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Storability of modified wet distillers grains with solubles

by

Guevara Che Nyendu

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Co-majors: Agricultural Engineering and Biorenewable Resources and Technology

Program of Study Committee:
Thomas J. Brumm, Major Professor
Carl J. Bern
D. Raj Raman
Daniel D. Loy

Iowa State University

Ames, Iowa

2011

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"In the light of the knowledge obtained, the happy achievement seems almost a matter of course, and any intelligent student can grasp it without too much trouble. But the years of anxious searching in the dark for a truth that one feels but cannot express, the intense desire and the alternations of confidence and exhaustion, and the final emergence into light – only those who have experienced it can appreciate it."

Albert Einstein

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CHAPTER 1. GENERAL INTRODUCTION

Thesis Organization

The study contained in this thesis is structured into four chapters. The first chapter presents a thesis organization and comprehensive introduction. The second chapter contains a review of literature. The third chapter contains a paper entitled "Storability of modified wet distillers grains with solubles" that is organized to meet the Journal of Stored Products Research publication requirements. The fourth chapter contains general conclusions.

Introduction

The negative environmental impacts of fossil-based fuels have drawn significant attention to renewable fuels, especially ethanol (Chisti, 2008). The demand for ethanol fuel as an alternative to and additive for fossil fuels has seen dramatic increase in the last decade. The primary feedstock for ethanol in the United States is corn (Biswas and Staff, 2001). Other potential feedstock such as sorghum, barley, wheat, rye and, cereals (Staff, 2010) are also used for ethanol production. The most common resultant co-product from processing these crops is distillers grains.

Ethanol plants consider distillers grains as a significant source of revenue through export and sale as a local feed. Profits from distillers grains contribute about 10 to 40% of an ethanol plant's entire revenue stream, depending on distillers grains sale price, corn feedstock price, natural gas price and other market conditions (Rosentrater and Kongar, 2009). In addition, distillers grains potential as lignocellulosic raw material (Noureddini and Byun,

2010) for biofuels might create a blossoming demand therefore maximizing the competitiveness and marketability of the co-product in the domestic and international market.

Generally, various starch-based crops are grown for ethanol but the most dominant source of starch is corn. Corn accounts for more than 90% of total ethanol production and most widely produced feed grain in the United States (USDA, 2010). US corn production is estimated to reach a record of 14.9 billion bushels in 2010 to 2011(Glauber, 2010). It is anticipated that by 2015, the portion of corn used for ethanol production will rise from its current value of 12% to 23% ([Runge and Senaure, 2007] and [Luchansky and Monks, 2009]). Commercialization of corn ethanol is possible because of vast knowledge of processing methods, infrastructure, economics and efficiency relative to other types of biomass. In 2009, the U.S. ethanol industry exported 8 million Mg (1 Mg = 1 metric ton = 1 tonne = 1000 kg) of distillers grains worth $1.6 billion (RFA, 2011b). China, Japan, Canada, Turkey and Mexico are among the top importers of distillers grains (Fox, 2009). China is currently the world's largest producer of meat for their internal markets and huge meat import from Canada (AAFC, 2010). These countries therefore have high demand for livestock feed such as distillers grains.

Primarily, there are two techniques for producing fuel ethanol using corn grain: wet mill process and, dry-grind process (Rosentrater and Kongar, 2009). The major differences between the processing methods are the techniques used for extracting starch, their respective products and, co-products. The wet mill process is more versatile than the dry milling process (Dale and Tyner, 2006); but to achieve this versatility, wet mills are more capital and resource intensive (Rosentrater and Kongar, 2009). Conventional wet milling is a complex

process designed for the recovery and purification of starch and several co-products: germ, gluten, fiber and steep liquor (Ramirez et al., 2008).

On the other hand, the dry-grind ethanol process is relatively simple (Klopfenstein et al., 2007), resulting in comparatively low investment and operational cost, yet achieving high ethanol yields (Rausch and Belyea, 2006). Therefore, dry-grind processing rapidly gained prevalence in the ethanol industry and accounted for 82% of the entire industry (RFA, 2007). The dry-grind process produces ethanol, distillers grains and carbon dioxide. The dry-grind production consist of a relatively simple sequence of operations, including grinding, cooking, liquefying, saccharifying, fermenting and distilling (Rosentrater and Kongar, 2009).

Distillers grains are co-products from ethanol production, with corn being the main feedstock in the Midwestern region of the United States (Birkelo et al., 2004). These feeds are rich in nutrient content (Rosentrater et al., 2005) because remaining nutrient after starch extraction is concentrated threefold in into the co-product (Klopfenstein et al., 2007). Distillers grains as feed are an economical source of both protein and energy (Klopfenstein, 1996). The abundance, nutritional profile, and favorable price of distillers grains has given cattle feeders the option to consider distillers grains as a feed source (Kinman et al., 2011). Livestock such as cattle, pigs, horses, sheep, goat, poultry and even pets have distillers grains inclusion in their feed. In the Midwest of the United States, distillers grains are a common feed input for beef and dairy cattle that is considered to effectively improve cattle performance and operation profitability (Klopfenstein et al., 2007).

Farm operations that include distillers grains in their feed attested that livestock performance on distillers grains outweighs other feeds such as corn. Cows fed distillers grains are found to have greater feed efficiency, higher milk yield and maintained milk

component concentration (Anderson et al., 2006). Inclusion of distillers grains in diets fed to pigs may also improve immune system activation (Stein and Shurson, 2009). Moreover, the wet form of distillers grains are rich in protein and energy relative to corn thus an excellent feed source for feedlot cattle (Schoonmaker et al., 2010). Extensive research dating back to the 1980s has demonstrated the effectiveness of wet distillers grains as a protein and energy supplement in feedlot diets ([Farlin, 1981], [Firkins et al., 1985] and [Schoonmaker et al., 2010]).

Despite the benefits derived from these co-products, wet distillers grains limitations typically have the tendency to deteriorate (dry matter loss) within a short time during storage. Nevertheless, research has suggested that high moisture distillers grains have higher energy value per unit dry matter than dried distillers grains (Loy and Strohbehn, 2007c). Traditionally, wet distillers grains are dried. This drying process, however, increases the energy costs incurred by the ethanol plant and may produce changes that reduce its nutritional value (Kinman et al., 2011). Several studies have been conducted to find suitable storage method for wet distillers grains so that the negative impacts of drying can be avoided. Chemical preservatives methods of retarding deterioration in wet distillers grains during storage is increasing in recent years. Among these chemical preservatives, CakeGuard™ (Alltech® Inc., USA) is the most common. This commercial product is suggested to reduce storage losses of wet feeds (Sommerfeldt, 2011).

In literature, “shelf-life” is widely used to describe storage time or storage life of wet distillers grains but unfortunately, this term is a misnomer. Storage life is a more appropriate term to describe the period of distillers grains storage. Storage life of wet distillers grains describes the allowable storage time when freshly discharged distillers grains are stored until

'significant' dry matter is lost and regarded unsafe or unusable for feeding and blending. 'Significant' because currently, no acceptable level or standard exists to describe deteriorated wet distillers grains safe feeding. Loss of dry matter (deterioration) is important because the wet feed prices are based on dry matter. Shelf-life which is commonly used in literature refers to feed quality. Wet distillers grains that has reached its "shelf-life" might still be safe, but quality can no longer be assured. Also, shelf-life is used to describe the quality of food for human consumption. On the other hand, both terms (storage life and shelf-life) are used interchangeably, however clear distinction is necessary to avoid misinterpretation.

Wet distillers grains storage loss could affect negatively the overall revenue generated by an ethanol plant and end user. Loss of this wet feed means loss of money and valuable resource. The likelihood of feeding or blending spoiled and contaminated distillers grains (e.g., mycotoxins) is also high which could inevitably end up in livestock product meant for human consumption. To enable storage loss prediction and retardation, it has become imperative to study wet distillers grain deterioration. The study of deterioration will serve as an acceptable measure to guide ethanol producers and end users as to when feed quality will be reduced or become unusable.

Objectives

The objective of this study was to determine the effect of CakeGuard preservative and temperature on modified wet distillers grains with solubles dry matter loss (as estimated by carbon dioxide evolution) during storage. Consequently, the hypotheses tested in this study were: 1) there is no CakeGuard treatment effect on modified wet distillers grains with

solubles dry matter loss during storage and, 2) there is no temperature effect on modified wet distillers grains with solubles dry matter loss during storage.

CHAPTER 2. REVIEW OF LITERATURE

The livestock feed industry has seen dramatic change due to increased supplies of feed (co)products such as distillers grains as a result of recent growth in the corn-ethanol industry in the United States (Hubbard et al., 2009). The supply of distillers grains from ethanol production creates tremendous opportunities as a potentially cheap and viable feed for livestock producers, particularly those in the beef and dairy industries (Buckmaster et al., 2008). Thanks to the high demand for protein both domestically and internationally. Distillers grains as a feed input for livestock may continue to gain significant importance if global patronage continue to rise.

Corn processing and refining: wet milling and dry-grind method

Ethanol production from grains yields various byproducts and co-products that are recovered and fed to cattle ([Schingoethe, 2007] and [Sasikala-Appukuttan et al., 2008]). At present, corn is the primary grain for ethanol production in the United States because of the high starch content, approximately two-third of a kernel (Loy and Miller, 2008). Processing a unit mass of corn into ethanol yields the following products (on an approximate equal mass basis): ethanol, distillers grains, and carbon dioxide (Saunders and Rosentrater, 2009). Generally, there are two techniques for producing fuel ethanol using corn grain: wet mill process and dry-grind process (Rosentrater and Kongar, 2009). These are detailed below:

Corn wet milling process

Wet mills are typically larger (Loy and Miller, 2008). The corn wet milling process (Figure 2.1) is very complex and produces a variety of products and co-products. The

individual kernels of corn are fractionated into components; starch, germ cake, fiber, gluten meal, crude oil and solubles (Gulati et al., 1996). Usually wet milling process requires high-quality (No.2 or better) corn, processed into variety of products intended for human use (Klopfenstein et al., 2007).

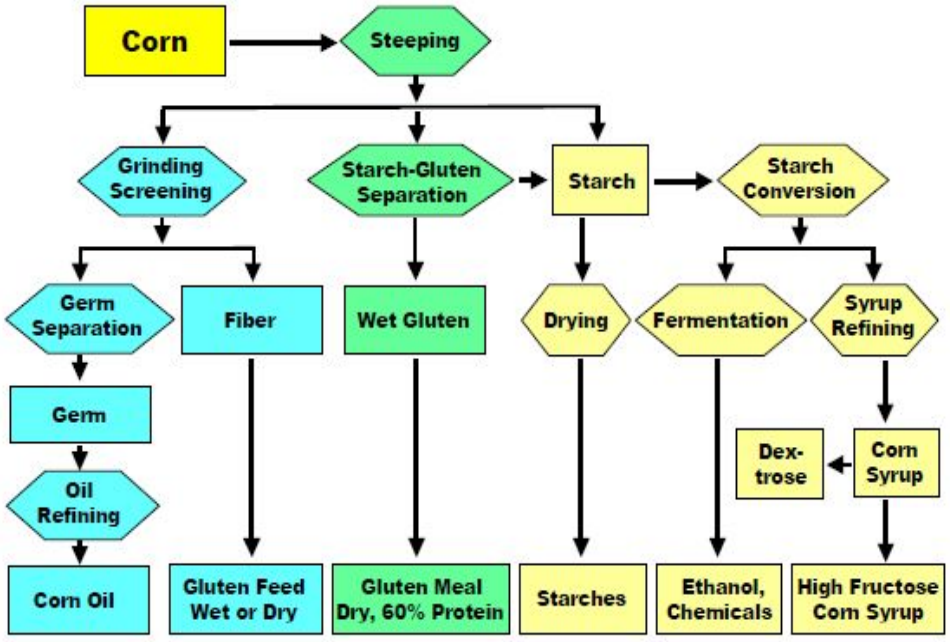

Figure 2.1. Wet milling ethanol process (Butzen and Hobbs, 2002).

In wet milling, corn is first cleaned to remove foreign material (sand, weeds, pieces of cob, and other cereal grains) and broken corn kernels ([Blanchard, 1992], [Johnson and May, 2003], [Watson and Eckhoff, 2004] and [Singh and Johnston, 2004]). The cleaned corn is then steeped with the objectives of softening the corn kernel, reducing or inhibiting the activity of undesirable microorganisms, and to assist in pure starch recovery ([Bartling, 1940]

and [Jackson and Shandera Jr, 1995]). Steeping is important because it plays an overall role in the efficiency of the wet milling process (Pérez et al., 2001).

Following the steeping process, kernels are then fractionated into kernel components of corn bran, starch, corn gluten meal (protein), germ, and soluble components corn (Klopfenstein et al., 2007). Wet milling produces corn gluten feed, corn gluten meal, and starch slurry (Ramirez et al., 2008) as the major feed products.

Corn dry-grind process

The corn dry-grind process is commonly referred to as the dry milling process (Kim et al., 2008a). This method is the most widely used in the U.S. for ethanol production (Kwiatkowski et al., 2006) with an annual production capacity of 50 billion Liters (13.23 billion gallons) (RFA, 2011a). The advantages of this processing technique include; low capital and energy investment costs (Rodríguez et al., 2010), relatively simple process (Rausch and Belyea, 2006) and high value co-products. Dry-grind process is designed to subject the entire corn kernel to fermentation (Rausch and Belyea, 2006) with the objective of maximizing the capital return per gallon of ethanol (Bothast and Schlicher, 2005).

In a dry-grind mill, the resultant feed co-products are distillers grains, distillers solubles and distillers grains plus solubles (Klopfenstein et al., 2007), which are excellent sources of supplemental proteins in livestock feed (Kim et al., 2008a). The nutrients in the corn kernels are concentrated threefold in the distillers grains (Klopfenstein et al., 2007). Apart from ethanol and co-product, carbon dioxide produced can be used for food processing or industrial purposes (Bothast and Schlicher, 2005). The major steps in the dry-grind ethanol

process (Figure 2.2) include; grain handling and milling, liquefaction and saccharification, fermentation, distillation and dehydration; and co-product recovery (Dale and Tyner, 2006).

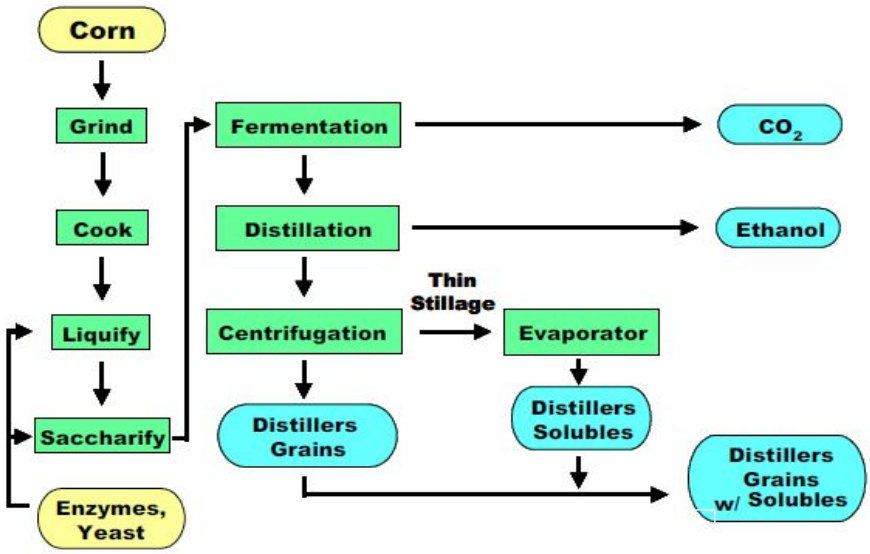

Figure 2.2. Dry-grind ethanol process (Butzen and Haefele, 2008).

Grain handling and grinding

Dried kernels brought into the plant are examined for quality. Following the quality examination the entire corn kernel (germ, endosperm and pericarp) is ground into a coarse flour through hammer mill (Bothast and Schlicher, 2005). The granular material is mixed with water to form slurry (Singh and Johnston, 2004). The slurry is commonly referred to as "mash" (Butzen and Haefele, 2008). The grinding increases the surface area of the corn, exposing starch and allowing more efficient hydrolysis to occur (Dale and Tyner, 2006).

