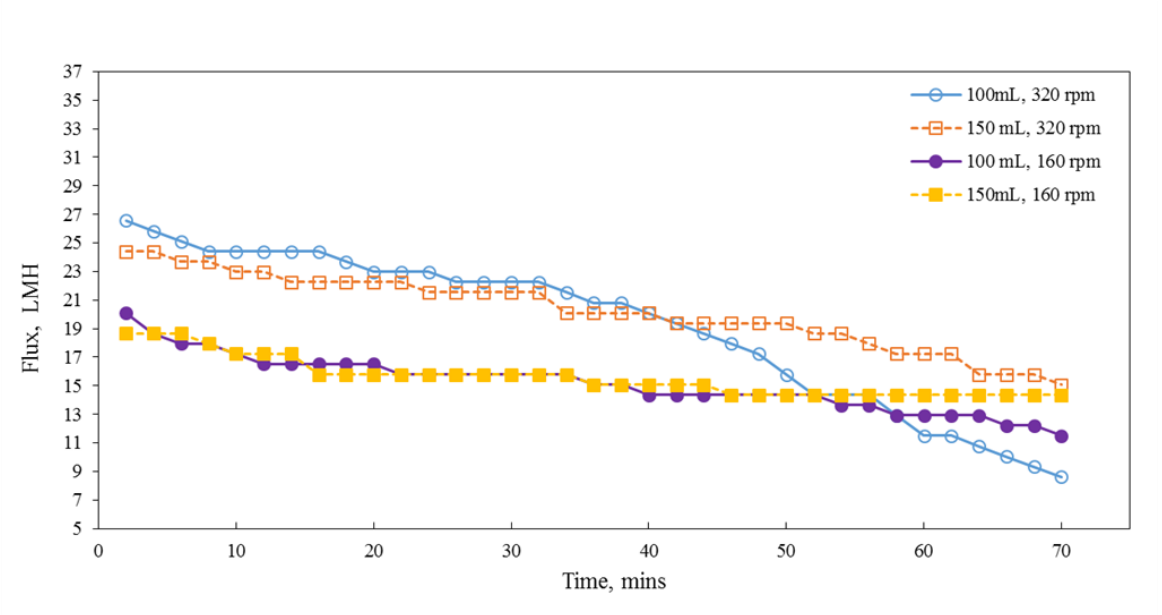

Figure 4.8 YM 10 membrane of flux affected by capacity and stirring speed for whole stillage

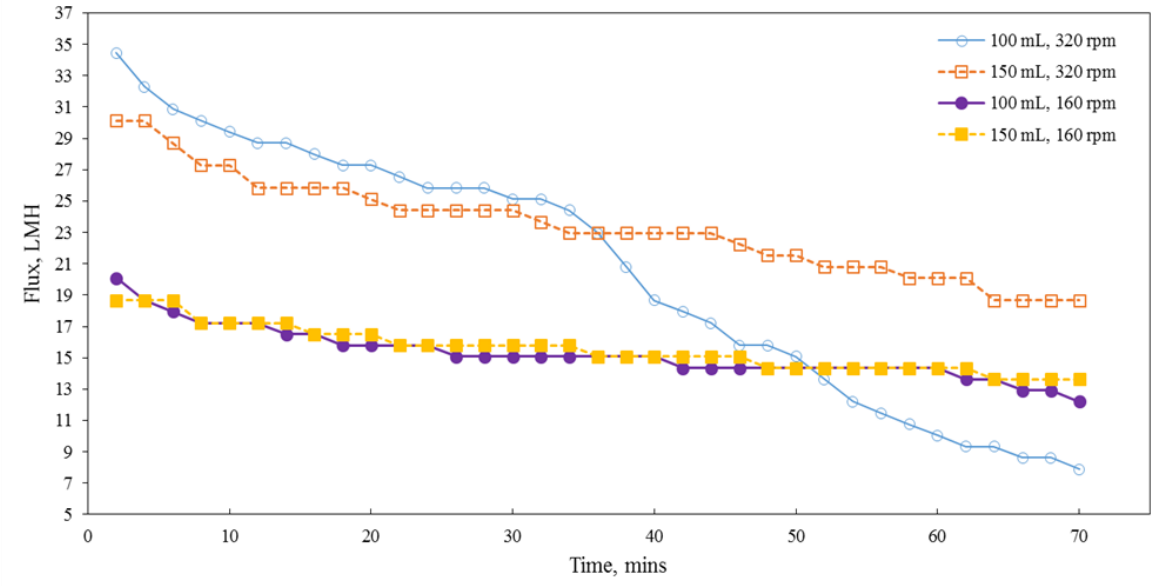

Figure 4.9 YM 100 membrane of flux affected by capacity and stirring speed for whole stillage

