



2950 Niles Road, St. Joseph, MI 49085-9659, USA
269.429.0300 fax 269.429.3852 hq@asabe.org www.asabe.org

An ASABE Meeting Presentation

Paper Number: 152184612

PHYSICAL AND CHEMICAL PROPERTIES OF WHOLE STILLAGE, THIN STILLAGE AND SYRUP

Lu Yang, Graduate Research Assistant

Department of Agricultural and Biosystems Engineering, Iowa State University,
3326 Elings Hall, Ames, IA 50010, yanglu@iastate.edu

Kurt A. Rosentrater, PhD.

Department of Agricultural and Biosystems Engineering, Iowa State University,
3327 Elings Hall, Ames, IA 50010, karosent@iastate.edu

**Written for presentation at the
2015 ASABE Annual International Meeting**

**Sponsored by ASABE
New Orleans, Louisiana**

July 26 – 29, 2015

Abstract. *The production of bio-based ethanol has been one of the fastest growing industries in the U.S. during the last decade. The generated co-products are currently fed to livestock. Whole stillage, thin stillage, and condensed distillers syrup are the major upstream materials used to produce coproduct feeds. The storability of whole stillage, thin stillage and syrup influences the economic and energetic balances of fuel ethanol production. But there are few investigations of the shelf life for those products, or how to measure these quantities. The objectives of this research were to test physical and chemical properties of whole stillage, thin stillage, and syrup, and determine storability and allowable shelf life for these materials as influenced by storage temperature levels. Using standard laboratory methods, several properties were determined, including moisture content, water activity, thermal properties (conductivity, resistivity, volumetric heat capacity, and diffusivity), color, crude protein, crude fat, crude fiber and CO₂ production.*

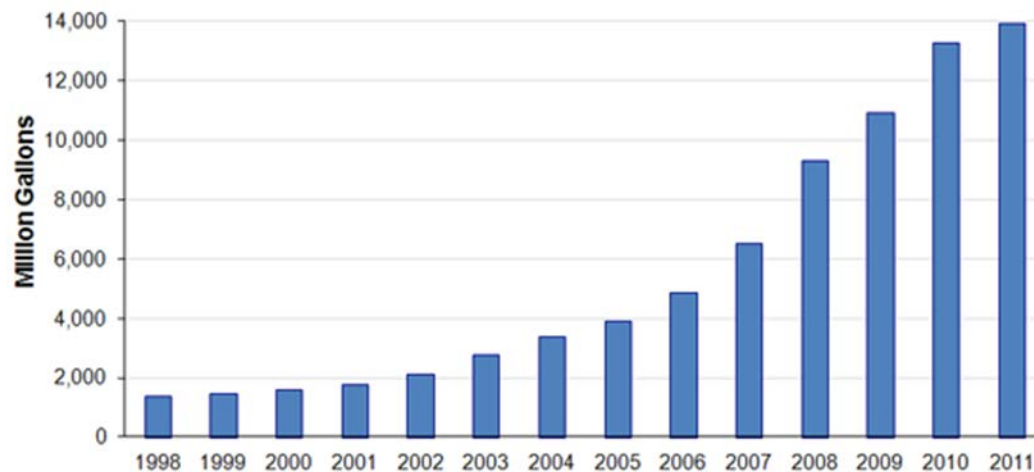
Keywords. *Corn, Ethanol, whole stillage, thin stillage, syrup.*

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Introduction

Fuel ethanol production is one of the fastest growing industries in the U.S. Many reasons, including fossil fuel exhaustion, less harmful environmental emission, widely available and easy use, and its renewable resource (West, 2014). According to U.S Department of Energy (2012), ethanol production in the US has risen from a near 2,000 million gallons per year in the 1998 to 14,000 million gallon per year in 2011. Furthermore, this dramatic increase will continue with production expected to be 20 billion gallons by 2020 (AgBioForum, 2008).

Chart 1. U.S. ethanol production, in millions of gallons, 1998–2011



Source: U.S. Department of Energy, Energy Efficiency & Renewable Energy, Alternative Fuels Data Center.

Corn-based material is the major raw material that the current energy industry utilizes to produce ethanol (Graboski, 2002). There are two distinct processes for the conversion of corn to ethanol, wet milling and dry grind. Wet milling process produces large amounts of corn and designed to produce 100 million or more gallons per year of ethanol (Graboski, 2002). The dry grind technology is the majority ethanol production in the U.S due to the low capital costs of construction and operation of ethanol plant (Singh, 2005).

Typically, dry grind process is designed to produce 30 to 50 million gallons per year of ethanol (Graboski, 2002). From figure 1, in the dry grind ethanol process, whole corn is first mixed with water, and cooked with enzyme (Singh, Rausch and Yang, etc. 2001). This mixture then liquefied to form as mash. The mash from cook tank is cooled down and then gluco-amylase is added to convert liquefied starch into simple sugars called dextrose during the saccharification process (Singh, 2005). Enzymes and yeast are added to the mash in fermentation processes. At the end of fermentation process, the resulting material (beer) consists of 10% alcohol, water and other solids that are not fermented, and then beer is transferred into distillation tank where ethanol is separated

from other residues which is called whole stillage(Singh, 2005). Whole stillage is sent to centrifugation process to remove excess liquid to form thin stillage, and thin stillage is then condensed by evaporation process (How ethanol is made, 2014). The condensed liquid is called syrup or condensed distillers soluble (CDS) and the remaining solids are referred as wet distillers' grains (WDG) (How ethanol is made, 2014). The mixture of WDG and CDS is called wet distillers' grains with soluble (WDGS), which is an important ingredients in animal feeding (Makkar, 2012). But usually, the residual coarse grain solids and CDS are mixed together and dried to produce distiller dried grain with solubles (DDGS) (Makkar, 2012). Due to the low moisture content and high fiber content of DDGS, it has a significantly longer shelf life and high quality material in livestock feeding (U.S. Grains Council, 2012).