Liquefaction and Saccharification

At this stage, the slurry is cooked with enzymes at approximately 160^{o}C using pressurized steam to break down the crystalline structure of starch granules (Singh and Johnston, 2004). Amylase (alpha-amylase) enzyme is added to break down starch polymers into short chain molecules (Singh and Johnston, 2004). The slurry is gelatinized by the alpha-amylase; a process referred to as liquefaction (Kim et al., 2008a). The slurry is further saccharified; hydrolysis of glucose using enzyme (Wang et al., 2007). Corn starch is made up of individual units of glucose, linked together in chains by alpha-1, 4 and alpha- 1, 6 linkages (Bothast and Schlicher, 2005).

Dale and Tyner (2006) described the role of enzymes in dry-milling as; alpha amylases as endozymes (enzymes that cause hydrolysis to occur randomly) that cleave alpha-1, 4 linkage hydrolyzing starch solutions into dextrin solutions. Gluco-amylase however consists of several different kinds of enzymes including: alpha-amylases, cellulases, and proteases. Gluco-amylase are exoamylytic (cleaves molecules in a stepwise manner) cleaving alpha-1, 4 and alpha-1, 6 linkages. Starch hydrolysis can be done using acid or enzymes. Acid hydrolysis is a random cleaving of the alpha-1, 4 and alpha-1, 6 linkages in starch over time ([Alexander, 1994] and [Dale and Tyner, 2006]). The acid hydrolysis reaction that occurs is:

$$(C_6H_{10}O_5)_n + H_2O\ (H+) \longrightarrow C_6H_{12}O_6$$

The enzymatic reaction is same as acid hydrolysis but an increased concentration of fermentable sugars are achieved. The enzymatic reaction that takes place and forms the basis of dry-grind ethanol process described is shown as:

$$(C_6H_{10}O_5)_n + H_2O \text{ (Alpha- + Gluco-amylase)} \longrightarrow C_6H_{12}O_6$$

The slurry is then held at an elevated temperature (~85^{o}C) for a short period of time, and cooled to approximately 32^{o}C (Berger and Singh, 2010). Cooking the slurry is either done using the traditional batch cooking or continuous method which is more energy efficient compared to the later (Butzen and Haefele, 2008). After saccharification process, the glucose rich stream is transferred to a fermentation vessel for yeast fermentation into ethanol (Kim et al., 2008a).

Fermentation

The fermentation of glucose into ethanol (alcohol) is achieved using yeasts called *Saccharomyces cerevisiae* (Butzen and Haefele, 2008). The yeasts, a type of fungi are able to metabolize glucose, disaccharides and trisaccharides for cell growth, repair, reproduction and alcohol production (Dale and Tyner, 2006). The fermentation process takes 48-72 hours in batch or continuous fashion with a final concentration of 10-12% (Bothast and Schlicher, 2005). Ethanol production from glucose is represented by the following equation (Singh et al., 2001):

$$C_6H_{12}O_6 + H_2O + \text{Yeast} \longrightarrow 2CO_2 + 2C_2H_5OH + H_20 + \text{Heat}$$

The resulting mixture after fermentation is called beer, which consists of ethanol, water, and solids that were not fermented (Rausch and Belyea, 2006). The fermentation process yields ethanol and carbon dioxide (CO_2). CO_2 produced is captured upon degasification and sold as a by-product or released into the atmosphere (Dale and Tyner,

2006). The beer is fed to a system consisting of two distillation columns and a stripping column (Singh et al., 2001).

Distillation and dehydration

Distillation is the process of separating the ethanol from the solids and water in the slurry (Butzen and Haefele, 2008). Fractional distillation is used to separate the various components of the slurry based on different boiling points. The resulting beer is flushed to separate the carbon dioxide (Singh et al., 2001). Through controlled sequential evaporations, condensations, re-evaporations, and re-condensations the ethanol content in the vapor is concentrated to higher levels (Dale and Tyner, 2006).

After distillation, the mixture is dehydrated to create fuel-grade ethanol (Bothast and Schlicher, 2005). Usually, conventional distillation/rectification methods can produce 95% pure (190 proof) ethanol (Butzen and Haefele, 2008). The 5% remaining water is removed using modern technology through molecular sieve system to produce absolute (100% or 200 proof) ethanol (Bothast and Schlicher, 2005). The final ethanol output is consumable therefore, it is denatured using gasoline to render it undrinkable (Butzen and Haefele, 2008).

Co-product recovery

Following distillation, the remaining alcohol-free slurry containing non-fermentable portions of the corn (protein, fat and fiber), along with yeast and other chemicals added to the fermentation (e.g., micronutrients and antibiotics) are collected from the distillation base and referred to as whole stillage (Singh and Johnston, 2004). Whole stillage is centrifuged to produce wet distillers grains (high density portion) and thin stillage. Using an evaporator, thin stillage is concentrated to form distillers syrup (Rausch and Belyea, 2006) or condensed

distillers solubles (Ganesan et al., 2006) or simply solubles. Solubles, the term that will be used in this work is a viscous, low-solid and high protein co-product stream which can be blended with wet distillers grains or dried distillers grains (Belyea et al., 1998).

For every bushel of corn, 17 pounds of DDGS (1 pound = 0.4536 kg) are generated via the dry-grind process (Bothast and Schlicher, 2005). Commonly, ethanol plants may mix the syrup with the wet grains to form wet distillers grains with solubles (WDGS) (Figure 2.3) also called 'wet cake' (Dale and Tyner, 2006).

Figure 2.3. Wet distillers grains with solubles from dry-grind ethanol process (Erickson et al., 2008b).

WDGS are approximately 65-70% moisture content (Erickson et al., 2008a). WDGS can be partially dried to ~50% moisture content (Schuster, 2011), called modified wet distillers grains with solubles (MWDGS) (Figure 2.4) or 10% moisture content called dried distillers grains with solubles (DDGS) (Erickson et al., 2008a) shown in Figure 2.5.

Figure 2.4. Modified wet distillers grains with solubles from dry-grind ethanol process (Loy et al., 2009).

Figure 2.5. Dried distillers grains with solubles from dry-grind ethanol process (Loy and Strohbehn, 2007d).

Recently, DDGS pelleting technology is another preservation method used in the ethanol industry. Pelleting is suggested to reduce shipping cost (both direct and indirect) by 89% and

sale cost by 96% (Rosentrater and Kongar, 2009). DDGS can also be compacted into cubes (Figure 2.6).The size of DDGS cubes shown below is 0.019 m (0.75 in).

Figure 2.6. Dried distillers grains with solubles in pellets (Rasby and Stalker, 2009).

Benefits and challenges of distillers grains utilization

The benefits of using WDGS such as high metabolizable energy, lower cost per unit dry matter compared to DDGS are many however, two major drawback exist: deterioration and transportation costs (Klopfenstein, 1996). High nutrient levels and high moisture of the wet co-product promotes rapid deterioration. Transporting of water raises transportation cost since less dry matter is moved per unit total mass moved. The various forms of distillers grains come with their unique merits and demerits. Moreover, studies have shown that the metabolizable energy value of distillers grains are consistently higher than that of corn (Trenkle, 2008). Klopfenstein (1996), found that the metabolizable energy value of wet distillers co-products was 132 to 174% that of corn when 40% dry matter was used for finishing cattle whereas the metabolizable energy value of DDGS was 130% that of corn.

Studies conducted by Erickson et al., (2005), there was 15 to 25% improvement in feed efficiency when 30 to 40% of corn grain was replaced with wet distillers grains. They found that the metabolizable energy value of wet distillers grains for finishing was 140 to 150% that of corn when medium levels (average of 17% of diet DM) was used and 130% of corn with 40% of DM.

DDGS are available throughout the year because they are easy to transport and have longer storage life due to less moisture content (Rosentrater and Kongar, 2009). High moisture WDGS are limited to short distances by transportation costs and have very short storage life. Protein destruction due to heat from drying is of great concern in DDGS compared to WDGS (Kaiser, 2008). Drying WDGS into DDGS denatures proteins and carbohydrate therefore reducing nutritional compositions (Ham et al., 1994) and energy content (Weiss et al., 2007). WDGS are highly palatable and they condition diets that are dry by maintaining homogeneity of feed particles (Kalscheur and Garcia, 2011). Other benefits of WDGS includes: lower cost per unit DM, higher energy concentration, mix well into a total livestock rations and moisture of the wet feed can reduce sorting when fed to cows (Weiss et al., 2007).

Economics of distillers grains

Distillers grains have become an important part of the ethanol plant profitability. However, the available supply and cost of competing commodity protein sources primarily corn, soybeans, and soy meal influence the market price of distillers co-products (Bothast and Schlicher, 2005). Some of the factors that heavily affect decisions relative to co-product feed pricing and inclusion rates include nutrient value of feeds, nutrient value of competing

feeds, consistency of product, reliability of supply, transportation, consistency of pricing and storage losses (Loy and Strohbehn, 2007b).

The price of the co-products are affected by moisture change since, water is regarded as insignificant in value. Fuel energy is required to dry WDGS to DDGS, therefore wet distillers co-products has been studied as a means of reducing energy and economic cost because of lower prices (Klopfenstein, 1996). As at June 2011, the average prices for distillers co-products per 0.907 Mg (1 ton) basis were; WDGS: ~$77, MWDGS: ~$102, DDGS: ~$210, corn gluten feed CGF (wet): ~$72, (CGF) (pelleted): ~$185 and corn gluten meal (CGM): ~$530 (Steevens and Sexten, 2011).

Transportation is a significant expense associated with distillers grains and feed inclusion rates. Transportation methods include rail, unit-train, export containers, trucks and barges. The mode of transportation depends on the proximity to the ethanol plants. Cost of transportation of distillers grains also depend on the form that is moved from one point to another. Typically, the transportation price of WDGS is higher due to higher moisture content (Vander Pol et al., 2006) taken into consideration how much dry matter is conveyed. Feedlots located 0 to 97 km (0 to 60 miles) from an ethanol plant feed 30% to 40% dry matter of WDGS to cattle, as the distance of feedlot increases from 97 to 161 km (60 to 100 miles), optimum inclusion ranges between 20% to 30% (Vander Pol et al., 2006).

A 2004 survey of ethanol plants, showed that ethanol plants pay $30 per 0.907 Mg (1 ton) to transport WDGS at a distance of 2494 km (1,550 miles) for rail ($0.0120/Mg-km or $0.0194/ton-mile) and $4 per 0.907 (1 ton) for 132 km (82 miles) using trucks ($0.0303/Mg-km or $0.0488/ ton-mile, or $2.5 times more than rail) (USDA, 2004). Co-products prices may also be influenced by seasonal variations especially summer and winter (Waterbury and

Mark, 2008). Seasonality price trend of WDGS and MWDGS is scanty however those of DDGS (Figure 2.7) can be applied. DDGS price trend in Figure 2.7. is a function of demand from cattle feeders (Waterbury and Mark, 2008).

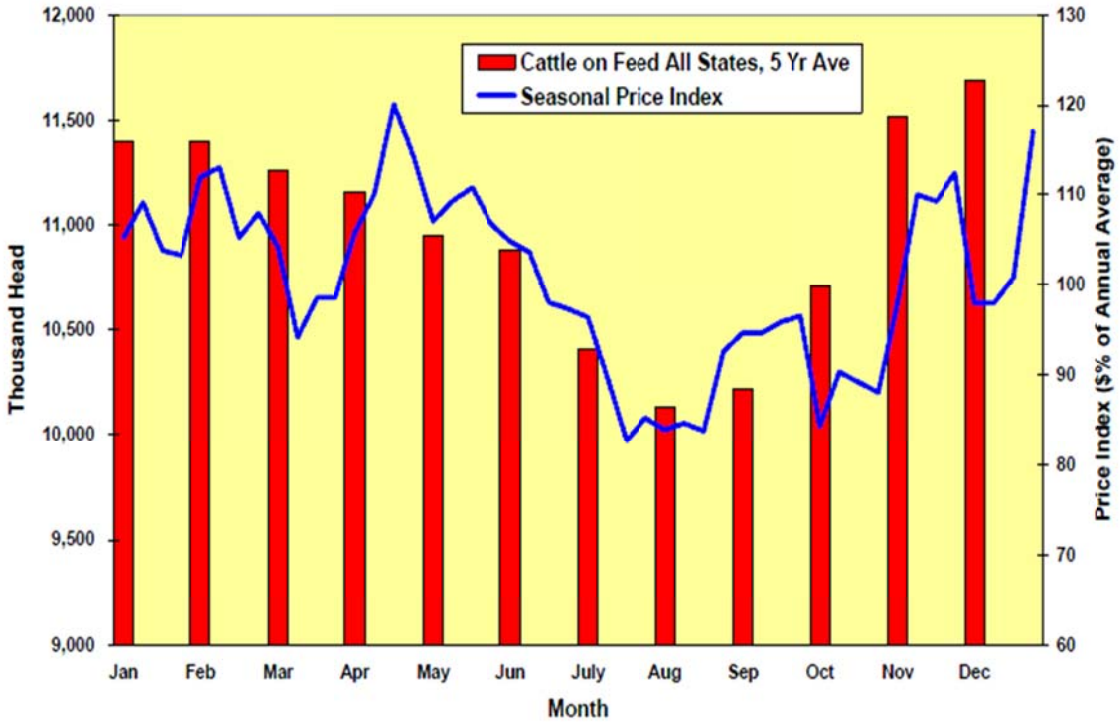

Figure 2.7. Seasonal price index of DDGS from 2003 to 2007 with cattle-on-feed by for the US ([AMS & University of Nebraska-Lincoln], [Waterbury and Mark, 2008] and [Erickson et al., 2008a]).

During summer months feedlots are less active and this can result in lower co-product prices (Christiansen, 2009). The variation in prices is a function of the number of cattle and feed demand. Several other factors could generally influence corn co-product pricing within individual plant.

Distillers grains composition

Distillers grains are rich in nutrient content (Rosentrater et al., 2005) because remaining nutrient after starch extraction is concentrated threefold in into the co-product (Klopfenstein et al., 2007). Therefore distillers grains are considered to be a good source of ruminally undegradable protein, energy, and readily digestible fiber ([Jones, 2007] and [Schingoethe, 2006]). The nutrient composition of distillers grains as with many co-products feeds, is influenced by several factors including type of grain used, grain quality, grinding procedures, extent of fermentation, drying conditions, quantity of solubles blended back with the distillers grains and particle separation (Kaiser, 2008).

Studies by Buckner et al. (2008b) showed that, dry matter and sulfur content for WDGS and MWDGS varied from plant to plant greatly though protein, fat and phosphorus remain fairly correlated. Kaiser (2005), also found that there was substantial variation in WDGS composition both within and across ethanol production facilities. The texture, color and odor of distillers grains vary among different plants. Variations exists from plant to plant even within a given plant (Klopfenstein et al., 2007). According to O'Connor (2007), DDGS quality is impacted by the design of the overall ethanol plant particularly; the design of the back end of the plant, centrifuges, evaporators and dryers.

WDGS on average contains 31.0% crude protein, 11.9% fat, 0.8% phosphorus and 0.77% sulfur on dry matter basis (Buckner et al., 2011). Distillers grains contain large amount of neutral detergent fiber (NDF) but low amounts of lignin (Schingoethe, 2006). Highly digestible fiber (Kaiser, 2008) and fat are excellent energy sources; therefore high fat levels in WDGS is desirable unless dietary inclusion is greater than 40% to 50% of diet DM

(Buckner et al., 2008b). Mineral concentrations of wet distillers grains are usually low; example 0.11% calcium, 0.43% phosphorus, 0.18% potassium (Rausch and Belyea, 2006).

The concentration of initial additives in distillers grains should inform livestock producers that mycotoxins contamination of corn is likely to have high concentration in the final co-product if present in the kernels. Mycotoxins are not degradable during the ethanol process (Garcia et al., 2008). Mycotoxins are poisonous compounds produced by molds under certain conditions (Bern et al., 2010). In addition, distillers grains in excess of minerals impact negatively on livestock performance; excess phosphorus and excess sulfur intake causes polioencephalomalacia ([NRC, 2001] and [Schingoethe et al., 2009]). Comprehensive and in-depth information on the chemical composition of distillers grains can be found in Rosentrater et al. (2005) work on ethanol processing residue properties.