4.4.2.2 Effect of Membrane Selection

Selection of a suitable membrane is important to achieve higher permeate flux. The fashions of permeate flux of YM 100 and flux of YM 10 performed similarity with the same stirring speed and volume capacity. At 320 rpm stirring speed, the permeate flux on YM 100 larger than the flux on YM 10 during the first 30-minute of filtration. However, at 160 rpm stirring speed, the permeate flux were similar during the 70 min experiment. Since the YM 100 membrane had larger pore size, it was expected to have higher flux YM 10 membrane at particle stirring speed and capacity size. YM 100 membrane required less stirring speed, thus reducing energy input but more solutes tended to pass through the membrane, reducing rejection of solids.

Table 4.5 Treatment factors effect on flux [a] in whole stillage

Factor	Flux
Membrane size (kDa)	0.04
Stirring speed (rpm)	< 0.0001
Volume capacity (mL)	0.05

[a] Probability (P) of the effects from treatment factor on flux

4.4.2.3 Flux Decline during Ultrafiltration

The membrane size had a significant effect ($P < 0.005$) on ultrafiltration flux (Table 4.7).In the whole stillage fraction obtained from the dry grind process, similar permeate flux profiles were observed for YM 10 and YM 100 membranes (Figure 4.10). The decrease range of flux for YM 100 membrane was from 29% to 77%, and the range for YM 10 membrane was 23% to 68% (Table 4.7). Rapid declines in permeate flux were observed during the first 10-minute of experiment. Flux decline has been attributed to gradual deposition of a gel layer is formed on a membrane surface. During 70-minute of operation, sharp flux decline on 320 rpm stirring speed was observed (Figure 4.10). Large stirring speed caused rapidly changes act to decrease the absolute value of flux. The permeate flux profiles for whole stillage were different for thin stillage filtration (Figure 4.6 and 4.10). Reasons for such differences in flux changes could be due to complex composition of thin stillage and whole stillage.

Table 4.6 Flux change on different experimental treatments in whole stillage

Treatments			Initial flux	Final flux	Change
Membrane size	Stirring speed (rpm)	Capacity (mL)			
YM 100	160	100	20.10	12.20	39%
		150	18.66	13.64	27%
	320	100	34.45	7.89	77%
		150	30.14	18.66	38%
YM 10	160	100	20.10	11.48	43%
		150	18.66	14.35	23%
	320	100	26.56	8.61	68%
		150	24.40	15.07	38%