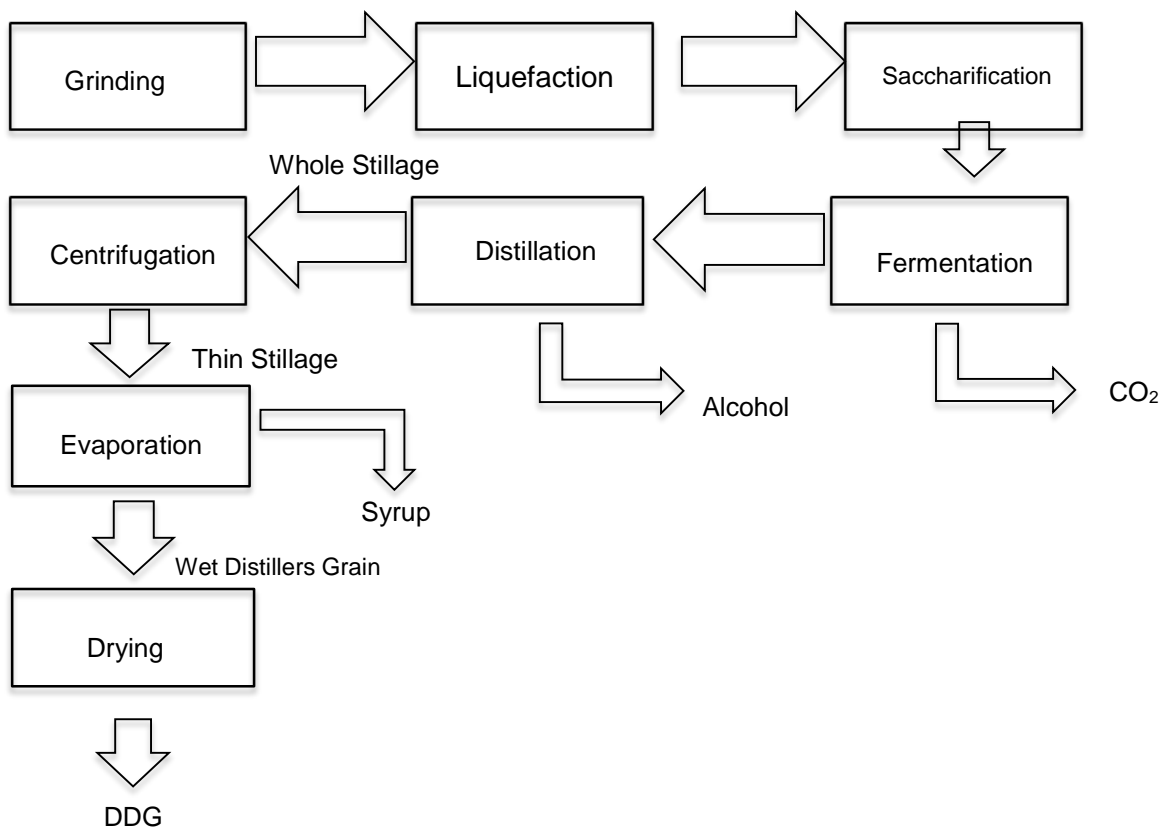


Figure1. Ethanol production process overview.

The major component in corn is starch, which takes about 62.0% (Shukla and Cheryan, 2001). The starch is converted into ethanol during the fermentation process, the rest of nutrients present in distillers grains which refer to whole stillage, thin stillage and distiller dried grain (DDG) (Erickson, Klopfenstein and Watson, 2007). Those by-products are the ideal supplements for animal feeding. But usually, DDGS is the primary product for

feed market. Since DDGS has longer shelf life and high dry matter, it can be shipped to and from any location in the country and can be stored under reasonable conditions (Clark, 2009). However, drying distiller grains requires energy, time and costs money; as a result DDGS are more expensive than other distiller grains (Gorden, 2008). In order to lower operation costs, WDG is considered as an alternative animal feeding ingredients, which has 30%-35% moisture content, are less efficient to ship due to its short shelf live (Dooley et al. 2008). As a consequence, WDGS are usually utilized by the farms in nearby an ethanol plant and with the ability to be delivered rapidly. However, the recent growth of fuel ethanol production resulted in an increased availability of ethanol by-products for animal feeding, thus the interest in using byproducts which contain relative high moisture content has also increased. Moreover, increases used of the intermediate products, like whole stillage, thin stillage and syrup in the marketplace could decrease environmental impacts such as greenhouse gas emission by drying process, and the overall energy cost of the fuel ethanol production, because drying could be reduced or even eliminated (Rosentrater and Lehman, 2008). Thus, the storability of whole stillage, thin stillage and syrup play a significant role in economic and energetic balances in animal feeding market. But there is a few work has studied the shelf life for those products. The objectives of this research was to measure the physical properties of these three products influenced by various temperatures.

Materials and Methods

2.1 Physical Properties Experimental Setup and Sampling

Freshly whole stillage, thin stillage and syrup were collected from Lincolnway Energy plant in Nevada, Iowa. Samples were placed into three individual 5-gallon plastic buckets with lids respectively. Homogenized, fresh whole stillage, thin stillage and syrup were distributed into 60 sterile, plastic (100x 15 mm) petri dishes. The sample in each dish had the same depth (10 mm). Groups of 20 petri dishes with lids containing whole stillage, thin stillage or syrup, were assigned to one of three incubations temperatures: 12°C, 22°C and 32°C. Ten-day incubations were conducted. At 1-day intervals, two randomly selected dishes for each distiller grain from each temperature treatment were analyzed for presence of mold, moisture content, water activity, thermal properties and color. Changes in the response variables were plotted with time to visual changes in mold, moisture content, water activity, thermal properties and color during the experiments.

In addition, the settling time was estimated for whole stillage, thin stillage and syrup. Each material was homogenized then placed into conical settling columns. Assigned six samples on horizontal lab table. At each hour, volume of top lay and bottom lay of each sample were recorded.

2.11 Initial Physical Characterizations

Samples of whole stillage, thin stillage and syrup were physically characterized at the outset of the experiment (t=0 day). For all physical properties, each was studied using three replicates. Moisture content was determined following Standard Method S352.2 (ASAE 2004), using a forced-convection laboratory oven (Heratherm, Thermo Scientific Inc., Odessa, TX) at 103°C for 24 hours. Water activity was measured by a calibrated water activity meter (Aqualab, Decagon Devices Inc., Pullman, WA). Thermal conductivity, resistivity, diffusivity and volumetric specific heat were determined with a thermal properties meter (KD2, Decagon Devices, Pullman, Wash). Color was measured using a spectrophotometer (CR-400 Chroma Meter, Konica Minolta, Ramsey, NJ) using the L-a-b opposable color scales (Hunter Associates Laboratory, 2002).

Table 1. Properties of the ethanol coproducts used in the study.

Property	Whole stillage		Thin stillage		Syrup	
	Mean	SD	Mean	SD	Mean	SD
Moisture content (%)	87	0.01	91.99	0.00	61.89	0.02
Conductivity (W/m K)	0.67	0.21	0.61	0.15	0.51	0.09
Resistivity (cm °C /W)	520.1	582.85	172.73	48.09	199.77	36.67
Diffusivity (mm ² /s)	0.13	0.04	0.14	0.02	0.14	0.01
Volumetric Specific heat (MJ/ m ³ K)	3.57	0.62	4.36	0.57	3.64	0.40
Hunter L	65.93	0.01	64.36	0.00	59.46	0.01
Hunter a	1.37	0.00	-0.70	0.01	5.65	0.01
Hunter b	41.82	0.01	42.64	0.01	41.97	0.00

2.12 Mold Development over Time

For the 18 dishes selected on each day, the presence of visible mold in the petri dish was assessed by inspection using the following progressive rating system: 0, no visible mold; 1, any visible mold (< 50% of surface colonized) and 2, extensive colonized (> 50% of surface colonized).