Demand and Utilization of distillers grains

The demand for distillers grains may continually rise with increasing ethanol production. Animal producers may purchase co-products for variety of reasons. However, the primary reason is economic (Rausch and Belyea, 2006). In 2005, over 70% of Iowa feedlot were using ethanol co-products whiles 27% of the cow-calf producers were incorporating them in their rations according to a survey conducted by the Iowa Beef Center (Loy and Strohbehn, 2007a). Distillers grains has received tremendous global patronage and countries including; Ireland, United Kingdom, Canada, Spain, Mexico, China, Taiwan, Japan and South Korea are among the top importers of distillers co-product (Shurson, 2006).

Kaiser (2005), reported that over 85% of U.S. fed distillers grains are consumed by dairy and beef cattle and incorporation of this feed into swine and poultry diets is increasing.

Anderson et al. (2006), observed that milk protein and fat yields were higher when lactating dairy cows were fed with WDGS and DDGS at 20% DM however, the effect of WDGS on yield was better. Results on WDGS from previous study by Schingoethe et al. (1999) showed similar trend to those obtained by Anderson et al. (2006). In general, research has shown that feeding distillers co-products results in an increase of average daily gain, feed efficiency and overall body weight and performance (Buckner et al., 2008a).

Eun et al. (2009), found that the effect of low and high level of DDGS on growth performance, digestibility, ruminal fermentation and carcass characteristics of beef steers growth was higher than those fed on ordinary barley grain ration. A combination of corn co-product feeds was observed to enhance performance in feedlot cattle.

Loza et al. (2010) experimented with 0%, 10%, 15%, 20%, 25% and 30% WDGS (dry matter basis) in diets containing 30% wet CGF (DM basis), dry-rolled corn and high-moisture corn-based finishing diets for beef cattle. They observed that inclusion of 30% wet CGF in the diet increased dry matter intake, average daily gain and growth to feed ratio when compared to diets with no co-products. Greater average daily gain was obtained at 15% to 20% inclusion rates combined with 30% wet CGF hence, concluded that combination of WDGS and WCGF in finishing diets resulted in similar or improved steer performance compared to corn.

Inclusion of corn co-products in livestock diet offer many feeding opportunities. New development might pave way for inclusion of distillers grains in humans diets. Liu et al. (2011) experimented on the quality of corn-bread incorporated with DDGS. They observed that moisture levels and texture were similar to corn-bread without DDGS however; DDGS-

corn-bread was slightly darker in appearance and DDGS inclusion rate beyond 25 g per 100 g DDGS showed decline in textural quality and batter elasticity.

Distillers grains storage and deterioration

The most important factors that greatly affect distillers grains (co-product in general) are storage methods, handling and, transportation especially with wet distillers grains (Loy and Strohbehn, 2007b). The ethanol industry and farm operations have faced many challenges storing wet distillers grains. The method of distillers grains storage depends on the form. DDGS will store well for a longer time because of less moisture compared to WDGS. The high moisture co-products will likely require large and sophisticated storage facilities to avoid exposure to weather effects, contamination and deterioration. Without proper storage, the right amount of moisture, nutrient and temperature will induce microbial activities leading to a rapid reduction of wet co-product storage life.

Deterioration of food products occurs as a result of insects, microorganisms, biochemical and physical changes ([Frazier and Westhoff, 1978] and [Jayas and Jeyamkondan, 2002]). Exposure of WDGS to air promotes aerobic decomposition therefore it is important to exclude air during storage. The limitations of WDGS prohibit long distance delivery. Therefore WDGS become an alternative to farm operations close to the ethanol plants. Basically, the two major reasons why storage has become relevant for distillers co-products are; ethanol plants prefer to deliver co-product semi-load quantities making it difficult for smaller feedlots to completely utilize in a short time and farm operations may want to use WDGS and MWDGS on seasonal basis due to price fluctuation (Erickson et al., 2008a).

Studies have shown that more than 34% of co-product users are willing to pay $1-$5 extra to extend storage life of co-products, 45% desire to pay more than $5 to extend storage life whereas 25% refused extra cost for storage life extension (Baskett et al., 2009). Some common storage and preservation practices include: bagging, piling and additives: use of chemical and biological preservatives (Nelson et al., 2009).

Bagging storage

Silage bags are commonly used for distillers grain storage and considered to be effective. Conventionally, the wet feeds are mixed or ensiled with forage materials before bagging. Strohbehn et al. (2008b) successfully stored 50.2% moisture MWDGS and WDG/dry hay (80%/20%) for 39 to 90 days respectively. Deterioration was however seen to occur at the edges in the silo bags (Figure 2.8).

Figure 2.8. Deteriorated surface of bagged modified wet distillers grains with solubles (Strohbehn et al., 2008b).

They also observed that after 90 days of storage, lactating cows responded well to the blended feed. Strohbehn et al. (2008a), stored 17.7% and 32.7% of MWDGS dry matter ration, in combination with haylages (hay + silage) for over 122 day period. Similar work by Erickson et al. (2008a) showed that WDGS store in bags successfully without any deterioration or compacting problems when moistures were increased by 50%. They discovered that storage of WDGS in bags under a pressure of 300 psi (1 psi = 6.895 kPa) or greater can result in bag bursting (Figure 2.9).

Figure 2.9. Split silo bag containing wet distillers grains due to compaction (Erickson et al., 2008a).

Strohbehn et al. (2008b) suggested that bagging cost is not competitive hence the need for more cost effective storage methods. Also, sample cost calculations estimate by Waterbury et al. (2009) using Co-Product STORE; a tool designed to quantify co-product storage for two storage methods (bunker and silo bags). They found that the as-is mixture

cost per 0.907 Mg (1 ton) with shrink was less for bag storage than bunker storage however, the dry matter mixture cost per 0.907 Mg (1 ton) with shrink was greater for silo bag storage method compared to the bunker method.

It should be noted that, a typical storage method will depend on several parameters including feed costs, equipment and structure costs and, other variable costs.

Fresh piles and bunker storage

The most common method of storing distillers grains is heaping on fields until fed or placed in bunkers due to large quantities that cannot be bagged. Fresh pile distillers grains are either stored covered, uncovered or heaped in a bunk (Nelson et al., 2009). According to a 2009 survey by Basket et al. (2009), about 80% of co-product users in Iowa and neighboring States indicated that fresh pile is their primary method of co-product storage whereas the remaining used piles with plastic or additives, upright silos, and forage sheds or silos as wet co-product storage practices. These methods of storing wet co-product may expose the feed to all kinds of environmental conditions, thus increasing susceptibility to contamination and deterioration if not fed quickly.

Studies conducted with wet distillers grains (WDG) stored in covered and uncovered bunker silo resulted in storage losses of 8.55% and 9.64% for a period of three months (Baskett et al., 2009). Erickson et al. (2008a), found that WDGS does not store properly in bunkers without forage (Figure 2.10) because high moisture content causes the pile to flow however MWDGS (Figure 2.11) will pile in bunkers without forage.

Figure 2.10. Wet distillers grains with soluble ensiled with straw in a bunker (Loy et al., 2009).

Figure 2.11. Modified wet distillers grains with solubles piled on field (Loy et al., 2009).

Additives

Additives include both biological and chemical preservatives that are commonly incorporated into wet feeds. Biological additives include; grass hay, corn stalk, wheat straw and soybean hulls. Ensiling with these biological materials is one of the options that has been successfully demonstrated ([Garcia and Kalscheur, 2004] and [Loy, 2008]). These materials are blended dry with the purpose of reducing moisture of the wet feed during storage. Schingoethe et al. (2006) combined WDGS at 0%, 15%, and 30% and soy hulls and concluded from their study that WDGS can be preserved by ensiling with soy hulls. A demonstration storage test showed that MWDGS alone did not store well as WDG/ dry hay blend. Dry matter loss due to deterioration and mold in MWDGS observed was 5.1% after 35 days (Strohbehn et al., 2008b)

Furthermore, chemical additives are becoming a common practice in the ethanol industries. Preservatives added at the ethanol processing plant can extend storage life (Loy, 2008). The common chemical preservatives in the U.S. markets are CakeGuard™ (Alltech® Inc., USA), ZeniPro® (Kemin Inc., USA) and Biomin® (Biomin America Inc., USA). The primary active ingredient found in these chemical preservatives is propionic acid. Chemical preservatives are noted to extend the storage life of high moisture co-products when added at the processing plant. The chemical balance and composition of most chemical preservative however will determine its potency.

Trials with chemical preservatives have indicated that visually, deterioration in untreated (no preservative) distillers grains (3.1%) was greater than in distillers grains treated with preservative (1.3%) when stored outdoor in open boxes (Walker and L. A. Forster,

2008). Similar research by (Kung, 2005), showed that preservatives are potential aerobic stabilizer of high moisture feeds.

Trials at Utica Energy indicated that wet distillers grains treated with CakeGuard at a low usage rate showed increased stability to 10 to 14 days and longer stability was achieved using higher rates (Sommerfeldt, 2011). It was observed in other trials that without the presence of CakeGuard, deterioration were observed in about three days when humid, and in dry conditions deterioration observations were made about seven days.

Drackley et al. (2004) studied the effect of preservative product (Zenipro) on wet distillers grains and found that there was apparent visual deterioration differences in treated and untreated (control) wet distillers grains yet, there was no clear difference in their analytical measures of yeast or mold count. In addition, they observed that treated wet distillers grains did not clump or harden.

Characteristics of CakeGuard preservative

In 2002, Alltech® senior application specialist, Michelle Stevens, researched and developed what later became known as CakeGuard (Sommerfeldt, 2011). The commercial product; is advertised as an easy to use, highly effective preservative that protect distillers grains from losses in palatability and nutritive value caused by molds growth as well as dangers of toxicity from mycotoxins (Alltech® Inc., USA). CakeGuard is marketed to extend storage life and increases marketing flexibility of distillers wet grains (Alltech® Inc., USA).

According to specification of the product, CakeGuard is a propionic acid-based mold inhibitor for distillers wet grains. The preservative has a pH of 5.0 and contains propionic acid, sodium benzoate, water, and potassium sorbate. It is a clear liquid with a pungent odor with a specific gravity of 1.14 g/ml. It is recommended that the product be stored in a closed

container away from heat and light. It has a life span of 36 months. The recommended dosage is 1.0 kg CakeGuard per Mg (1 Mg = 1 metric ton = 1 tonne = 1000 kg) of distillers wet grains.

Factors that influence distillers grains deterioration during storage

Short storage life or 'shelf life' of wet distillers grains is the common term associated with the wet feed due to rapid rate of deterioration. In reflection, deterioration of wet co-product is of a major concern. The mechanism of deterioration of wet co-products is not well understood however high moisture, concentrated nutrient and presence of oxygen are suggested to be the major factors that promote rapid deterioration of wet co-product.

According to Fawole (1969), intrinsic biological activities like respiration and sprouting and extrinsic biological activities like mold and yeast are two factors that affect stored grain. The mechanism of deterioration is probably a complex system of interactions of microorganisms, environmental conditions and respiration of biological materials. As a result of this rationality, it has been suggested that deterioration of a biological material can only be obtained by separating microbial activities ([Milner and Geddes, 1945] and [Steele, 1967]). However, it will be impossible for biological degradation to occur without microorganisms. Research has shown that deterioration is mostly affected by moisture; temperature, microorganisms (mold and yeast) and relative humidity.

Moisture and Temperature

Microbial survival and activities can be influenced by moisture and temperature variations. The moisture content and temperature will influence and even direct events that occur during storage and may sometimes lead to deterioration and self-heating (Mills, 1989).

Marks and Stroshine (1995), observed that the storability of corn was reduced with high moisture content during corn storage tests. High moisture content influences the rate of deterioration of biological materials. High moisture affects grain storability whereas low moisture increases the time period for corn storage (Al-Yahya, 1996). The original condition of a material is probably the most important factor affecting it storage. The problem with moisture can be eliminated by drying however, it attracts energy cost.

Relative humidity

Relative humidity is the ratio of the absolute humidity of air to the maximum possible absolute humidity of that air, at the same temperature (Bern et al., 2010). This implies that, relative humidity (and therefore moisture) is dependent on temperature. An air sample with relative humidity level of 50% will increase five degrees in temperature from 25^{o}C to 30^{o}C if its relative humidity decrease to 38% however, if the temperature of the air sample is decreased five degrees, from 25^{o}C to 20^{o}C, the relative humidity level will increase to 69% (Mills, 1989). Relative humidity determines the rate of moisture lost or gained by a material thus affecting the rate of microbial activities. Biological organisms that cause stored products to deteriorate require different levels of relative humidity for normal development (Mills, 1989). Generally the level for bacteria for any stored plant material is above 90% and for deterioration molds it is above 70% (Mills, 1989).

Microorganisms

Microbes of different kinds under various conditions can grow on all kinds of biological substrates and contribute to deterioration. Exposure of wet distillers grains to aerobic conditions is another critical factor that affects deterioration thus storage life. Molds

may grow if under normal storage condition temperature between 68^{o}F (20^{o}C) and 86^{o}F (30^{o}C) is maintained for extended time (Garcia et al., 2008). The ideal growing condition for mold ranges between 13% to 18% moisture (Garcia et al., 2008). Yeast can grow effectively at neutral pH however yeasts are able to grow at a pH of 5 or lower and in the presence of sugars, organic acids and other easily metabolized carbon sources (Kurtzman, 2006). At sufficiently low pH, growth is stopped.

Carbon dioxide evolution as a measure of deterioration (dry matter loss)

All biological materials are made up of two components: water and dry matter. Loss of dry matter occurs as a result of glucose oxidation in the presence of other variables such as moisture, temperature and microorganisms (Bern et al., 2002). Carbon dioxide evolution as a result of glucose oxidation due to fungal activities represent a usable indicator for dry matter loss-DML (Bern et al., 2002). DML can be determined in two ways: indirect method by measuring evolved carbon dioxide and direct method by weight difference.

Two general methods of indirect method of measuring CO_2 from grains during storage as an indication for DML exists ([Milner and Geddes, 1945] and [Fawole, 1969]). Firstly, the quantity of CO_2 generated within inter-seeds atmosphere in a sealed container for a period during storage can be measured. It is however recommended that such containment should not exceed 96 hours to prevent accumulation of quantities of CO_2 that decrease microbial activity ([Bailey, 1921] and [Fawole, 1969]). Secondly, and probably the most common, continuous or intermittent aeration is applied to seeds and the exiting air is analyzed for evolved CO_2 ([Bailey, 1921] and [Fawole, 1969]).

Steele (1967), Steele et al. (1969), Fernandez et al. (1985), Friday et al. (1989), Al-Yahya (1993), used various CO_2 evolution techniques measurement to quantify DML of stored grains. The aeration method was used by Steele (1967) on his work; deterioration of damaged shelled corn as measured by carbon dioxide production which follow previous deterioration studies on grain deterioration. Al-Yahya et al. (1993) used similar method of aeration for his work on fungicide–treated high moisture corn for DML measurement. White et al. (2010), also applied the aeration method to quantify DML on ozone-treated high-moisture corn.

As CO_2 evolution is an accepted tool for quantifying dry matter loss of aerobic decomposition of biological materials (Chitrakar et al., 2006), this study on storability of MWDGS followed the method of aeration to quantify DML during storage.

CHAPTER 3. STORABILITY OF MODIFIED WET DISTILLERS GRAINS WITH SOLUBLES

A paper to be submitted to Journal of Stored Products Research

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Abstract

Distillers grains, a high-quality co-product from dry-grind ethanol production processes, is widely uses as a livestock feed both locally and internationally. However, the wet form of distillers grains deteriorates (i.e., undergoes dry matter loss-DML) rapidly during storage which affects overall management and utilization. There are several active research initiatives aimed at developing alternative preservation methods to retard storage losses thus extending the wet feed storage life to meet the fundamental principle of availability of feed during scarcity. And yet, data on wet distillers grains DML is highly limited. This study investigated the effect of 0.1% w/w CakeGuard™ preservative (propionic acid-based) and temperature (10°C, 20°C and 30°C) on modified wet distillers grains with solubles (MWDGS) DML under aerobic conditions during storage for 21 days. There was significant difference in DML with preservative treatment at 20°C and 30°C. Effect of temperature treatment was significant. Preservative and temperature interaction effect on DML was

significant. Treated MWDGS DML after 21 days averaged 3.12%, 16.8% and 19.3% DML whereas untreated samples averaged 3.22%, 21.4% and 28.0% DML at 10^{o}C, 20^{o}C and 30^{o}C respectively. Overall, the preservative helped maintain appearance and texture of the wet feed within the storage period. While further research is obviously necessary to define criteria for predicting storage losses, this study serves as a foundation for future investigations.