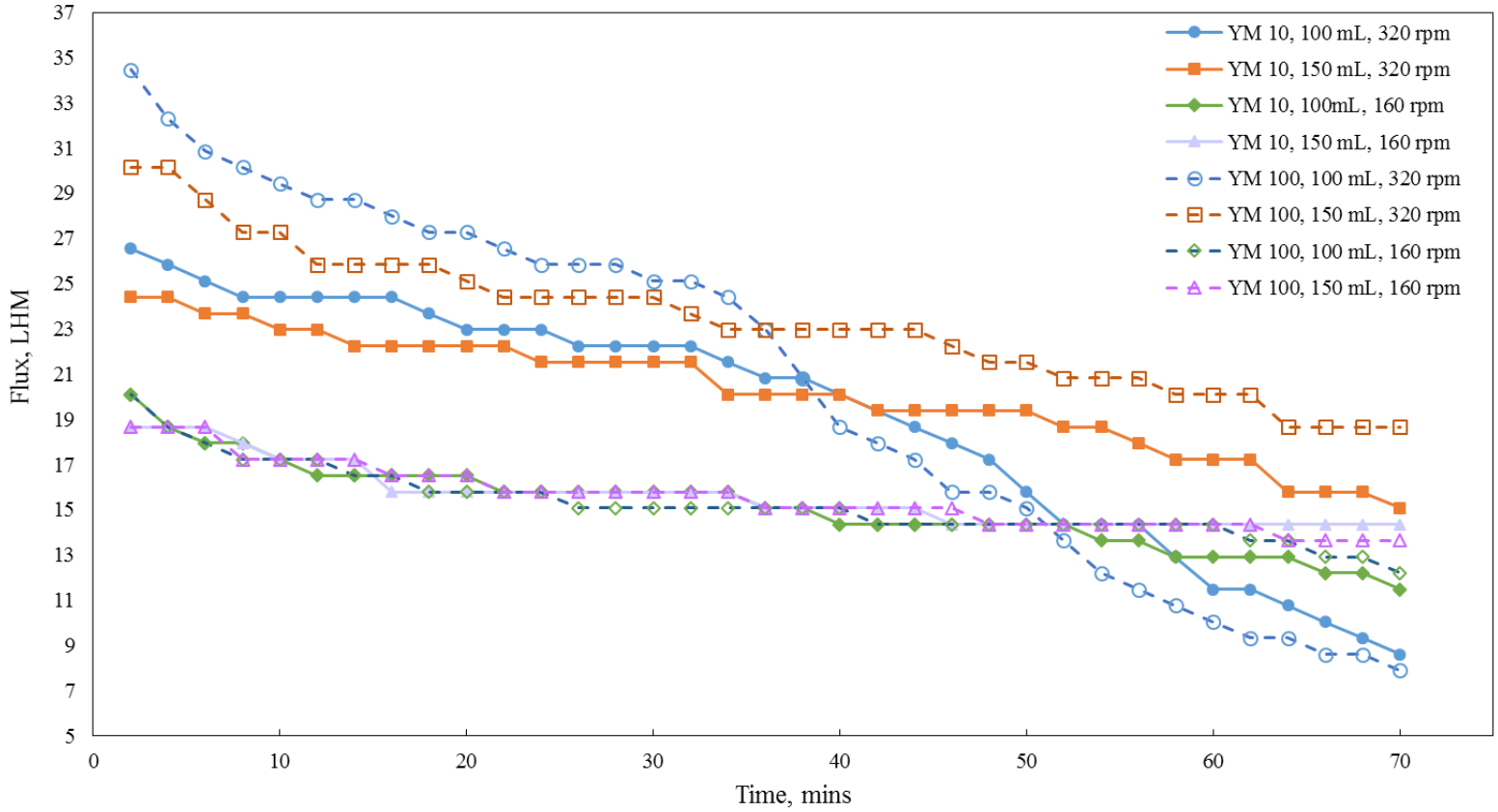

Figure 4.10 Whole stillage permeate flux profile of thin stillage at 25°C for two membranes; stirring speed; volume capacity for YM 10 and YM 100 membranes were 160 rpm and 320 rpm, 100 mL and 150 mL, respectively.

Table 4.7 Treatment factors effect on flux [a] in whole stillage

Factor	Flux
Membrane size (kDa)	0.04
Stirring speed (rpm)	< 0.0001
Volume capacity (mL)	0.05

[a] Probability (P) of the effects from treatment factor on flux

4.4.2.4 Retentate Recovery

The treatment used in the study included two pore size membranes (YM 10 with pore size of 10 kDa and YM 100 with pore size 100 kDa,, two stirring speeds (160 rpm and 320 rpm), and two volume capacities (100 mL and 150 mL). Membrane size had a significant effect ($P < 0.05$) on solid recovery (Table 4.9). Table 4.8 demonstrates the solid recovery following the ultrafiltration for each treatment evaluated. The recovery percent for YM 100 membrane was ranging from 75.2% to 82.5%, however, the solid recovery for YM 10 kDa was ranging from 79.6% to 89.3%. The relatively high yield with the YM 10 membrane showed that small pore size membrane was better in rejecting solid during the ultrafiltration process.