2.13 Moisture Content over Time

Each day, 18 randomly selected samples were placed on metal dishes. Moisture content was determined following Standard Method S352.2 (ASAE 2004), using a forced-convection laboratory oven (Heratherm, Thermo Scientific Inc., Odessa, TX) at 103°C for 24 hours.

2.14 Water Activity over Time

Each day, 18 randomly selected samples were placed on plastic dishes. The sample in each dish had the same depth (1 mm). Water activity was measured by a calibrated water activity meter (Aqualab, Decagon Devices Inc., Pullman, WA).

2.15 Color Changes over Time

Each day, 18 randomly selected samples were placed on metal dishes. Color was measured using a spectrophotometer using the L-a-b opposable color scales (Hunter Associates Laboratory, 2002). To measure color, each dish containing a sample was placed under the machine's sample observation port, and two reflectance spectra measurements were collected.

2.16 Thermal Properties over Time

Each day, the 18 randomly selected samples were poured into 50mL glass beakers. The sample in each beaker had the same depth (40mL). Thermal properties were determined with a thermal properties meter (KD2, Decagon Devices, Pullman, WA).

2.17 Settling Testing over time

Each material was homogenized then placed into two conical settling columns. The Six conical settling columns were assigned on the horizontal lab able. Each hour, volume of top lay and bottom lay of each column was recorded.

2.2 Solvita® CO₂ Production Testing

2.21 Solvita® Testing Procedure

Five ml samples were poured into Solvita® glass jars and a Solvita® paddle was inserted into each bottle, and its lid was screwed on tightly. For each material, two glass jars were assigned to one of two incubations temperatures: 25°C and 35°C. Five –day incubations were conducted. Solvita® color numbers were read 24 hours after paddles had been inserted and the values were converted to percent CO₂ which provided by Solvita®. Each day, duplication of data for each distiller grain was recorded from each temperature treatment. During the five-day incubation period microbes' activity would increase and the rate of CO₂ production would increase.

Results and Discussion

Observing visible mold is a method to measure the development of mold growth. Temperature had significant treatment effects on mold ratings. When samples were held at 32°C, mold first appeared at day 5 in both whole stillage and thin stillage. In the 22°C treatment, whole stillage got mold at 5th day, and first mold appeared in thin

stillage was at 6th day. Compared with the 32°C and 22°C treatments, samples had a longer shelf life in 12°C. The appearance of mold in whole stillage and thin stillage was between 8 and 9 days. No mold was found on surface of syrup at three temperature conditions during the 10-day experiment.

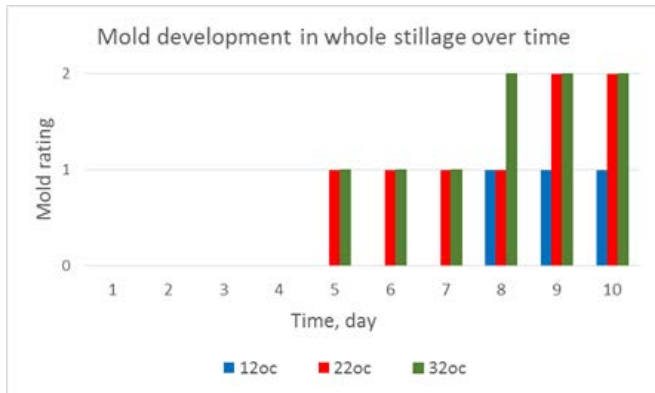


Figure 2. Mold development in whole stillage



Figure 3. Mold development in thin stillage

Moisture content indicates the amount of water in the sample. Moisture content is related to the freshness and stability for the storage of the material. Whole stillage and thin stillage had high moisture content, which was 87% and 92% respectively, however syrup sample contained relatively low water, which was 62% in the initial testing. Moisture content in the samples decreased over the 10-day observation. The samples in the 32°C treatment had the lowest moisture, due to the highest temperature causing the greatest evaporation.

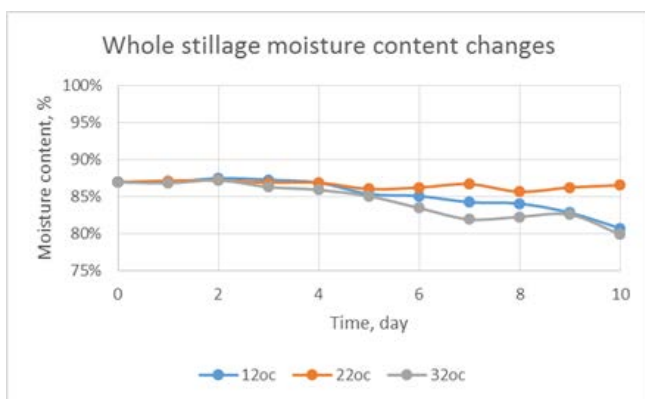


Figure 4. Moisture changes in whole stillage

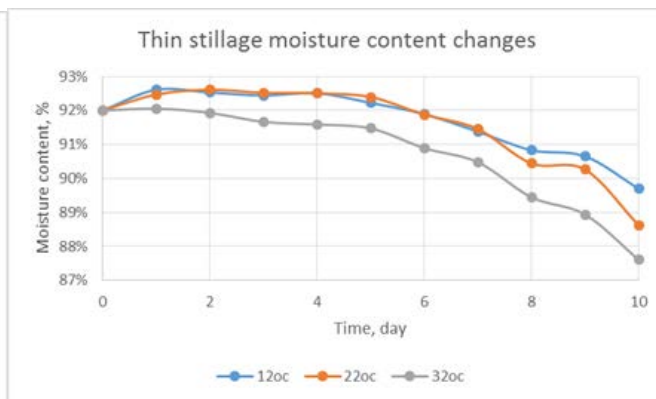


Figure 5. Moisture changes in thin stillage

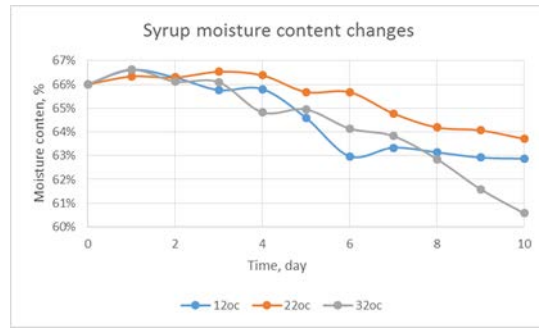


Figure 6. Moisture changes in syrup

Water activity measures the amount of “free” water available for use by microorganisms and chemical agents, and is therefore a measure of a material’s susceptibility to spoilage. Thus, products with no free water $aw=0.0$ are not at risk for degeneration, while materials with free water ($aw=1.0$) are at high risk for rapid spoilage (Rosentrater and Lehman, 2008). Both whole stillage and thin stillage had water activity values of 0.99 in initial testing. Thus, it showed that they were very susceptible to have mold growth. Additionally, the water activity of syrup was 0.92, which means it is easily spoiled by microorganisms. In general, water activities in three materials decreased over the 10-day experiment, the “free” water in whole stillage and thin stillage were still high, which ranged from 0.96 to 0.98 at the end of the experiment. Water activity in syrup changed from 0.92 to around 0.9.