Keywords: Wet distillers grains; Deterioration, Storage life; CakeGuard™ preservative

Introduction

Increasing demand for renewable and sustainable energy fuels has led to expanding corn-based ethanol production in the United States especially across the Midwest. Ethanol is becoming important as an alternative to fossil fuels. As a consequence, large quantities of co-products are generated, most commonly distillers grains obtained from processing cereal grains into ethanol (Wu, 1989). Corn is the predominant feedstock for ethanol production (Kim and Dale, 2004) because on an industrial scale, it is currently the most economical feedstock that can be converted (Rosentrater et al., 2005). Feeding wet distillers grains with solubles results in better performance than dried distillers grains with solubles (Klopfenstein et al., 2007). Other benefits includes: maintain nutrients in corn growing areas, saves drying costs and promotes beef industry.

Distillers grains have become the most economically attractive feed input for livestock globally. The supply of distillers grains is growing yearly. In 2010, 32.5 million Mg (1 Mg = 1 metric ton = 1 tonne = 1000 kg) of distillers grains was produced compared to 2.3 million Mg in 1999 (RFA, 2011c). Distillers grains export and domestic market contribute

substantially to the economic viability of ethanol manufacturing generally dried distillers grains account for 10 to 40% of an ethanol plant's entire revenue stream (Rosentrater and Kongar, 2009). Continuing high levels of ethanol production will make distillers grains available to the feed industry. However, availability and utilization of distillers grains are influenced by many factors including supply and demand, compositional variation, handling, transportation and storage.

Although wet distillers grains are cheaper than dried distillers grains on both wet and dry matter basis, high moisture content and concentrated nutrients promote rapid deterioration (dry matter loss- DML) of the wet co-product during storage (Bern et al., 2008). These limitations engender challenges to the overall management of the wet feed and therefore affect how wet distillers grains are handled, transported, stored and how much they cost feed buyers (Mathews and McConnell, 2009). Generally, corn distillers grains are often fed wet to avoid drying costs (Birkelo et al., 2004). Therefore, information on deterioration is a priority if decreased dependence on drying wet distillers grains, which represents as much as 30% of energy cost and 50% of natural gas usage in a typical dry mill ethanol plant (Loy, 2009), is to be achieved.

Distillers grains production

Distillers grains are co-products from ethanol production, with corn being the main feedstock in the Midwestern region of the United States (Birkelo et al., 2004). Generally, corn can be converted into ethanol by either wet milling or dry-grind processing ([Singh et al., 2001] and [Belyea et al., 2004]) depending on the pretreatment method. Wet-mills are capital intensive (Rausch and Belyea, 2006) and are designed to produce a variety of

products, therefore the plants are complex and larger (Gallagher et al., 2005). The purpose of wet milling is to fractionate the kernel into its constituents (Jackson and Shandera Jr, 1995) which results in starch, crude oil, protein, fiber, corn gluten meal and corn gluten feed ([Voloch et al., 1984], [Ladisch and Svarczkopf, 1991], [Matz, 1991] and [Gulati et al., 1996]).

Alternatively, the dry grind ethanol process is relatively simple (Rausch and Belyea, 2006), and has low capital and energy investment costs (Rodríguez et al., 2010). It is currently the predominant corn processing method in the ethanol industry. In 2006, dry grind ethanol refineries accounted for 82% of production capacity, and wet mills 18% (RFA, 2007). Dried distillers grains with solubles (DDGS) and wet distillers grains with solubles (WDGS) are the major co-products of the dry-grind ethanol facilities (Kim et al., 2008b). Corn dry-grind processing (Figure 3.1) is designed to subject the entire corn kernel to fermentation (Rausch and Belyea, 2006).

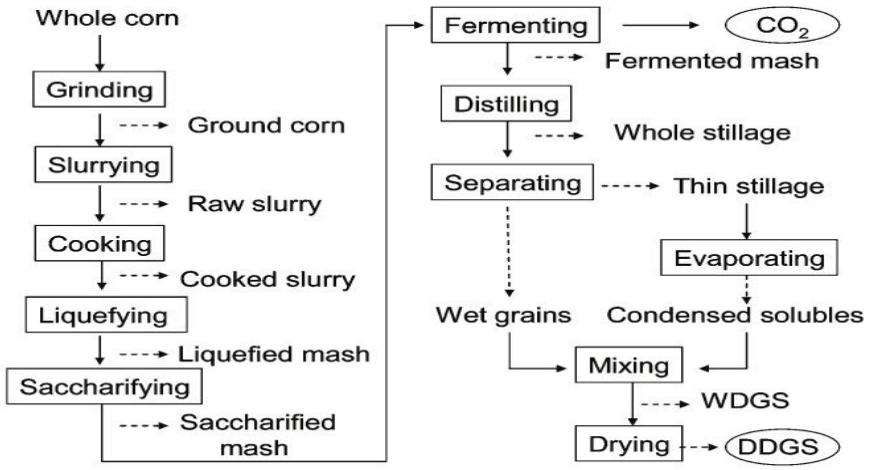

Figure 3.1. Schematic diagram of conventional dry grind ethanol production from corn (Liu, 2011).

The major steps involved in the dry-grind method include dry-grinding, liquefaction, saccharification, fermentation, distillation and co-product recovery (Liu, 2011). The entire corn kernel is ground and slurried with water and alpha-amylase enzyme to form "mash" (Butzen and Haefele, 2008). The mash is held at an elevated temperature (~85^{o}C) for a short period and cooled to 32^{o}C (Berger and Singh, 2010).

Following this step, glucoamylase enzyme and yeast are added for simultaneous saccharification and fermentation in a fermentation vessel (Berger and Singh, 2010). During this process, simple sugars are converted into ethanol and carbon dioxide by the action of yeast and heat (Kwiatkowski et al., 2006).

Ethanol is recovered from the mash by distillation leaving the non-volatile components called whole stillage (Bothast and Schlicher, 2005). Whole stillage is centrifuged to produce a liquid fraction called thin stillage and a solid fraction called wet distillers grains (the high density portion) (Kim et al., 2008b). The thin stillage is processed through a series of evaporators and concentrated into condensed distillers solubles (CDS), commonly referred to as "syrup" (Ganesan et al., 2006).

Wet distillers grains are usually blended with the syrup to form wet distillers grains with solubles (WDGS) (Kaiser, 2008). WDGS has a moisture content (all moistures are % wet basis) of about 65% and is sometimes dried to dried distillers grains with solubles (DDGS) with a moisture content of 10 to 12% with a goal of increasing storage life (Butzen and Haefele, 2008). Some ethanol plants may produce modified wet distillers grains with solubles (MWDGS) by partially drying WDGS to ~50-55% moisture (Perrin et al., 2009) or to a given moisture content upon customer request.

Distillers grains nutrient profile

The resultant feed co-products: distillers grains, distillers solubles and distillers grains plus solubles from the dry-grind method (Klopfenstein et al., 2007), are excellent sources of supplemental proteins in livestock feed (Kim et al., 2008a). Distillers grains are a good source of energy, protein, fiber, and phosphorus (Schroeder, 2010) and contain an average of 31% protein, 12% fat, and 0.8% phosphorus on dry matter basis (Buckner et al., 2008b).

WDGS and MWDGS, have high metabolizable energy concentration, mix well into total livestock rations (Weiss et al., 2007) and are highly palatable (Kalscheur and Garcia, 2011). They enhance feed efficiency (Vander Pol et al., 2006) and have the tendency to reduce acidosis occurrences than do low-roughage diets (Trenkle, 2008).

Distillers grains storage and storage life

For years, development work on wet distillers grains has been focused on methods of improving and extending wet co-products storage life. Bagging in silage bags is the conventional method for storing wet feeds (Erickson et al., 2008b). Fresh pile distillers grains are either stored covered, uncovered or heaped in a bunk (Nelson et al., 2009). Studies have shown that wet distillers grains can be successfully stored by ensiling ([Garcia and Kalscheur, 2004] and [Loy, 2008].

Treating distillers grains with chemical preservative as an alternative to other preservative methods is increasing. Johnson and Huber (1987), studied the effect of 0, 1.57%, 3.14% and 4.71% (dry matter basis) ammonia on wet distiilers grains. They observed that wet distillers grains treated with a low ammonia dosage were less stable and deteriorated faster than untreated grains. However, intermediate and high ammonia dosages reduced mold

growth and spoilage losses. Nofsinger et al. (1983) observed significant effect of sorbic acid, potassium sorbate and ammonia on wet distillers grains DML whereas propionic acid did not reduce dry matter loss.

Many chemical preservatives, however, contain propionic acid as the active ingredient. Propionic acid or other organic acids may be effective in inhibiting mold growth and extending storage life of wet feeds (Uslu et al., 2009) however, scientific documentation of such results are difficult to find (Schroeder, 2010). Also, very limited published data exists on aerobic microbes suspected to catalyze spoilage of WDGS (Rosentrater and Lehman, 2008). WDGS utilization as livestock feed is increasing and becoming common, however, due to deterioration challenges, feeding of the wet co-product is limited. WDGS are usually seen to mold when exposed to air for 3 to 14 days (Erickson et al., 2008a) and typical by seven days in the tropics (Tjardes and Wright, 2002).

Although creating DDGS is a solution which may increase storage life, research has shown that drying reduces energy digestibility in DDGS (Weiss et al., 2007), and heat denatures nutrients therefore reducing the feeding value of DDGS ([Klopfenstein, 1991] and [Klopfenstein, 1996]). Increased use of WDGS (versus DDGS) could reduce the overall energy cost of corn ethanol production, and subsequent environmental impacts such as greenhouse gas emissions by drying operations (Rosentrater and Lehman, 2008).

Carbon dioxide evolution as a measure of dry matter loss

Carbon dioxide (CO_2) evolution from biological materials has been studied as a useful measure of aerobic decomposition (Chitrakar et al., 2006). Various studies have established that dry matter loss as estimated by carbon dioxide emission from grains is an

indicator of deterioration ([Saul and Steele, 1966], [Steele, 1967], [Steele et al., 1969] and [Bern et al., 2002]). Data on corn DML as estimated by carbon dioxide evolution from fungal activities during storage (Saul and Steele, 1966), with results from other researchers, form the basis for an ASABE Standard for estimating allowable storage time for shelled corn based on moisture content and temperature ([Bern et al., 2002], [ASABE, 2006] and [Moog et al., 2008]).

The techniques employed by these researchers involved capturing and weighing evolved CO_2 present in aeration air ([Steele, 1967], [Steele et al., 1969], [Fernandez et al., 1985], [Al-Yahya et al., 1993], [Dugba et al., 1996]) or determining CO_2 concentration evolved in aeration air ([Wilcke et al., 1993], [Ng et al., 1998] and [Chitrakar et al., 2006]).

Withstanding the fact that seed grains and wet distillers grains have different physical and chemical properties (Nofsinger et al., 1983), the concept of seed grains (corn) deterioration will help elucidate and serve as a guide to study deterioration in wet distillers grains.

Problem statement

Wet distillers grains deteriorate rapidly therefore it is difficult to store for extended periods. The wet feed storage losses (dry matter loss) are not properly developed. It is very difficult to find scientific documentation results on wet distillers grains deterioration, thus creating a huge gap in the knowledge base. A standard on WDGS deterioration is unavailable to guide livestock safe feeding limit. Deteriorated WDGS poses a threat to livestock as it can result in off-flavors, toxins, discoloration, rotting and formation of pathogenic or allergenic

propagules ([Chelkowski, 1991], [Bigelis, 1992], [Gravesen et al., 1994], [Tipples, 1995] and [Filtenborg et al., 1996]).

Predicting and retarding wet distillers grains deterioration will enable efficient utilization and thus avoiding wastage and allowing profit maximization for ethanol producers since extra cost of drying will be reduced. In addition, research on wet distillers grains DML will lead to an acceptable standard which will serve an informative tool for predicting storage losses and a measure against deterioration. Quantification of wet distillers grains dry matter loss is therefore crucial.

Objectives

The objectives of this study were:

1. To quantify deterioration (dry matter loss, as estimated by carbon dioxide evolution) in MWDGS during storage as a function of preservative (CakeGuard: propionic acid-based) treatment and temperature.
2. To evaluate the efficacy of the commercial product on MWDGS dry matter loss.

Materials and Methods

Overview

An experiment was conducted to determine the effect of CakeGuard (Alltech® Inc., USA) preservative and temperature on MWDGS dry matter loss during storage. 1 mL of diluted preservative was applied to 50-g samples. Treated and untreated samples were placed in autoclaved glass columns and aerated for a period of 21 days in environmental chambers set at 10^{o}C, 20^{o}C, and 30^{o}C. Storage temperatures were chosen to mimic environmental

conditions. The experiment was designed as a two-way factorial treatment structure with repetitions considered as blocks. Details on the procedures used follow:

Sample collection

A 50-kg MWDGS lot was obtained from the Lincolnway Energy ethanol plant west of Nevada, Iowa on March 3rd, 2011 as it was released from the dryer. The sample was sealed in a plastic air-tight tub and transported about 10 km (6.1 miles) back to Iowa State University and stored at 5^{o}C until tested.

Moisture content determination

Moisture content was determined using 5 g of fresh MWDGS, air-dried in triplicate for 24 h in an oven set at 60^{o}C (Wilken et al., 2008). The initial MWDGS moisture content was 41% wet basis (w.b.).

Material preparation

In this study, 15 kg of MWDGS from the 50 kg sample collected was uniformly mixed in a covered cement mixer for 15 minutes. The mixed sample was divided into eighteen 50 g sub samples and each randomly assigned to preservative treatments, 0% and 0.1% respectively. and environmental chambers set at 10^{o}C, 20^{o}C and 30^{o}C. The environmental chambers were also randomly assigned to temperature. Two preservative treatments; untreated MWDGS (control) and preservative treated MWDGS all in triplicate were used.

The manufacturers recommended dosage for preservative application was 1 kg preservative per Mg (1 Mg = 1 metric ton = 1 tonne = 1000 kg) of wet distillers grains

(Alltech® Inc., USA), to achieve 0.1% (w/w) treatment level. In our case, 50 g of MWDGS was used therefore the preservative was diluted with distilled water in the ratio of 1:50. Treated samples were prepared by evenly applying 1 ml of diluted preservative using a pipette to the individual 50 g samples. After each preservative application, the mixture was stirred immediately and thoroughly by hand for three minutes sequentially and covered to prevent moisture loss. The same amount of distilled water was added to the untreated MWDGS samples and mixed thoroughly.

Experimental setup

Sample storage and aeration

The prepared samples were carefully placed in individual glass columns 1 m in length with internal and external diameter of 47.2 and 54.2 mm, respectively. Two pieces of plastic spongy kitchen scrubber (Re-think It, Inc., FL, USA) (Figure 3.2) were placed between samples in each glass column to aid aeration and prevent clogging which could restrict airflow.

Figure 3.2. Spongy kitchen scrubber placed between MWDGS samples in glass column.

The glass columns were tightly closed with stoppers, sealed with duct tape, and mounted vertically in the 10°C, 20°C and 30°C environmental chambers (Model I-35-L, Percival Scientific, Inc., Boone, IA, USA) (Figure 3.3).