Table 4.8 Solid recovery from various experimental treatments in whole stillage

Treatments			Recovery (%)
Membrane Size	Stirring speed (rpm)	Capacity (mL)	
YM 100	160	100	82.36
		150	75.19
	320	100	82.5
		150	74.94
YM 10	160	100	89.31
		150	79.63
	320	100	84.15
		150	88.58

Table 4.9 Treatment factors effect on solid recovery [a] in whole stillage

Factor	Solid Recovery [a]
Membrane size (kDa)	0.031
Stirring speed (rpm)	0.485
Volume capacity (mL)	0.462

[a] Probability (*P*) of the effect from treatment factor on solid recovery

4.5 Conclusion

The membrane size, stirring speed and volume capacity had a significant effect ($P < 0.05$) on flux during the ultrafiltration. The flux increased by 30% maximum as siring speed increased from 160 to 320 rpm for YM 10 membrane (10KDa) in both thin stillage and whole stillage samples. Permeate flux for large volume capacity (150 mL) decreased relatively in steady fashion in both whole stillage and thin stillage samples during 70-min filtration process. The effect of membrane size on solid recovery was significant ($P < 0.05$). The solid recovery for YM 100 membrane in whole stillage ranged from 75% to 83%, and 74% to 84% for thin stillage, however, the YM 10 kDa was ranging from 80% to 90% in whole stillage, and 84% to 90%. Retentate products from ultrafiltration could be further used as an ingredient to feed animals, and the permeate stream could be recycled in dry grind plants to help in reducing process water requirement.

4.6 References

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CHAPTER 5: GENERAL CONCLUSION

In this study, the storage temperature, moisture content, and water activity are significantly impacted shelf life of distillers grains. The effects of moisture content and water activity on thin stillage and whole stillage were significant. The thin stillage and whole stillage samples studied had high moisture contents of 92% (w.b.) and 87% (w.b.) respectively, and water activity o 0.99; the high water content caused samples at risk to rapid spoilage. In the case of syrup sample, it had both relative low moisture content of 62% and water activity of 0.92. Relative low moisture content and water activity marked no visible mold was observed during the experiment. Storage temperature effect on shelf live was also significant. Both thin stillage and whole stillage samples were observed with mold growth at 32^{o}C after five days storage. However, at 25^{o}C treatment, mold growth was observed in whole stillage after five days storage and in thin stillage after six days.

As expected, water content and temperature are two most important parameters that influence deterioration during storage. Samples with high water content could be easily susceptible to rapid spoilage during storage, especially in warm and hot weather seasons.

From this study, the flux increased by 30% maximum as stirring speed increased from 160 to 320 rpm for YM 10 membrane (10KDa) in both thin stillage and whole stillage samples. Higher stirring speed probably caused an increased in mass transfer rate resulting in high permeate flux. The flux of permeate stream with YM 100 membrane was larger than the YM 10 at the beginning of the experiment. Since the YM 100 membrane had a larger pore size and more solutes tended to pass through the membrane.

As the particle started to deposit on the surface, the overall flux decreased over the experiment. The overall solid recovery of YM 100 for both whole stillage and thin stillage was around 80%, while the recovery of YM 10 could reach to 90%. The relatively high yield with YM 10 showed that small pore size membrane was better in rejecting solid in ultrafiltration process.

Distillers grains is becoming a promising livestock feed due to high nutrient contents and reliability of supply. Therefore, it is therefore important that future studies should consider the mechanism of deterioration, nutrient contents, and microbial ecology and how they influence the deterioration. Also the continued investigation of concentrate and analyze the valuable content of distillers grains which may prove to be highly beneficial in ethanol plants.

CHAPTER 6: RECOMMENDATIONS FOR FUTURE RESEARCH

Future studies should be considered on understanding factors that promote rapid deterioration in distillers grains and possible ways of concentrating and storing valuable contents. The following investigations are essential to distillers grains storage and utilization.

1. Explanation of microbial culture during storage and toxicity level is needed to evaluate harmfulness of feeding limits of deteriorated of whole stillage and thin stillage.
2. Different high-temperature levels should be studied on syrup.
3. Various preservation additives and treatment levels should be studied on whole stillage and thin stillage.
4. A higher capacity of CO_2 test kit should be provided to measure a wider range of CO_2 evolution during future studies.
5. Different high-pressure levels should be provided to ultrafiltration process and determine the optimum transmembrane pressure based on the membrane performance.
6. The permeate streams could be processed further using nanofiltration or reverse osmosis processes,
7. The retentate and permeate streams produced could be tested for solid compositions such as protein, ash or fat, and quantified carbohydrate.