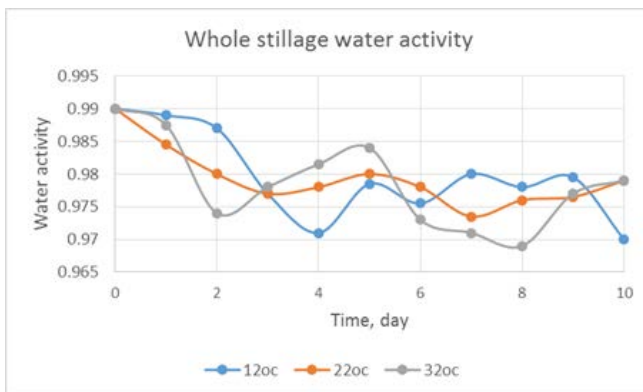


Figure 7. Water activity changes in whole stillage

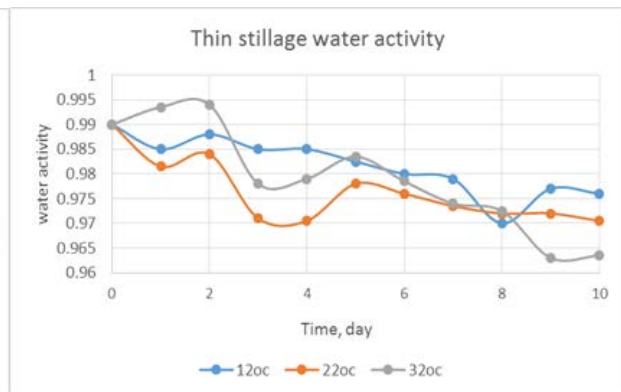


Figure 8. Water activity changes in thin stillage

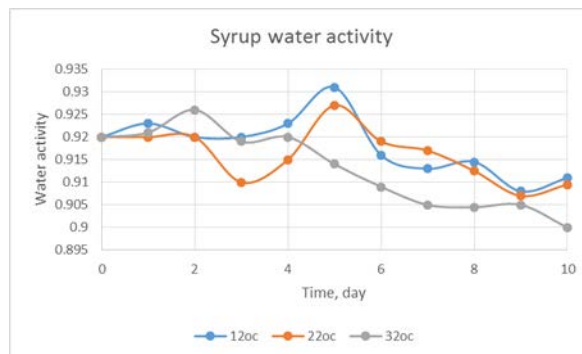


Figure 9. Water activity changes in syrup

Color appeared to change over time as well, but these changes were not as straightforward as the mold development data. From the results, Hunter L did not predict any of the microbial growth, but the significant changes in Hunter a and b related to microbial activity data in both whole stillage and thin stillage. In whole stillage, Hunter a had a significant increase at $t=8$ days when the ambient temperature was 12°C , and Hunter b had a dramatic increase at the 8th day. Similar changes could be found when samples were held in 22°C and 32°C treatments. From the result of thin stillage, Hunter a and b had a significant increase when the samples were held at 32°C . The presence of the mold on surface could cause visual color changes in the visual observation of the samples.