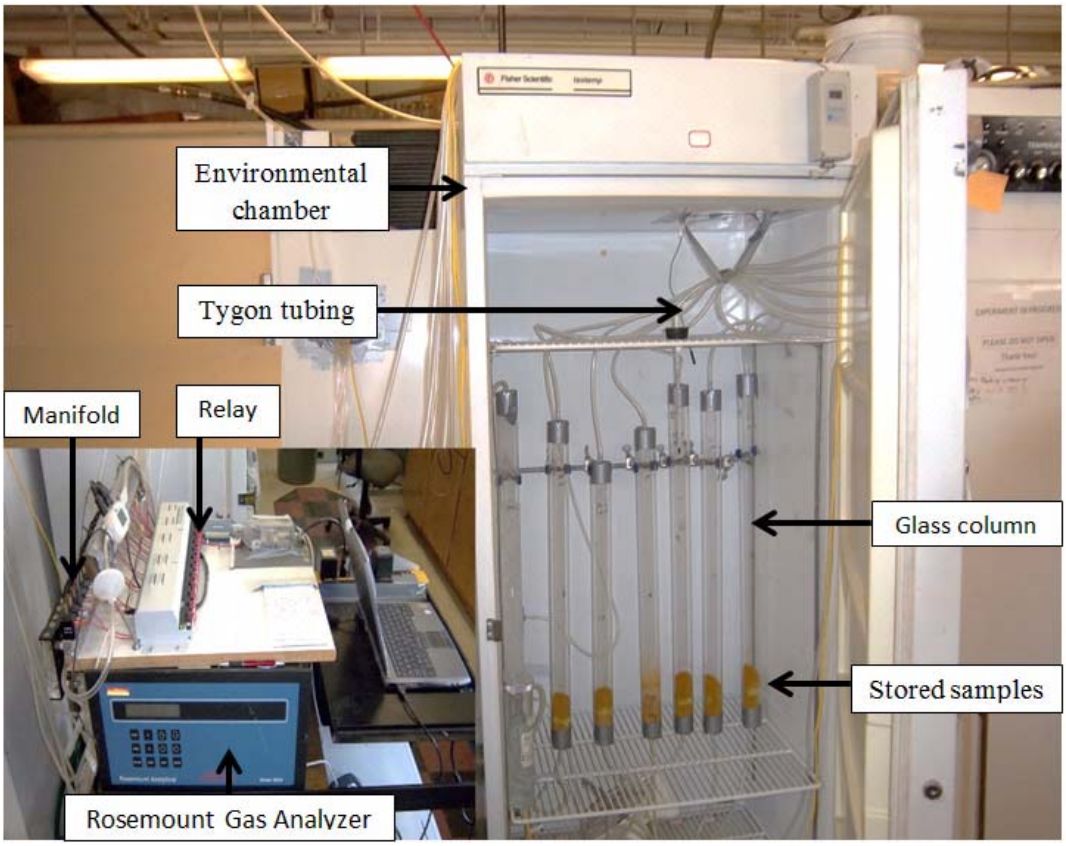

Figure 3.3. Environmental chamber showing the arrangement of the glass columns containing modified wet distillers grains with solubles.

Tygon tubing (Saint-Gobain Performance Plastics Corporation, Akron, OH, USA), 6.35 mm internal and 9.53 mm external diameter (Figure 3.3) was connected to the inlet and outlet ports made in the stoppers at both ends of each glass columns. Two Gast models, DOA-P135-AA and ROA-P151-AA oil-less diaphragm pump (Gast Manufacturing Inc.,

Benton Harbor, MI, USA) provided airflow distribution via the glass columns at a target rate of 0.47 Std L min^{-1} (9.4 m^3 min^{-1} m^{-3} of MWDGS, and 11.7 cfm bu^{-1} by volume of MWDGS). Standard conditions were defined as 21.1^{o}C (70^{o}F) and 101.3 kPa (14.7 psia, 1 bar). Airflow rates were controlled by valves connected to PM-1000 flowmeters (Matheson Instruments, Montgomeryville, PA, USA).

The accuracy of airflow rates through the flowmeters were monitored by a TSI 4100 Series High Performance Linear OEM Electronic Flowmeter (TSI Inc., Shoreview, MN, USA). The exiting air from the samples was distributed and directed to a non-dispersive infrared gas analyzer using a single station, bar manifold (Model CAT 335-000-N-G, Ingersoll Rand, Bryan, OH, USA) assembled as stack. Each manifold has a three-way solenoid valve.

Humidification and temperature

Humidification, at 100% is necessary to condition the stored samples at constant moisture content and temperature. Therefore, ambient air was drawn and bubbled through a glass column filled with distilled water connected in series to the samples. While there is no standard data on equilibrium moisture content for wet distillers grains above 40% moisture, the equilibrium moisture content for dried distillers grains with 10 to 25% solubles ranged from 23.1% w.b. to 39.8% w.b. for equilibrium relative humidities of 90% for all temperatures used in this study (ASABE, 2007).

An HIH-4000-001 (Honeywell Inc., 1998-2004) relative humidity sensor and LM 35 (National Semiconductor Corporation, USA) temperature sensors were used to monitor the

desired levels of relative humidity and temperature respectively of air passing through the samples in each environmental chamber.

Carbon dioxide production and measurement

Carbon dioxide (CO_2) and water produced within MWDGS samples during storage as a result of microbial respiration were carried out by the air passing through each glass column. The water was allowed to condense before reaching both flowmeters and manifolds. One empty column was used to measure the ambient CO_2 to correct for CO_2 produced in the samples. The system was checked regularly for air leaks. Figure 3.4 shows the complete experimental setup of the carbon dioxide monitoring system.

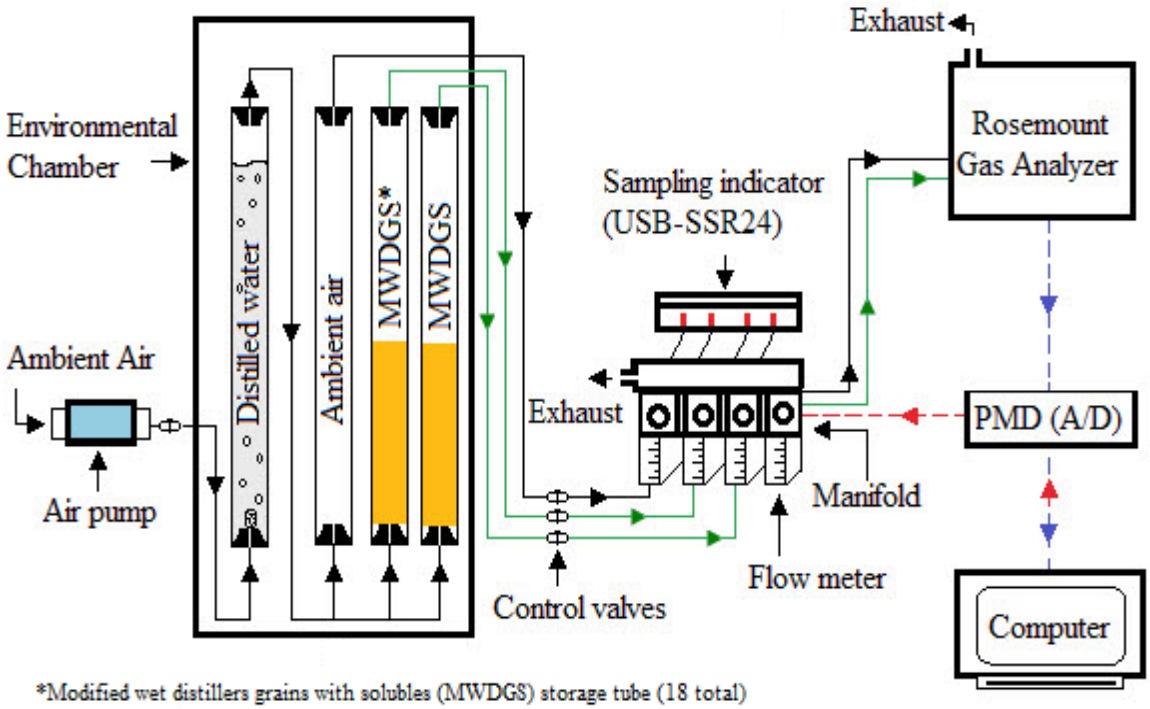

Figure 3.4. Schematic diagram of carbon dioxide evolution monitoring system.

A Visual Basic (VB) program with a Microsoft Access (Microsoft Corporation, Redmond, WA, USA) interface was used to control the manifolds sequentially at 5-min intervals. This allowed enough time to purge the manifold and gas analyzer lines prior to each measurement.

Air directed by the manifolds from each column entered a non-dispersive infrared gas analyzer (Rosemount Analytical Model 880A, Emerson Process Management, Orville, OH, USA) with ±1% full scale (±15 ppm) precision which analyzed and measured CO_2 in the air exiting the stored samples. The non-dispersive infrared gas analyzer was calibrated according to the manufacturer's specification for each experimental repetition. Light-emitting diode (LED) indicators on a high-drive, full speed, solid state relay (SSR) board, USB-SSR24 (Measurement Computing™) allowed visual observation of the state of the relays therefore showed which specific glass column and corresponding manifold port sampled at a particular time.

Data collection and dry matter loss calculation

After sampling a particular glass column, the concentration of CO_2 in the evolving air was recorded correspondingly with the glass column description, date, time, temperature and relative humidity. Analog data signals read from the CO_2, temperature, relative humidity and airflow measuring devices were converted to digital signal using an analog-to-digital (AD) signal converter PMD; Personal Measuring Device (PMD) (Measurement Computing™).

Dry matter loss was computed using the averaged corrected CO_2 evolved from the stored samples after 21 days. Percent cumulative DML was computed based on the premise

that; overall biological degradation was by glucose oxidation (Bern et al., 2002). The stoichiometric relationship is expressed as:

$$C_6H_{12}O_6 + 6O_2 \longrightarrow CO_2 + 6H_2O + 2835 \text{ kJ mol}^{-1}$$

The equation used to convert measured CO_2 to DML is shown below:

Equation 1. Conversion of evolved CO_2 into percent cumulative dry matter loss (DML).

% Cumulative DML =

$$\left[\sum_{i-1}^{n}\left\{CO_2 \times {Q}/{10^6} \times {P}/{(R \times T)} \times MW_{CO_2} \times (t_i - t_{i-1})\right\}\right] \times \left({MW_{glucose}}/{(6 \times MW_{CO_2})}\right) \times {1}/{DM_{sample}} \times 100$$

where

$\sum_{i-1}^{n}$ = cumulative summation of the parameters in the bracket ({ })

n = number of independent measurements

CO_2 = carbon dioxide evolved, ppm

Q = normalized airflow rate to a target of 0.47, Std L min^{-1} (example; if 0.55 Std L min^{-1} produces 400 ppm CO_2, then 0.47 Std L min^{-1} will produce 468 ppm CO_2)

P = absolute pressure of gas, 1 atm

R = universal gas constant, 0.0821 L atm K^{-1} mol^{-1}

T = Temperature at which sample was stored, K

MW_{CO2} = molecular weight of CO_2, 44 g mol^{-1}

t_i = time of i-th CO_2 measurement, min; i=1, 2, ……..,n,……

t_{i-1} = time prior to i-th measurement, min; i=1, 2, ……..,n,……

$MW_{glucose}$ = molecular weight of glucose ($C_6H_{12}O_6$), 180 g mol^{-1}

DM_{sample} = dry matter of sample used, g

Statistical analysis

A two-way factorial ANOVA using the PROC MIXED procedure of Statistical Analysis Systems (SAS for Windows, version 9.2, SAS Inst., Cary, NC, USA) was used to determine the significance of preservative treatment and temperature main effects on DML. Lsmeans statement in PROC MIXED procedure was used to calculate the means of main effects and potential interactions. Tukey post-hoc test was used for group comparison. The main and interaction effects were determined at $\propto \leq 0.05$.

Results

The effects of two-level preservative treatment; 0 and 0.1% (w/w) at 10^{o}C, 20^{o}C and 30^{o}C on MWDGS DML after three repetitions for 21 days storage period are shown in Figure 3.5, below. The results indicated a highly significant main effect of preservative on DML (p-value = 0.0015). The main effect of temperature treatment on DML was highly significant (p-value = 0.0003). There was significant interaction effect (Figure 3.5) observed between preservative and temperature treatment (p-value = 0.0356).

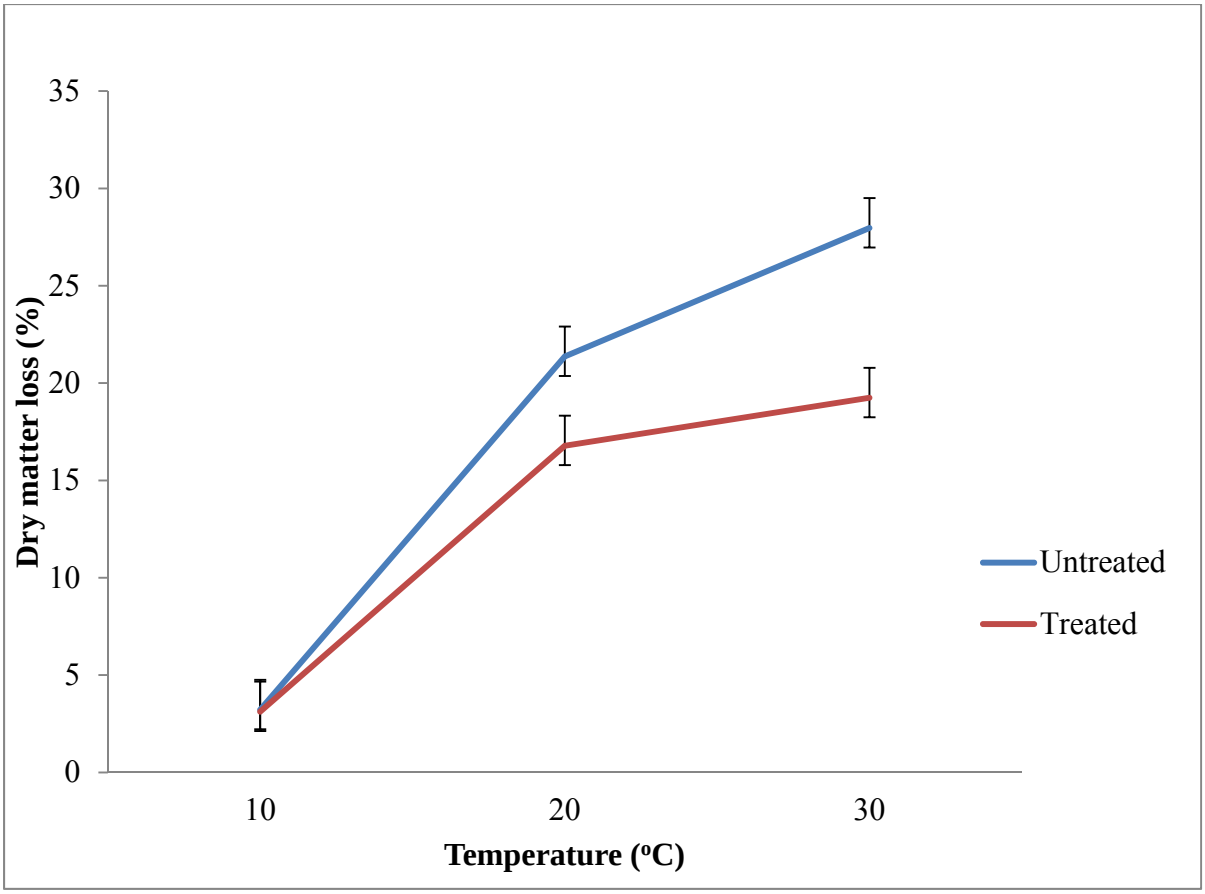

Figure 3.5. Effects of temperature and preservative treatment interaction on modified wet distillers grains with solubles treated with 0.1% (w/w) CakeGuard preservative at 10°C, 20°C and 30°C.

There was no statistically significant DML difference between preservative treatments at 10°C (p-value = 0.9678). Treatment with preservative significantly reduced DML at 20°C (p-value = 0.0496) moreover at 30°C preservative effect on DML reduction was highly significant (p-value = 0.0004). Comparison of the least square means of significant effects on percent DML are summarized in Table 3.1.

Table 3.1. Percent dry matter loss in CakeGuard preservative treated and untreated modified wet distillers grains with solubles after 21 days of storage.

		Preservative Level		
		Untreated (0% w/w)	**Treated (0.1% w/w)**	**Mean**
Temperature (^{o}C)	**10**	3.22^{d}	3.12^{d}	3.17
	20	21.4^{b}	16.8^{c}	19.1
	30	28.0^{a}	19.3^{bc}	23.7
Mean		17.5	13.1	

$^{a-e}$Data with different letter are significantly different (Tukey; $\propto \leq 0.05$)

The effects of preservative treatment and temperature on DML for each experimental repetition are illustrated in Figure 3.6. DML values for each repetition (rep) are averages of three samples stored for 21 days.

