



# The effect of storage temperature and percentage of condensed distillers solubles on the shelf-life of distillers wet grains stored aerobically



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## ABSTRACT

Distillers wet grains with solubles (DWGS) are widely used as a supplemental protein source in North American cattle rations. However, due to its short shelf-life, its use is limited to feedlots within close proximity of an ethanol production plant. It is known that the shelf-life of DWGS in the summer diminishes drastically compared to in the winter. This effect is primarily due to temperature, which drives mold growth in high moisture feedstocks. The purpose of this study was to understand and quantify the effect of temperature (10, 20, and 25 °C) and condensed distillers solubles (CDS) levels [0%, 20% and 30%] on the shelf-life of DWGS under warm and cool aerobic storage conditions. Sample conditions which indicate shelf-life and product deterioration such as moisture content (m.c.), pH, fat acidity (FA), fungal growth and mycotoxin levels were measured in the DWGS samples before and after seven days of storage under three temperature levels (10, 20, and 25 °C). It was found that changes in temperature had the most significant effect on sample conditions ( $P < 0.05$ ). After seven days of storage, m.c. and water activity ( $a_w$ ) decreased with increase in temperature. In comparison, it was determined that FA, pH, fungal growth and mycotoxin levels increased with temperature after seven days of storage. Also, FA and CFU increased with increase in CDS level in DWGS. It was concluded that after seven days of storage at both warm and cool aerobic storage conditions, FA, aflatoxin, fumonisin and zearalenone levels increased. DWGS deteriorated less at 10 °C than at 20 °C and 25 °C, likewise less deterioration in 0% CDS than in 20% and 30% was noted.

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## 1. Introduction

Distillers Wet Grains with Solubles (DWGS), a co-product from the ethanol production dry grinding process, is a good source of energy, protein, vitamins, and minerals (Ham and Stock, 1994; Ganesan and Rosentrater, 2008). During the production of ethanol, once fermented beer is distilled, whole stillage containing the non-fermentable portions of corn grain is centrifuged to separate insoluble solids from liquids known as thin stillage. Thin stillage is further condensed by removing water using evaporators to syrup known as condensed distillers solubles (CDS) which has about 35–40% solids content (Kingsly et al., 2010). In 2012, 41% of distillers grains produced were sold as a wet commodity (RFA,

2013). As an animal feed, both wet and dried distillers grains, were utilized in beef cattle (48%) and dairy cattle (31%) feed rations (RFA, 2013). Although DWGS is a cheaper feed option when compared with distillers dried grains with solubles (DDGS), there are concerns with its very short shelf-life. The shelf-life is defined as the time during which the product will remain safe; be certain to retain desired sensory, chemical, physical and microbiological characters (IFST, 1993). DWGS cannot be stored longer than 3–4 days in warm weather (20 and 25 °C) and 5–7 days in cold weather (10 °C) (Johnson and Huber, 1987). Without proper storage and handling, DWGS can harbor multiple types of fungi, some of which produce mycotoxins and thus make it unsafe for use as a feed ingredient. Mycotoxins are secondary metabolites produced by fungi that can grow on corn kernels during storage and are toxic and carcinogenic (Shotwell, 1977; Cleveland et al., 2003). Common stored grain mycotoxins include aflatoxin, fumonisin and zearalenone; the FDA has set guidance levels for these in grain. Aflatoxin can be no more than 20 ppb in lactating dairy feeds and up to

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300 ppb for finishing (feedlot) beef cattle (FDA Compliance, 1979). Fumonisin levels must not exceed 60 ppm for ruminants less than 3 months old being raised for slaughter and 30 ppm for breeding ruminants (U.S. FDA, 2000). For zearalenone, no FDA guidance levels are available. There is a major limitation in feeding distillers grains wet because farmers do not have the means of preserving quality in storage beyond one week, thus increasing frequency of trucking it in and therefore delivery cost. Additionally, the short shelf-life of DWGS limits its market to feedlots that are close to ethanol plants and are able to frequently consume large amounts. For ethanol plants, eliminating drying of DWGS reduces production cost and thereby increases profits. Increasing the shelf-life of DWGS provides more feedlots with an option of feeding distillers grains wet and access to a higher quality low-cost feed in comparison to the dried product, DDGS. Additionally, saving from not drying DWGS would benefit fuel ethanol plants and improve the overall profitability of U.S. biofuels production from corn.

Distillers wet grains (DWG) are more susceptible to mold growth than whole corn kernels because the pericarp of the grain has been completely disrupted (Garcia et al., 2008). If used as a feed, mold contaminated grains can decrease the productivity and negatively affect the health of the animal (Garcia et al., 2008). There have been limited studies conducted that investigated the shelf-life of DWGS. A review of the literature revealed that extension manuscripts have given approximate shelf-life of DWGS in warm and cool weather and it appears that most of the shelf-life indicated was based on anecdotal evidence from farmers in the field or feeding trial experiments rather than well documented experiments (Lardy, 2003; Garcia et al., 2008). A recent study on the flowability of DDGS indicated that there is a significant interaction effect between CDS level and ambient drying temperatures for flow parameters (Bhadra et al., 2012). It was suspected that there might also be differences in the shelf-life of DWGS with different levels of solubles, which would change the nutrient profile and moisture content (m.c.) of DWGS and hence its shelf-life. Jay et al. (2005) states that range of water activity ( $a_w$ ) at which microbial growth occurs is greatest at optimum temperature for growth, and the presence of nutrients, such as CDS, increases the range of  $a_w$  for which the organism can thrive. It was expected that colony forming units (CFU), pH and fat acidity (FA) would indicate levels of deterioration and what changes are caused by deterioration in storage, while m.c. and  $a_w$  might explain differences among the sample treatments. Moisture and microbial analysis should correlate with the levels of CDS blended with DWG. The objective of this study was to understand the effect of storage temperature (warm and cool season weather) and percentage CDS inclusion on the shelf-life of DWG under aerobic storage conditions after seven days.