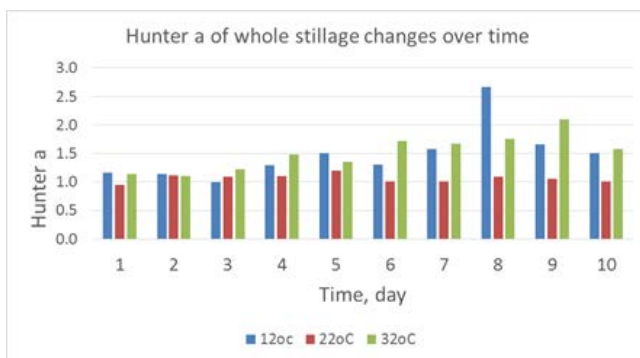


Figure 10. Hunter a changes in whole stillage

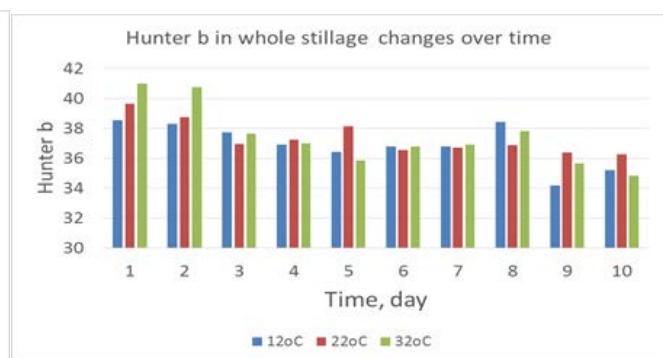


Figure 11. Hunter a changes in whole stillage

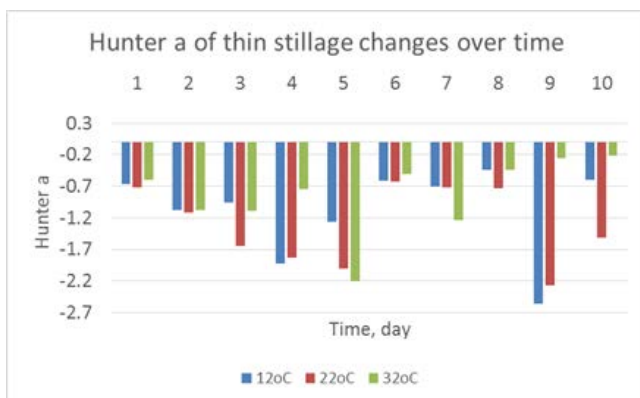


Figure 12. Hunter b changes in whole stillage

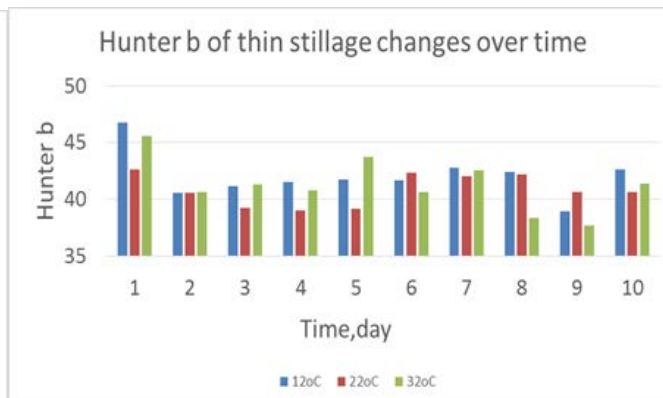


Figure 13. Hunter b changes in whole stillage

Settling happened after 1 hour in whole stillage and thin stillage samples. Top volume of these two materials increased as the time change. However, the separation quantity of whole stillage and thin stillage decreased after 7 hours. No separation happened in syrup during the 72 hour observation. Compared with whole stillage and syrup, thin stillage had the greatest separation rate.