For the purpose of descriptive analysis, we monitored the trend of DML (Figure 3.7) over time using the averaged cumulative DML after the three experimental repetitions. The trend of DML shows the variation of DML accumulation during storage. The time series reveals a consistent decrease in preservative treated MWDGS dry matter loss at 20^{o}C and 30^{o}C.

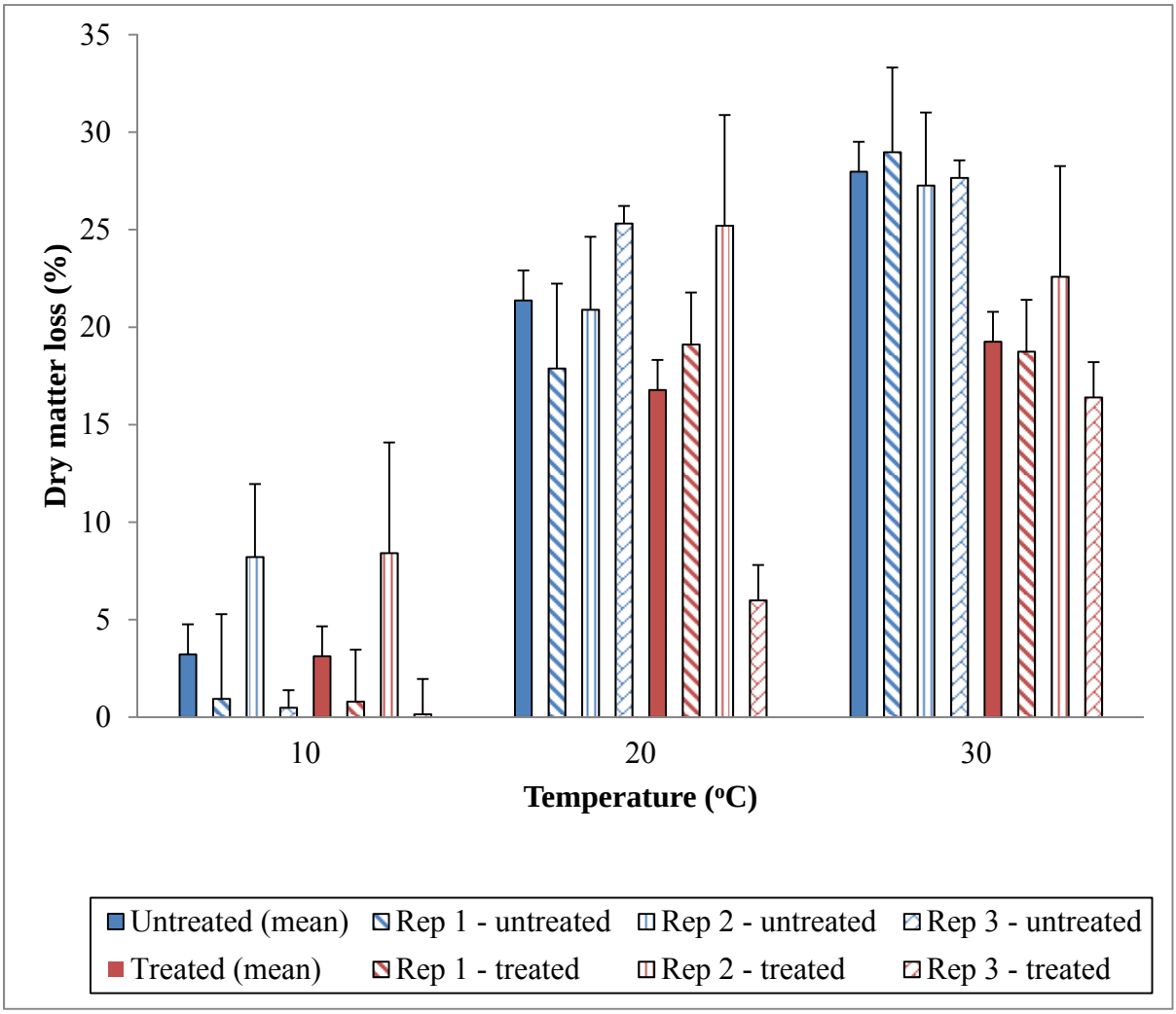

Figure 3.6. Effects of preservative and temperature on modified wet distillers grains with solubles dry matter loss (DML) for 21 days with DML for each repetition and mean DML for the repetitions.

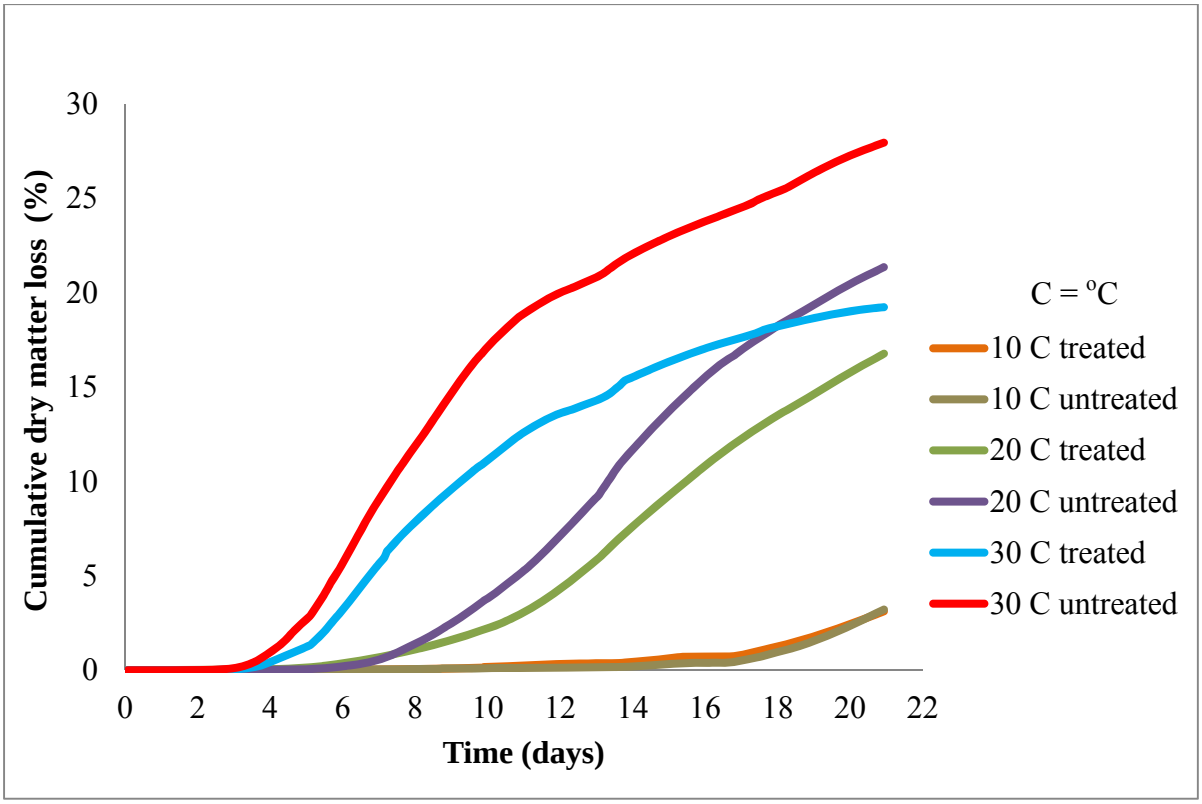

Figure 3.7. Dry matter loss rates in untreated and preservative treated stored modified wet distillers grains with solubles as a function of temperature for 21 days.

Discussion

The aim of this study was to quantify DML in MWDGS and to determine the effects of preservative and temperature treatments on DML during storage for 21 days. One minor problem occurred during the repetition of the experiment. One of the environmental chambers failed after the second repetition and needed replacement before the third repetition. The two-way factorial allowed us to analyze the differences in the means of absolute values for the data collected despite the unfortunate and unexpected circumstance.

The rapid rate of deterioration of wet distillers grains is the most critical issue with regards to storage life and it is often undesirable due to quantity and quality lost. DML occurs as a direct result of microbial activity. The presence of residual nutrients and high moisture content of the wet feed create an environment which favors growth of spoilage microorganisms. DML at the end of the 21 days storage was highly influenced by preservative and temperature treatment especially at 30^{o}C.

There was consistent increase in DML with increasing temperature (Figure 3.5) irrespective of preservative treatment. At 10^{o}C, 20^{o}C and 30^{o}C; DML loss with CakeGuard (propionic acid-based) preservative treatment observed were 3.12%, 16.8%, 19.3% and DML for untreated MWDGS were 3.22%, 21.4% and 28.0% respectively from original 29 g dry matter sample. Temperature effect on DML was significant at all levels except at preservative treatment at 20^{o}C and 30^{o}C (Table 3.1.). The data illustrates that the lowest DML occurred at 10^{o}C. This is expected because low temperatures slow down microbial metabolism whereas high temperatures facilitate rapid metabolism as evident at 30^{o}C. According to Al-Yahya (1996), low temperature have very little effect on grain storability, whereas higher temperature increases grain rate deterioration.

Considering the impact of deterioration on wet distillers grains by seasonal variation, this study shows that DML during hot weather season can occur very rapidly and in significant amounts compared to cold weather seasons (Figure 3.5). However preservative application can appreciably reduce the quantity of DML when stored for 21 days. From Figure 3.7, it can be estimated that the amount of DML in three weeks at 10^{o}C will occur in less than one week when stored at 30^{o}C. Storage strategies should therefore aim at preventing exposure of wet distillers grains to aerobic conditions in hot weather seasons, as the presence

of air which favors aerobic decomposers will hasten the rate of DML. It is however important to note that, inconsistencies of wet distillers grains such as initial quality of corn kernels, compositional variation, processing methods, storage conditions, biological factors, prevailing atmospheric conditions and time of the year could cause a general departure pattern in DML.

From the results (Table 3.1.), it is evident that more DML occurred in the untreated samples compared to the preservative treated samples at all temperature levels. On the contrary, Nofsinger et al. (1983) observed that propionic acid favored more DML when wet distillers grains were treated at a rate of 0.5%, 0.25% and 1% (w/w) than untreated wet distillers grains stored at 30^{o}C. The inhibitory effect of CakeGuard preservative against the growth of spoilage microorganisms maybe due to the chemical composition (a combination of propionic acid, sodium benzoate and potassium sorbate), therefore has a higher potency in reducing DML compared to propionic acid effect as observed by Nofsinger et al. (1983).

In our study, even though the rate of DML (Figure 3.7) accelerated rapidly in less than a week at 30^{o}C and in about a week at 20^{o}C, the quantitative differences in DML at these temperature levels undoubtedly was due to the inhibitory effect of the preservative used. The low recommended rate of the preservative application (0.1% w/w) seems effective in reducing storage losses at ambient temperatures. Nevertheless interaction effect observed indicates that preservative and temperature have a synergetic relationship on DML. This interdependency shows that preservative treatment will perform differently at different temperature treatments levels and vice versa

Visual observations on mold formation were made during storage. Mold colonies appeared within an average of five days on both preservative treated and untreated samples at

30°C. At 20°C mold colonies began forming seven days on untreated samples and 10 days in treated samples respectively. At 21 days, there was no obvious mold growth on preservative treated sample at 10°C and a few observed on untreated samples. Lyberg et al. (2008), studied the biochemical and microbial properties on cereals mix fermented with whey and wet wheat distillers grains and found that higher temperatures favor microbial growth. Different microbial coloration was visually observed between preservative treated and untreated samples during the period of monitoring CO_2 evolution. These could possibly be due to different types of spoilage microbes.

At the end of the storage period, the preservative treated MWDGS showed considerable reduction in molds and were moderately stabilized (less clumps) than the untreated samples at 20 and 30°C whiles at 10°C both preservative treated and untreated samples appeared completely undegraded. According to Uslu et al. (2009), propionic acid at levels between 0.1% and 1% (w/w) inhibits mold growth. Geetha et al. (2009), treated barley distillers grains with 0%, 0.5%, 1.0%, 1.5% and 2.5% (w/v) propionic acid and found that mold count was significantly lower at both 2% and 2.5% percent treatment level but they fail to report storage losses. Visual observation of preservative treatment may prove deceptive as one might think that, reduction of mold due to treatment could necessarily lead to significant retardation of DML.

The final averaged moisture content in the samples was found to increase at 10°C and 20°C but decreased slightly at 30°C. The increased moisture content apparently was due to either microbial activities or aerating with 100% relative humidity. From Figure 3.6, DML during the second repetition was higher than both first and third repetitions. The differences may be caused by some accidental error during the experiment. The variation in moisture

content (42% w.b. in this study) of wet distillers grains which can reach as high as 70% w.b. before they are sold, unsurprisingly may result in even higher DML within a shorter time under the storage conditions investigated. In addition to these specific findings, it is important to know that storage temperature may influence the action of preservatives (weak acids). It was reported that ambient storage temperature decreases acid survival more than storage at cold temperatures ([Skandamis and Nychas, 2000] and [Beales, 2004]). This could be a reason for the high DML; 16.8% and 19.3% of samples treated with preservative at 20 and 30^{o}C respectively.

Future research

Additional testing and analysis of numerous loads of wet distillers grains DML during storage is needed to allow for precise estimation (using mathematical models) of the mean values and DML estimates. Studies have shown that the effectiveness of propionic acid-based preservatives increases with higher application rates ([Kung Jr et al., 1998], [Kung Jr et al., 2000] and [Kung Jr, 2010]), therefore it is recommended to study the effect of higher application rates of CakeGuard preservative on wet distillers grains DML beyond 21 days. Furthermore, the effect of including solubles on wet distillers grains DML and factors affecting aerobic stability such as microbial ecology should be studied.

Conclusions

From this research, we found significant differences in DML between preservative-treated and untreated MWDGS at 20^{o}C and 30^{o}C. The effect of temperature on DML was

also significant. Although preservative treatment was not significant at 10^{o}C, it recorded the lowest DML whereas the highest dry matter losses occurred at 30^{o}C. Storage at 10^{o}C appeared to have extended MWDGS storage life for 21 days. It might be reasonable to suggest the feasibility of cold storage method over other methods however; special reference to the economic feasibility is needed. The results suggest that, treating MWDGS with preservative during winter seasons will not be useful. However, the observation of appreciable performance of preservative treatment at 20^{o}C and 30^{o}C informs that using CakeGuard preservative during summer months will reduce storage losses considerably.

Finally, it may be difficult to apply the results obtained on a larger scope to predict future DML occurrences because of potential variations in wet distillers grains from plant to plant. However, the findings in this study have established that CakeGuard preservative is effective in reducing DML and overall, visual observation showed that the preservative is effective in stabilizing, conditioning and retarding mold growth. Therefore a rational response to the concerns of wet distillers grains storage losses can be addressed using CakeGuard preservative for 21 days.

Acknowledgement

We are grateful for the partial financial support from the USDA Higher Education Challenge Grant Award #2006-38411-17034.

CHAPTER 4. GENERAL CONCLUSIONS

In this study, the storage temperature and preservative use both significantly impacted dry matter loss from modified wet distillers grains with solubles (MWDGS) (p-value = 0.0003 and p-value = 0.0015 respectively). The effect of preservative on DML at 10^{o}C was not significant (p-value = 0.9678), whereas the effect of preservative on DML at 20^{o}C and 30^{o}C were highly significant (p-value = 0.0496 and p-value = 0.0004 respectively). Temperature effect on DML was also significant. Interaction effect of preservative and temperature treatment on DML was significant. The DML observed in MWDGS samples after 21 days storage with preservative treatment at 10^{o}C, 20^{o}C and 30^{o}C were 3.12%, 16.8% and 19.3% respectively. Untreated samples at 10^{o}C, 20^{o}C and 30^{o}C resulted in a DML of 3.22%, 21.4% and 28.0% respectively.

As expected, storage temperature forms one of the most important parameters that influence deterioration. Losses of the magnitudes observed under ambient temperatures imitated, indicates the need for exclusion of air during storage to prevent aerobic conditions which could boost the rate of DML. The encouraging performance of preservative on storage losses retardation at moderate (20^{o}C) and high (30^{o}C) temperature compared to preservative treatment at 10^{o}C makes suitable and useful for preserving wet distillers grains in hot weather conditions. These findings are vital to both the ethanol industry and farm operations as the most storage losses occur during warm weather conditions and very rapidly.