## 2. Materials and methods

In the area of food safety, shelf-life can be determined by various methods, one of which is referred to as the direct method. For this method a product is stored under predetermined conditions for a given period of time longer than the anticipated shelf-life. Evaluation of items such as  $a_w$ , pH and oxygen availability are used to indicate product related spoilage (NZFSA, 2005). Therefore, this method of determining shelf-life was a key component in the design of the test performed in this study.

### 2.1. Sample preparation and storage tests

DWG and CDS were obtained in 5 gallon plastic buckets from a local ethanol plant in Indiana (New Energy Corp, South Bend, Indiana). The samples received were immediately stored in a walk

in cold freezer at  $-20\text{ }^{\circ}\text{C}$  to prevent spoilage and maintain sample integrity until storage tests could be conducted. In preparation for testing, samples were placed in a walk in cooler at  $4\text{ }^{\circ}\text{C}$ , for 24 h, to allow them to thaw. To produce DWGS, the CDS and DWG were mixed in the container, and then divided into three sterilized buckets, and filled by alternating between each bucket. Each bucket was filled with 2000 g of DWG. CDS was incorporated by weight to yield 0%, 20% and 30% inclusion and then blended with a hand mixer. Samples were separated out and randomized within CDS blends for the test conditions. Any product that was not used was placed back in the cold freezer at  $-20\text{ }^{\circ}\text{C}$ .

Experiments were conducted using a  $3 \times 3$  full-factorial design, with three storage temperatures (10, 20, and  $25\text{ }^{\circ}\text{C}$ ) and three CDS levels (0, 20 and 30%), yielding a total of nine treatment combinations. These treatment combinations were implemented using a completely randomized design. DWGS samples were prepared in three replications, thus yielding  $3 \times 3 \times 3 = 27$  experimental runs. Sample properties were determined before and after the storage tests using three replicate measurements for each treatment combination.

DWG samples with three different additions of CDS each with a total of 125 g were stored in  $4.7 \times 10^{-4}\text{ m}^3$  (16 oz) glass Mason jars for seven days at the three different temperatures in three Percival Scientific Biological Incubator (Percival Scientific Inc., 1-36VL, Boone, Iowa), mimicking summer and winter temperatures. DWGS blends were placed in glass mason jars and stored without lids under aerobic conditions at the given temperature conditions. Samples were stored for seven days, being the anticipated length of shelf-life for DWGS stored at cool winter temperatures.

To determine the level of deterioration on DWGS, changes in m.c.,  $a_w$ , mycotoxin levels, colony forming units (CFU) counts, pH, and FA before and after seven days of storage were measured.

### 2.2. Pre and post analysis of stored samples

The analyses conducted before and after storage were completed using sub-samples of DWGS from the replicate blends and from the storage jars, respectively. Analyses were conducted in three replications for each test. The moisture contents of sub-samples of DWGS before and after the storage tests were determined by the NFTA 2.2.2.5 method (Shreve et al., 2006) where 2 g of the sample were placed in an air oven for 3 h at  $105\text{ }^{\circ}\text{C}$ . All moistures were determined on a dry basis. Water activity of sub-samples of DWG and CDS blends before and after the storage tests were measured using an AquaLab Water Activity meter (Series 3, AquaLab, Pullman, WA). The pH was measured with a probe pH meter (pH Electrode LE427, Mettler Toledo, Columbus, OH) used for solids. Fat Acidity values were measured by titration according to AACC standard 02-01 and 02-03 (AACC, 1995) and expressed as the milligrams of potassium hydroxide (KOH) required to neutralize the free fatty acids from 100 g of grain (mg KOH/100 g). The method involved extracting free fatty acids from milled DWGS using purified toluene and titrating with a  $\text{CO}_2$  free standard solution of 0.0178N KOH. The reported values are an average of the three titration replicates.

Additionally, the susceptibility to deterioration for the DWGS blends prior to storage was determined by measuring the carbon dioxide ( $\text{CO}_2$ ) given off by fungi feeding on DWGS according to Moog et al. (2004). This test was used to determine the comparative susceptibility to spoilage of the various DWGS blends. This involved the use of  $\text{CO}_2$  indicator paddles to determine the quantities of  $\text{CO}_2$  produced after incubating. Methods from Moog et al. (2004) were followed, described here in brief: DWGS samples (100 g) were stored for 72 h at the given conditions in similar  $4.7 \times 10^{-4}\text{ m}^3$  (16 oz) Mason jars. A slit was placed in the top of the Mason jar lid and

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