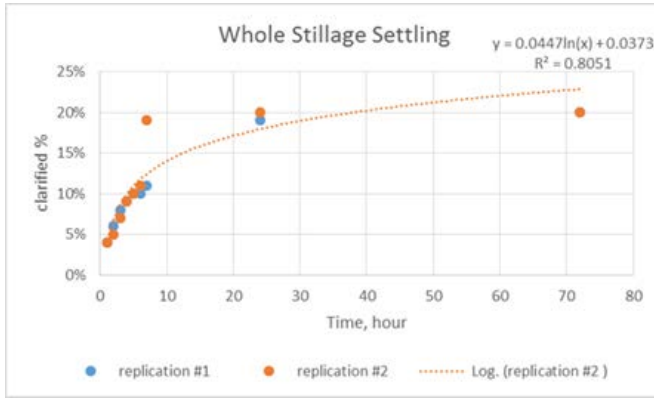


Figure 14. Top volume changes in whole stillage

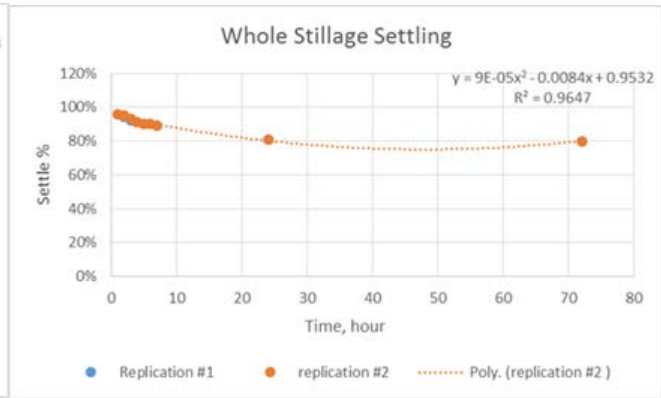


Figure 15. Bottom volume changes in whole stillage

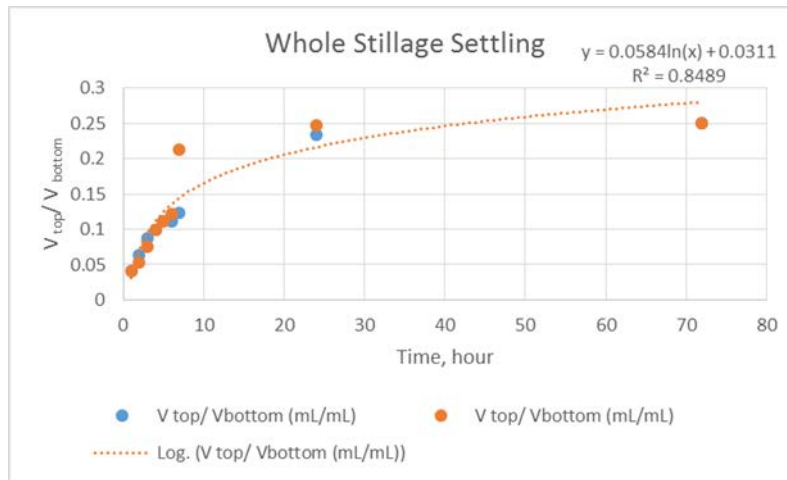


Figure 16. Top volume vs. bottom volume changes in whole stillage

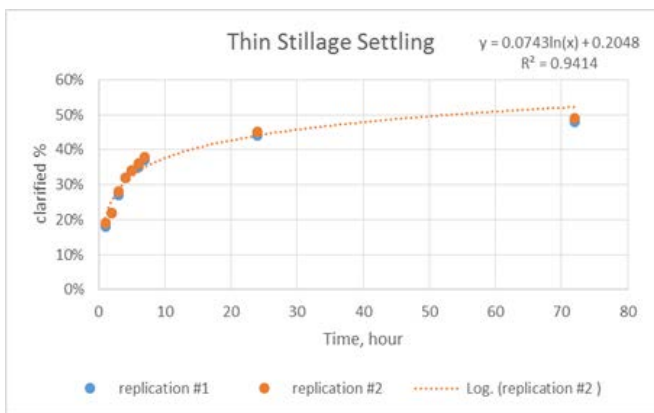


Figure 17. Top volume changes in thin stillage

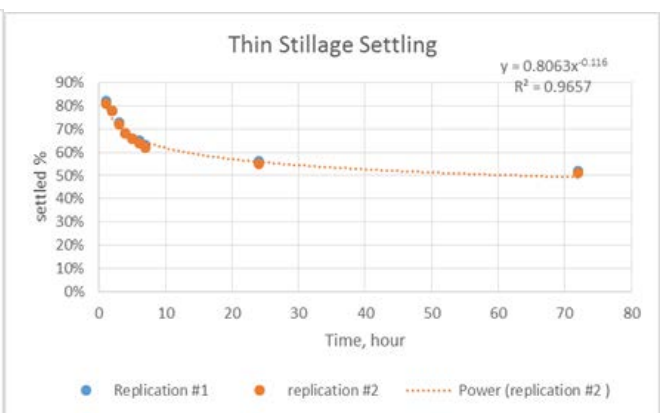


Figure 18. Bottom volume changes in thin stillage

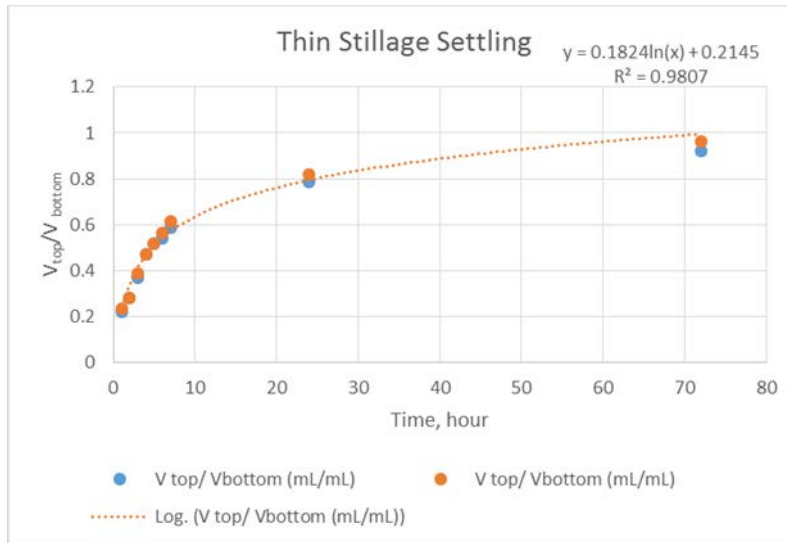


Figure 19. Top volume vs. bottom volume changes in thin stillage

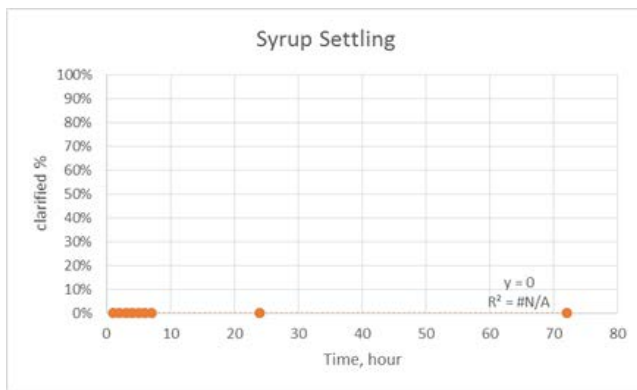


Figure 20. Top volume changes in syrup

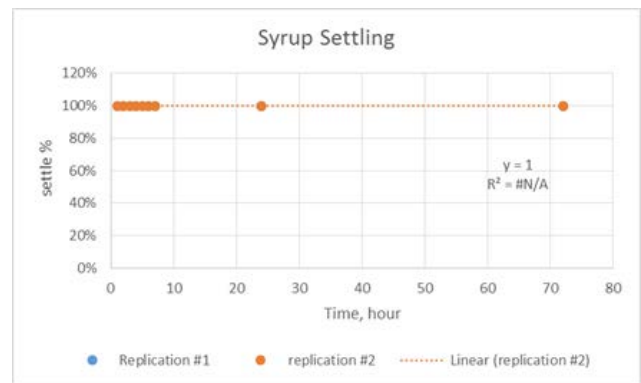


Figure 21. Bottom volume changes in syrup

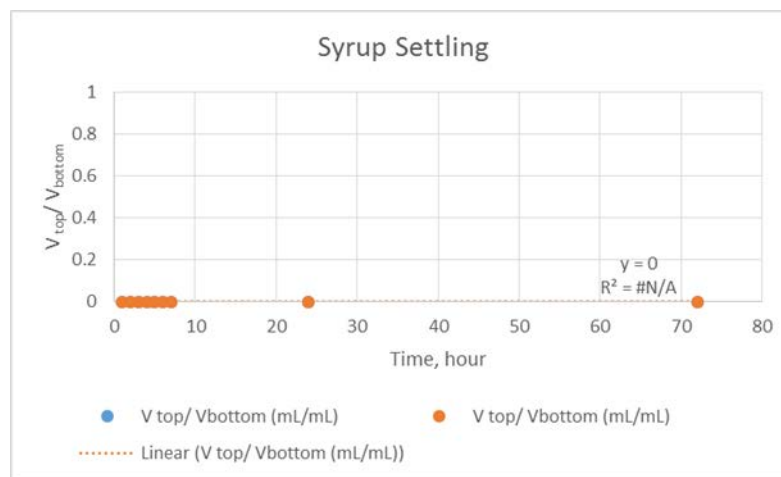


Figure 22. Top volume vs. bottom volume changes syrup

Figure 23 shows storage time versus percentages of CO₂ for thin stillage at 25°C and 35°C treatments from day 1 to day 5. As storage time gets large, the Solvita® color number increased in both two temperature conditions, which means the CO₂ production increased. At 35°C temperature condition, the Solvita® color number reached to 5 at day 2, while Solvita® color number approached to 5 at day 5 in 25°C condition. It indicated that higher temperature caused greater microbes' activities. Figure 25 shows CO₂ changes over time in whole stillage samples. Things happened in whole stillage was similar to thin stillage, but CO₂ percentage in whole stillage at 25°C was higher than samples at 35°C at the end of day 1. At the end of day 5, whole stillage samples in both temperature conditions reached to 5. Figure 27 shows the CO₂ changes from day 1 to day 5 in syrup at 25°C and 35°C. The Solvita® color number reached to 5 at day 2 in syrup samples at 35°C. Syrup samples at both temperature condition got to 5 at the end of day 3.