Furthermore, for DML reaching 3% in MWDGS (42% m.c. used in this study) at 10^{o}C and more than 15% at 20^{o}C and 30^{o}C in 21 days raises concern about how much DML that can occur within the shortest time frame in wet feeds. Moisture content of wet distillers

grains sometimes can reach as high as 70% w.b. therefore higher levels of DML may occur at this moisture content level during storage especially in warm weather seasons. The effect of solubles added to wet distillers grains could be a potential promoter of deterioration. This should be checked in future studies as it may be the case.

Wet distillers grains; a kind of feed which is becoming the most prominent and immediately promising livestock feed constitute a major revenue stream of an ethanol plant, which is also affordable for farm operations because of lower cost compared to dried distillers grains. It is therefore crucial that further studies be directed towards understanding the mechanism of deterioration and factors such as nutrient profile, pH and microbial ecology and how they influence deterioration. Based on the results from this experiment, continued investigation of varying CakeGuard preservative application rates which may prove to be highly beneficial in extending the storage time of these invaluable feeds over 21 days is recommended.

RECOMMENDATIONS FOR FUTURE RESEARCH

Future studies should be centered on expanding knowledge on factors that promote rapid deterioration in wet distillers grains and possible ways to avert this issue. The following investigations are crucial to wet distillers grains storage and utilization:

1. Direct DML method should be used and compared with CO_2 method.
2. DML of numerous wet distillers grains samples should be collected and analyzed over time with more replications. This will allow for estimation of the mean values and also the variation of DML estimates.
3. Future studies should focus on the effects on wet distillers grains deterioration with and without solubles.
4. Different preservative treatment levels should be studied on wet distillers grains DML.
5. Elucidation of microbial culture during storage and toxicity levels is needed to determine unsafe feeding limits of deteriorated wet distillers grains.
6. Effect of CakeGuard-treated wet distillers grains on livestock dry matter intake, gain, milk yield and, overall performance needs to be researched.
7. A higher capacity Rosemount Infrared Gas Analyzer should be provided to measure a wider range of CO_2 evolution during future studies.

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APPENDIX A. STATISTICAL RESULTS

A. SAS CODES

NB: Repetitions are considered as blocks.

```
Data DML;
Input Rep Temp Trt $ DML;
Datalines;
```

1	10	U	0.88
1	10	U	1.08
1	10	U	0.86
1	10	T	0.97
1	10	T	0.63
1	10	T	0.80
1	20	U	17.80
1	20	U	18.10
1	20	U	17.76
1	20	T	20.55
1	20	T	19.44
1	20	T	17.39
1	30	U	28.96
1	30	U	28.98
1	30	U	28.99
1	30	T	9.51
1	30	T	20.15
1	30	T	26.58
2	10	U	10.94
2	10	U	8.48
2	10	U	5.24
2	10	T	2.42
2	10	T	13.05
2	10	T	9.79
2	20	U	22.79
2	20	U	19.82
2	20	U	20.09
2	20	T	24.59
2	20	T	25.42
2	20	T	25.62
2	30	U	28.44
2	30	U	25.51
2	30	U	27.87

2	30	T	21.48
2	30	T	18.79
2	30	T	27.50
3	10	U	0.26
3	10	U	0.27
3	10	U	0.94
3	10	T	0.34
3	10	T	0.05
3	10	T	0.07
3	20	U	24.99
3	20	U	25.97
3	20	U	25.01
3	20	T	9.63
3	20	T	5.60
3	20	T	2.77
3	30	U	27.88
3	30	U	27.01
3	30	U	28.10
3	30	T	9.10
3	30	T	25.35
3	30	T	14.75

```
run;

Proc Mixed data=DML method=type3;
class rep temp trt;
model DML = rep temp trt trt*temp/outp=predicted residual;
random rep*temp;
lsmeans trt*temp temp trt/adjust=Tukey pdiff;
run;

proc plot data=predicted;
plot studentresid*pred;
run;

proc univariate data=predicted normal plot;
var resid;
run;
```

B. SAS OUTPUT

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The Mixed Procedure

Model Information

Data Set	WORK.DML
Dependent Variable	DML
Covariance Structure	Variance Components
Estimation Method	Type 3
Residual Variance Method	Factor
Fixed Effects SE Method	Model-Based
Degrees of Freedom Method	Containment

Class Level Information

Class	Levels	Values
Rep	3	1 2 3
Temp	3	10 20 30
Trt	2	T U

Dimensions

Covariance Parameters	2
Columns in X	15
Columns in Z	9
Subjects	1

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The Mixed Procedure

Dimensions

Max Obs Per Subject	54

Number of Observations

Number of Observations Read	54
Number of Observations Used	54
Number of Observations Not Used	0

Type 3 Analysis of Variance

Source	DF	Sum of Squares	Mean Square	Expected Mean Square	Error Term
Rep	2	355.099744	177.549872	Var(Residual) + 6 Var(Rep*Temp) + Q(Rep)	MS(Rep*Temp)
Temp	2	4147.155544	2073.577772	Var(Residual) + 6 Var(Rep*Temp) + Q(Temp,Temp*Trt)	MS(Rep*Temp)
Trt	1	269.697452	269.697452	Var(Residual) + Q(Trt,Temp*Trt)	MS(Residual)
Temp*Trt	2	167.802115	83.901057	Var(Residual) + Q(Temp*Trt)	MS(Residual)
Rep*Temp	4	77.146344	19.286586	Var(Residual) + 6 Var(Rep*Temp)	MS(Residual)
Residual	42	974.925533	23.212513	Var(Residual)	.

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The Mixed Procedure

Type 3 Analysis of Variance

Source	Error DF	F Value	Pr > F
Rep	4	9.21	0.0319
Temp	4	107.51	0.0003
Trt	42	11.62	0.0015
Temp*Trt	42	3.61	0.0356

Rep*Temp	42	0.83	0.5132
Residual	.	.	.

Covariance Parameter Estimates

Cov Parm	Estimate
Rep*Temp	-0.6543
Residual	23.2125

Fit Statistics

-2 Res Log Likelihood	292.3
AIC (smaller is better)	296.3

The Mixed Procedure

Fit Statistics

AICC (smaller is better)	296.6
BIC (smaller is better)	296.7

Type 3 Tests of Fixed Effects

Effect	Num DF	Den DF	F Value	Pr > F
Rep	2	4	9.21	0.0319
Temp	2	4	107.51	0.0003
Trt	1	42	11.62	0.0015
Temp*Trt	2	42	3.61	0.0356

Least Squares Means

Effect	Trt	Temp	Estimate	Standard Error	DF	t Value	Pr > |t|
Temp*Trt	T	10	3.1244	1.5366	42	2.03	0.0484
Temp*Trt	U	10	3.2167	1.5366	42	2.09	0.0424
Temp*Trt	T	20	16.7789	1.5366	42	10.92	<.0001
Temp*Trt	U	20	21.3700	1.5366	42	13.91	<.0001

The Mixed Procedure

Least Squares Means

Effect	Trt	Temp	Estimate	Standard Error	DF	t Value	Pr > |t|
Temp*Trt	T	30	19.2456	1.5366	42	12.52	<.0001
Temp*Trt	U	30	27.9711	1.5366	42	18.20	<.0001
Temp		10	3.1706	1.0351	4	3.06	0.0375
Temp		20	19.0744	1.0351	4	18.43	<.0001
Temp		30	23.6083	1.0351	4	22.81	<.0001
Trt	T		13.0496	0.8871	42	14.71	<.0001
Trt	U		17.5193	0.8871	42	19.75	<.0001

Differences of Least Squares Means

Effect	Trt	Temp	_Trt	_Temp	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
Temp*Trt	T	10	U	10	-0.09222	2.2712	42	-0.04	0.9678	Tukey-Kramer
Temp*Trt	T	10	T	20	-13.6544	2.1730	42	-6.28	<.0001	Tukey-Kramer
Temp*Trt	T	10	U	20	-18.2456	2.1730	42	-8.40	<.0001	Tukey-Kramer
Temp*Trt	T	10	T	30	-16.1211	2.1730	42	-7.42	<.0001	Tukey-Kramer
Temp*Trt	T	10	U	30	-24.8467	2.1730	42	-11.43	<.0001	Tukey-Kramer
Temp*Trt	U	10	T	20	-13.5622	2.1730	42	-6.24	<.0001	Tukey-Kramer
Temp*Trt	U	10	U	20	-18.1533	2.1730	42	-8.35	<.0001	Tukey-Kramer

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The Mixed Procedure

Differences of Least Squares Means

Effect	Trt	Temp	_Trt	_Temp	Estimate	Standard Error	DF	t Value	Pr > |t|	Adjustment
Temp*Trt	U	10	T	30	-16.0289	2.1730	42	-7.38	<.0001	Tukey-Kramer
Temp*Trt	U	10	U	30	-24.7544	2.1730	42	-11.39	<.0001	Tukey-Kramer
Temp*Trt	T	20	U	20	-4.5911	2.2712	42	-2.02	0.0496	Tukey-Kramer
Temp*Trt	T	20	T	30	-2.4667	2.1730	42	-1.14	0.2628	Tukey-Kramer
Temp*Trt	T	20	U	30	-11.1922	2.1730	42	-5.15	<.0001	Tukey-Kramer
Temp*Trt	U	20	T	30	2.1244	2.1730	42	0.98	0.3339	Tukey-Kramer
Temp*Trt	U	20	U	30	-6.6011	2.1730	42	-3.04	0.0041	Tukey-Kramer
Temp*Trt	T	30	U	30	-8.7256	2.2712	42	-3.84	0.0004	Tukey-Kramer
Temp		10		20	-15.9039	1.4639	4	-10.86	0.0004	Tukey
Temp		10		30	-20.4378	1.4639	4	-13.96	0.0002	Tukey
Temp		20		30	-4.5339	1.4639	4	-3.10	0.0363	Tukey
Trt	T		U		-4.4696	1.3113	42	-3.41	0.0015	Tukey-Kramer

Differences of Least Squares Means

Effect	Trt	Temp	_Trt	_Temp	Adj P
Temp*Trt	T	10	U	10	1.0000
Temp*Trt	T	10	T	20	<.0001
Temp*Trt	T	10	U	20	<.0001
Temp*Trt	T	10	T	30	<.0001

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The Mixed Procedure

Differences of Least Squares Means

Effect	Trt	Temp	_Trt	_Temp	Adj P
Temp*Trt	T	10	U	30	<.0001
Temp*Trt	U	10	T	20	<.0001
Temp*Trt	U	10	U	20	<.0001
Temp*Trt	U	10	T	30	<.0001
Temp*Trt	U	10	U	30	<.0001
Temp*Trt	T	20	U	20	0.3479
Temp*Trt	T	20	T	30	0.8639
Temp*Trt	T	20	U	30	<.0001
Temp*Trt	U	20	T	30	0.9225
Temp*Trt	U	20	U	30	0.0440
Temp*Trt	T	30	U	30	0.0051
Temp		10		20	0.0009
Temp		10		30	0.0003
Temp		20		30	0.0762
Trt	T		U		0.0015

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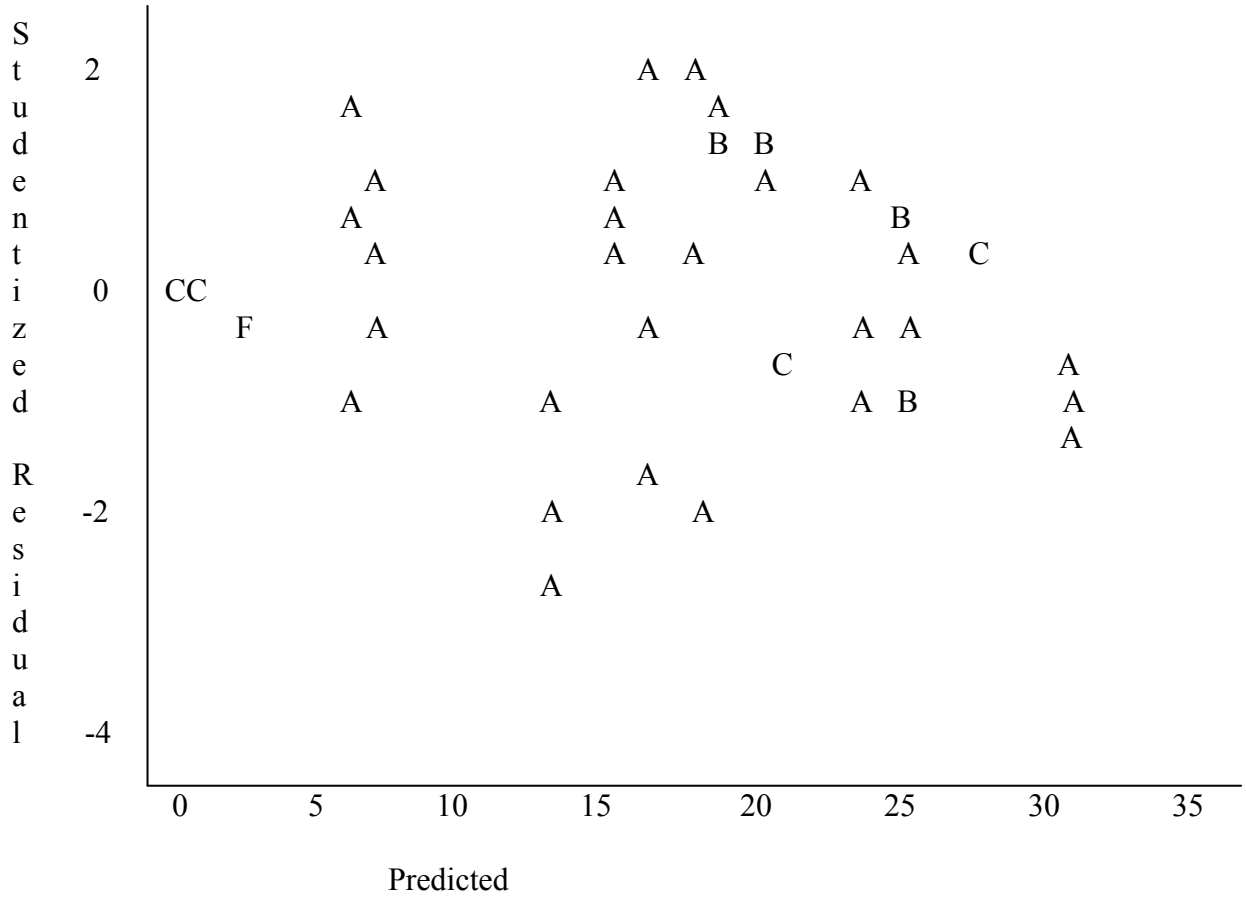
Plot of StudentResid*Pred. Legend: A = 1 obs, B = 2 obs, etc.