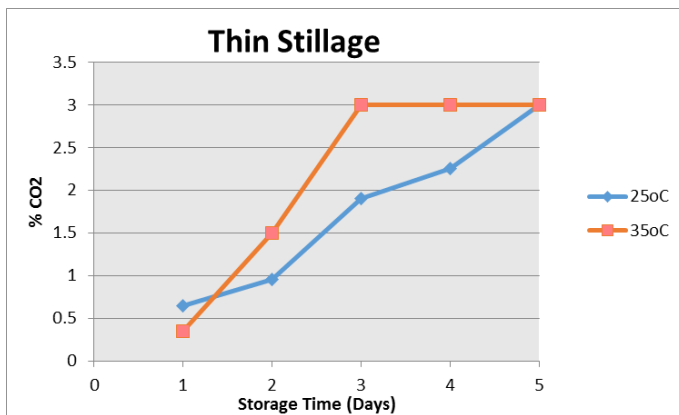


Figure 23. % CO₂ vs. storage time of thin stillage

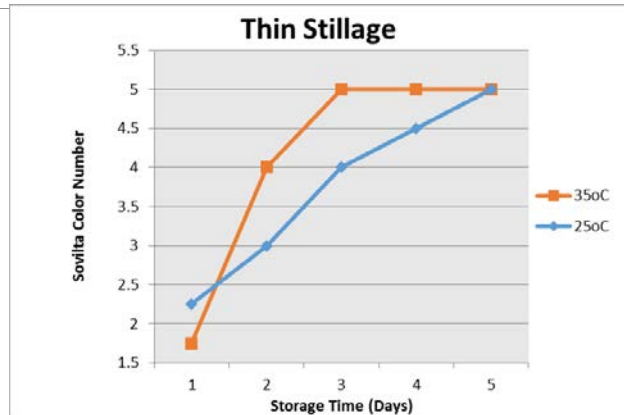


Figure 24. % Solvita number vs. storage time of thin stillage

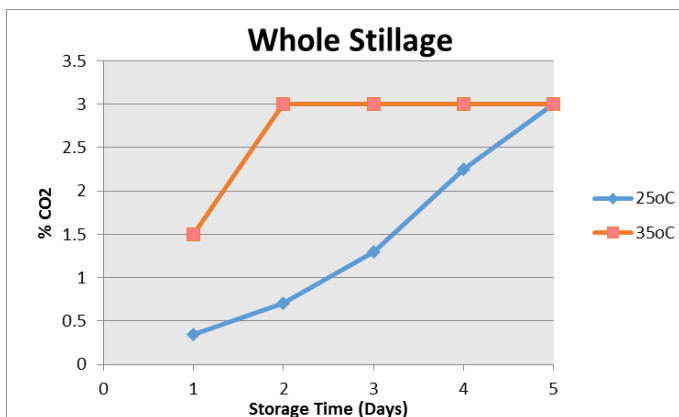


Figure 25. % CO₂ vs. storage time of whole stillage

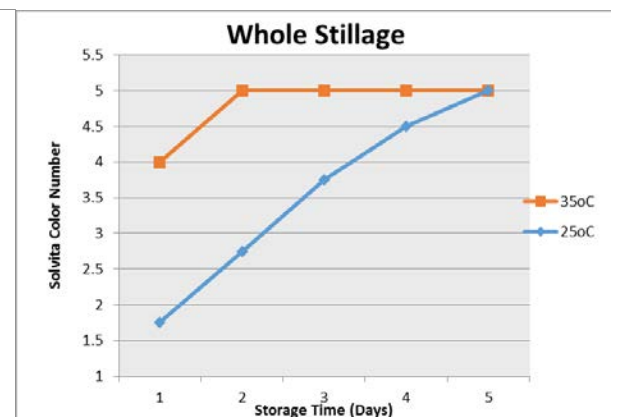


Figure 26. % Solvita® number vs. storage time of whole stillage

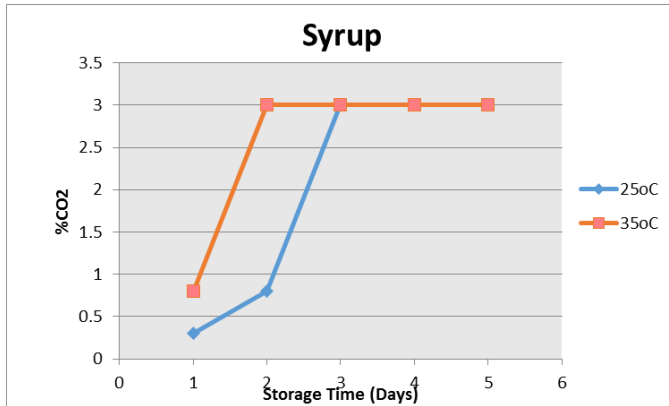


Figure 27. % CO₂ vs. storage time of syrup

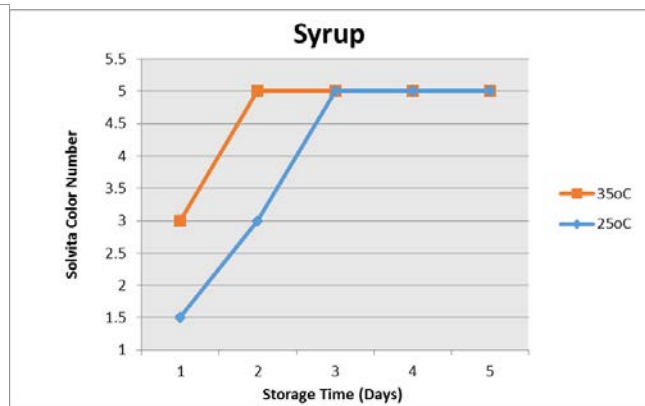


Figure 28. % Solvita® number vs. storage time of syrup

Conclusions

Many studies have investigated the properties of distiller's grains (DWG) and distiller dried grain with soluble (DDGS), but few have examined whole stillage, thin stillage and syrup. The thin stillage and whole stillage samples studied had high moisture contents of 92% (w.b) and 87% (w.b) respectively, and water activity of 0.99; the high water content made samples easily susceptible to rapid spoilage. Also, high temperature increased microorganisms' activities. Both thin stillage and whole stillage samples showed mold growth at day 5 in the 32°C treatment. Due to the high moisture content, thin stillage had the greatest separation rate in settling experiment. In comparison with the water content in thin stillage and whole stillage, syrup had relative low moisture content of 62% and water activity of 0.92. Relative low water content caused no separation to happen in syrup and no visible mold was found on the surface of syrup. Solvita testing showed that high temperature treatment caused high CO₂ production in all samples. The information about thermal properties and color parameters are important to design of equipment, processes, and facilities to handle, store and utilize these products.

Acknowledgements

We thank Lincolnway Energy for providing samples.

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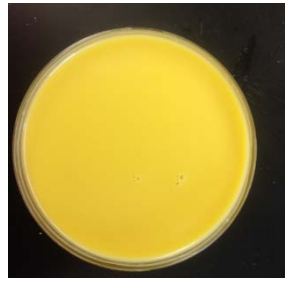
Appendix

The visible mold was evaluated by inspection using a subjective rating system.