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The UNIVARIATE Procedure
Variable: Resid (Residual)

Moments

N	54	Sum Weights	54
Mean	0	Sum Observations	0
Std Deviation	4.52805905	Variance	20.5033188
Skewness	-0.1873664	Kurtosis	-0.2055179
Uncorrected SS	1086.6759	Corrected SS	1086.6759
Coeff Variation	.	Std Error Mean	0.61619079

Basic Statistical Measures

Location		Variability	
Mean	0.00000	Std Deviation	4.52806
Median	-0.44084	Variance	20.50332
Mode	.	Range	20.48709
		Interquartile Range	6.15651

The UNIVARIATE Procedure
Variable: Resid (Residual)

Tests for Location: Mu0=0

Test	-Statistic-		-----p Value------	
Student's t	t	0	Pr > |t|	1.0000
Sign	M	-3	Pr >= |M|	0.4966
Signed Rank	S	23.5	Pr >= |S|	0.8418

Tests for Normality

Test	--Statistic---		-----p Value------	
Shapiro-Wilk	W	0.984808	Pr < W	0.7224
Kolmogorov-Smirnov	D	0.078873	Pr > D	>0.1500
Cramer-von Mises	W-Sq	0.054674	Pr > W-Sq	>0.2500
Anderson-Darling	A-Sq	0.299945	Pr > A-Sq	>0.2500

Quantiles (Definition 5)

Quantile	Estimate
100% Max	8.92813
99%	8.92813

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The UNIVARIATE Procedure
Variable: Resid (Residual)

Quantiles (Definition 5)

Quantile	Estimate
95%	7.04993
90%	6.06993
75% Q3	3.52003
50% Median	-0.44084
25% Q1	-2.63647
10%	-4.93346
5%	-8.63489
1%	-11.55896
0% Min	-11.55896

Extreme Observations

------Lowest------		-----Highest-----	
Value	Obs	Value	Obs
-11.55896	48	6.08993	45
-8.72896	47	6.78003	23
-8.63489	16	7.04993	44
-7.32187	52	8.43511	18

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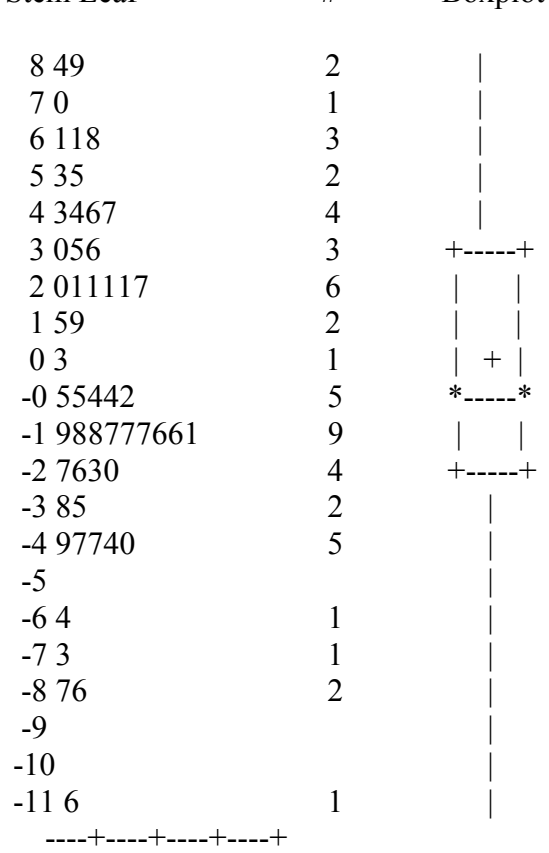
The UNIVARIATE Procedure
Variable: Resid (Residual)

Extreme Observations

------Lowest------		-----Highest-----	
Value	Obs	Value	Obs
-6.38547	32	8.92813	53

The UNIVARIATE Procedure
Variable: Resid (Residual)



Stem Leaf	#	Boxplot
8 49	2	
7 0	1	
6 118	3	
5 35	2	
4 3467	4	
3 056	3	
2 011117	6	
1 59	2	
0 3	1	
-0 55442	5	
-1 988777661	9	
-2 7630	4	
-3 85	2	
-4 97740	5	
-5		
-6 4	1	
-7 3	1	
-8 76	2	
-9		
-10		
-11 6	1	

----+----+----+----+

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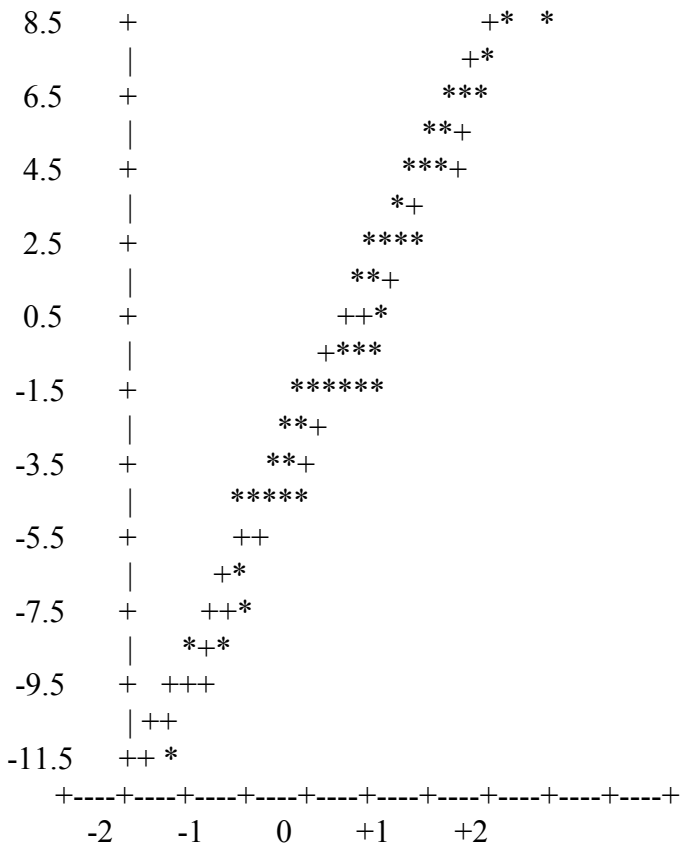
The UNIVARIATE Procedure
Variable: Resid (Residual)

Normal Probability Plot



APPENDIX B. MODIFIED WET DISTILLERS GRAINS WITH SOLUBLES DRY MATTER LOSS FOR 21 DAYS USING DIRECT DRY MATTER DETERMINATION METHOD

		Preservative Level		
		Untreated (0% w/w)	**Treated (0.1% w/w)**	**Mean**
Temperature (^{o}C)	**10**	4.90	4.66	4.78
	20	23.3	19.4	21.4
	30	31.7	21.9	26.8
Mean		20.0	15.3	

APPENDIX C. EXPERIMENTAL SETUP AND COMPONENTS

Figure B1. Diagram showing carbon dioxide measurement setup.

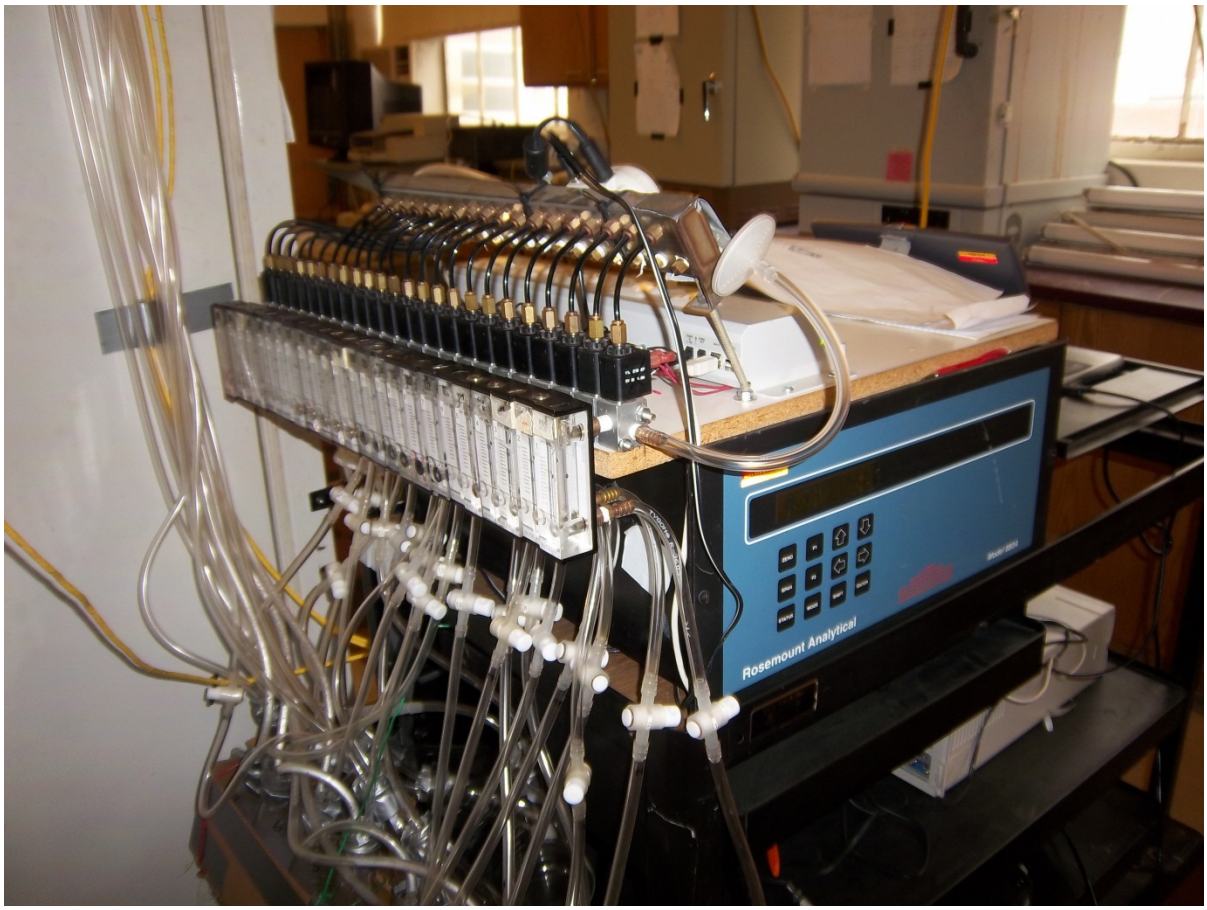


Figure B2. Rosemount gas analyzer, flow meter, stacks of manifolds and condenser components of carbon dioxide measurement setup.

APPENDIX D. VISUAL OBSERVATIONS MADE DURING MODIFIED WET DISTILLERS GRAINS STORAGE

NB: First three samples are preservative treated whereas the last three samples are untreated samples. Order of arrangement follows thought all the environmental chambers.

Figure C1. Day 0; samples stored at 10^{o}C.

Figure C2. Day 7: samples stored at 10^{o}C.

Figure C3. Day 14: samples stored at 10^{o}C.

Figure C4. Day 21: samples stored at 10^{o}C.

Figure C5. Day 0: samples stored at 20^{o}C.

Figure C6. Day 7: samples stored at 20^{o}C.

Figure C7. Day 14: samples stored at 20^{o}C.

Figure C8. Day 21: samples stored at 20^{o}C.

Figure C9. Day 0: samples stored at 30^{o}C.

Figure C10. Day 7: samples stored at 30^{o}C.

Figure C11. Day 14: samples stored at 30^{o}C.

Figure C12. Day 21: samples stored at 30^{o}C.

APPENDIX E. CARBON DIOXIDE SOFTWARE OPERATING INSTRUCTIONS

The carbon dioxide software is a computer program used in this study that controls the peripherals of the carbon dioxide monitoring, records and stores measured data. The program is coded in Visual Basic (VB) with Microsoft Access 2002 interface designed to automatically control 24 sample outputs automated manifolds (ports), record temperature, relative humidity and, airflow rate measurement. The program also controls a high-drive, full speed, solid state relay (SSR) board, USB-SSR24 (Measurement Computing™) incorporated in the setup to indicate which port is sampling at a particular time. Communication between the software and the peripherals is made possible by a PMD. The PMD converts analog signal to digital signal to enable data to be read and stored in the computer.

The following procedures should be followed prior to using the software on a new computer.

i. Install InstaCal software (Measurement Computing Corporation). This software assigns the USB-SSR24 and USB-1408-FS board numbers. The program will not run without InstaCal.

   USB-1408-FS should be assigned to board number 0 and configured to Single Ended and the Extension clock type set to continuous. USB-SSR24 assigned to board number 1. All other configurations in InstaCal are automatically configured.

ii. Run the software and enable macro before configuring and operating the it.

Software Interface

The software interface is divided into four major sections and subsections:

a. Tables

i. Channels

ii. Formulas

iii. Measures

iv. Ports

b. Queries

i. Data

c. Forms

i. Channels

ii. Data

iii. Formulas

iv. Main

v. Ports

vi. Settings

d. Modules

i. Constants

ii. Error Handling

iii. General

iv. PMDAux (Personal Measurement Device Auxiliary)

v. PMDLib (Personal Measurement Device Library)

vi. Record Handling

Channels

The channel comprises of the parameters being measured and their relative position (channels) on the PMD. The parameters are carbon dioxide (CO_2), airflow, temperature and, relative humidity. The channel shows the data output position on the PMD; channel name is where you input the name of the parameter measured and, channel formula column contains formula to calculate the value of each parameter. The columns can be expanded using "Click to add" button. All inputs can be edited according to the users' preference. Note however that the value 16384 (2^14) should be used with USB-1408-FS and 4096 (2^12) should be used for USB-1208 LS.

Formulas

This table or form is designed for the user to input dry matter loss, carbon dioxide equivalent formula and weight of material used. The user can choose to ignore this section if data analysis is to be done manually.

Measures

The measure table displays the sample identification (id), date and time sampling was completed, port number and all raw values measured.

Ports

The user can manage and select which port numbers measure by assigning names. To correct for ambient carbon dioxide, the control ports should be selected for all samples in a particular environmental chamber. Example assume port 1 is set to measure ambient CO_2 and port 2 to 5 are set to measure samples CO_2 evolution in a particular environmental chamber, then control for port number 1 to 5 should be set to 1. Individual ports intended for use must be selected.

Main

The main form is a user-friendly and interactive interface where the program gives feedback on measurements being made. The user has the opportunity to select sampling intervals based on hours, minutes and seconds. Autorefresh allows the program to automatically refresh and displays current measured and stored data. The user can choose to manually refresh the program (recommended). The user can start and stop the program anytime using the start and stop button. It is advisable not to select 'Simulate Measures' when the program is running.

The blue time bar indicates the sampling time remaining. This form also displays last sampled data of all parameters, port number, date and, time. Setting button takes the user to channel and port setting. Below these settings data collected are arranged from new to old. To delete any entry, highlight the particular cell and press delete on the keyboard.

Settings

This form takes the user to channel and port settings.

Queries

This form displays all measured data as can be seen on as can be seen on the main form.

Modules

Each module contains the program codes. The user should not attempt to modify them.

Parameter description on the main form

- Id: Identifies each measurement with a specific number in increasing order relative to recent measured data.
- Date/time: records the time and the date sampling of a particular port was completed.
- Port: displays port number
- CO_2: displays measured carbon dioxide from the Rosemount Infrared Analyzer in ppm (parts per million).
- Airflow: displays reading from the TSI airflow meter in Standard Liters per minutes (SLPM).
- Temperature: displays output of LM 35 temperature sensor in ^{o}C.
- Relative humidity: displays output of HIH 4000 relative humidity sensor in %
- Control: applies the ambient carbon dioxide port to each corresponding sample
- Sampling interval: the duration allowed for switching port and relays.
- Starting time: time a particular sampling time begins.
- Next sample on: time to begin measuring next sample.
- Last sample Data: display last sample readings.
- Start: Initiate the program to run.
- Stop: kills the program.

  After the end of data collection, stop and close the program or save the file. The data is stored in the file and can be accessed on any computer.

Troubleshooting

a. A/D device not found error message displayed

Solution:

1. Stop the program.
2. Completely turn off the power of the whole system. Turn power on again and allow enough time for each board (relay and PMD) to properly assign to channels in InstaCal.
3. Run the program.

b. Reading outputs are negative values

Solution:

1. Stop the program.
2. Turn off LPS 25 Series 25 Watts power supply and turn it on again or
3. Completely turn off the power of the whole system. Turn power on again and allow enough time for board assignment in InstaCal.
4. Run the program.

c. **No reading from port/ports**

Solution:

1. Check if the airflow rate is high enough to produce any reading.
2. Check if tubing is properly connected and there is no kink. If problem still exist go to solution 3 and 4.
3. Check for TSI 4100 Series High Performance Linear OEM Electronic Flowmeter (airflow meter) blockage. Avoid condensation or particles from reaching the

airflow meter. If blocked, either unscrew the top of the airflow meter and flush air through to remove particle or completely change the affected airflow meter.

4. If airflow meter is not blocked but there is no reading, then the automatic manifold (Model CAT 335-000-N-G, Ingersoll Rand, Bryan, OH, USA) air distribution circuitry has malfunctioned.

   Remove the relay connecting wires and open the top the manifold unit that malfunctioned. Remove and replace the black enclosure (conducting element). If problem still persists, remove and replace the bronze-colored tube in the unit holder.

d. **Avoid water or dust on any component of the setup**

NB: Complete program codes and description can be found in Del Campo's (2010) work on deterioration of corn cobs.