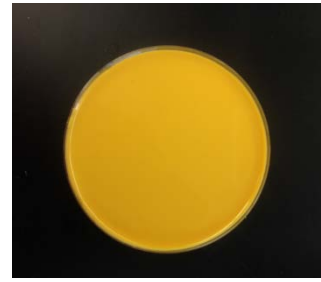
No visible mold----- 0



Whole Stillage



Thin Stillage



Syrup

Any visible mold (< 50% of surface colonized)-----1

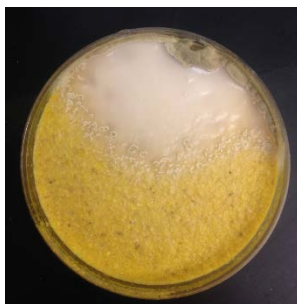


Whole Stillage

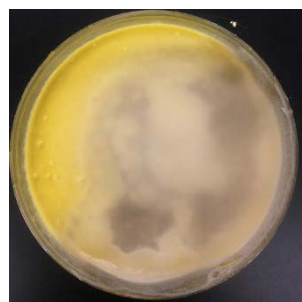


Thin Stillage

Extensive mold (> 50% of surface colonized)-----2



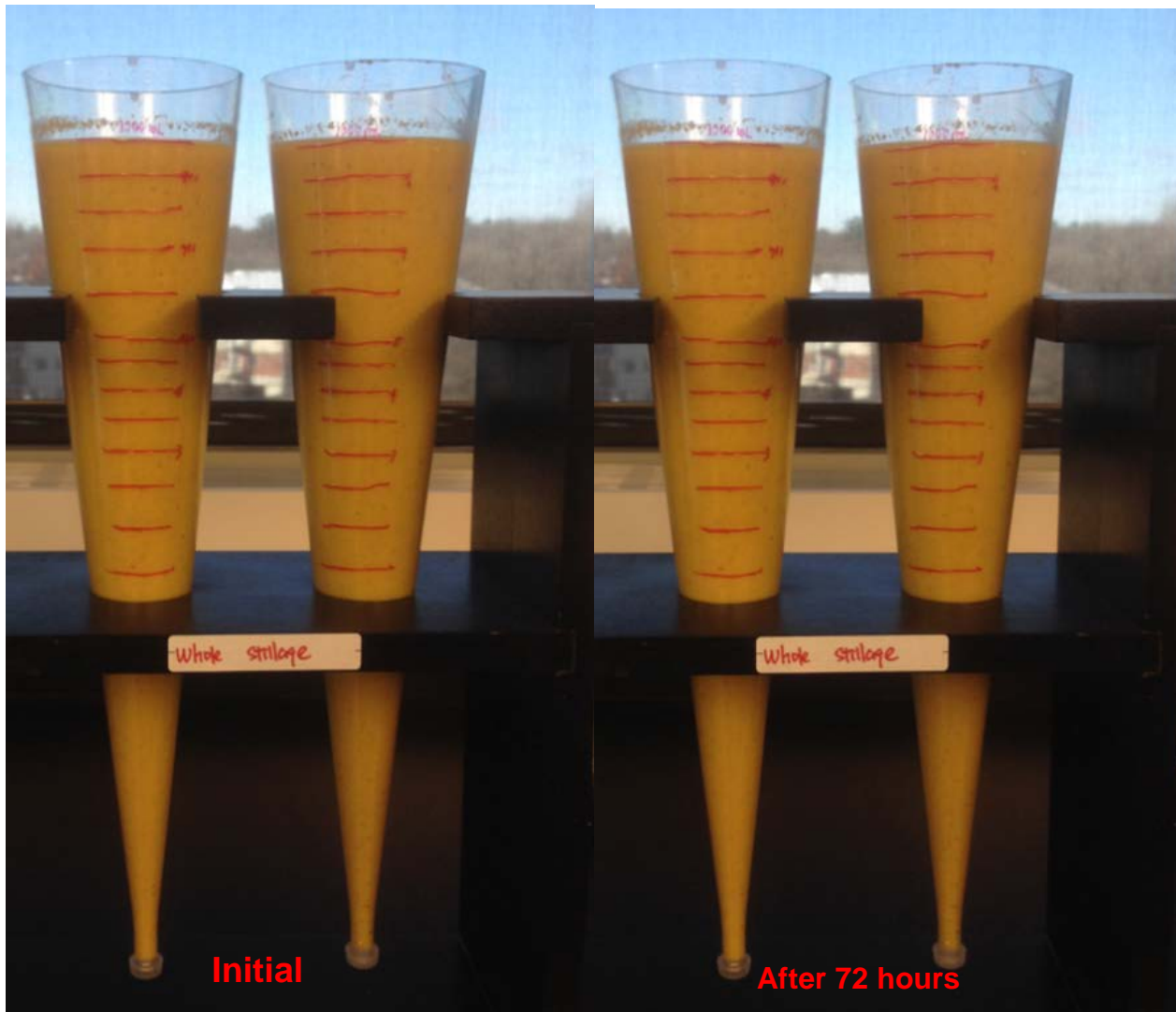
Whole Stillage



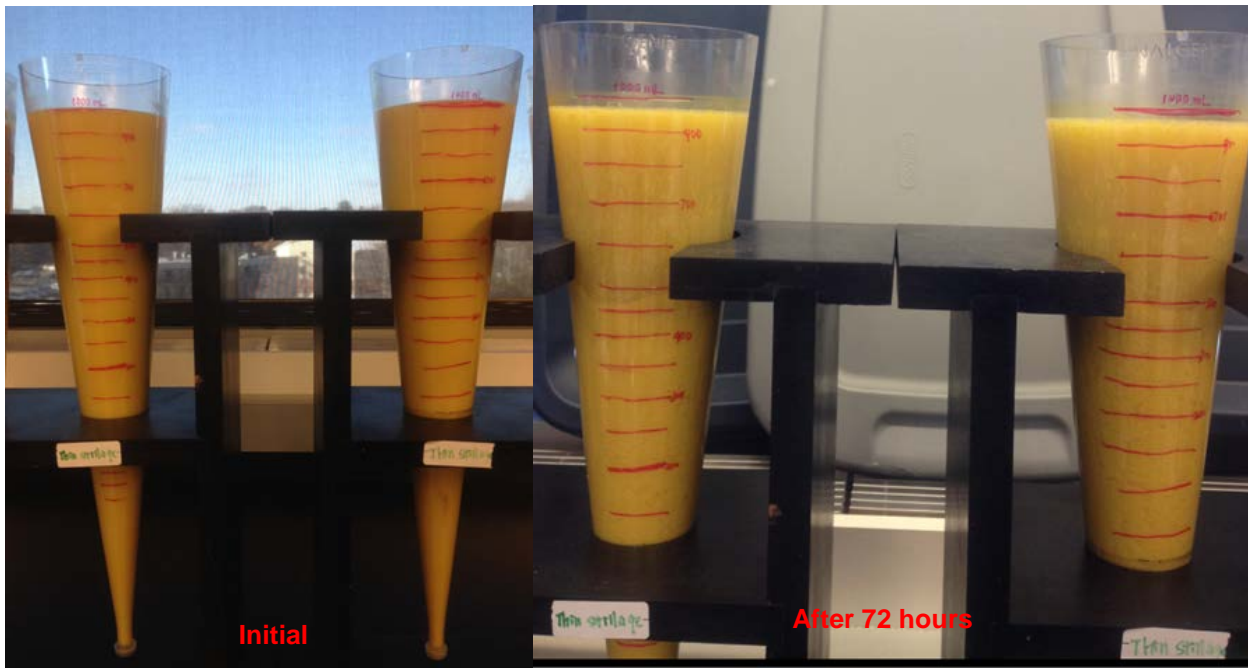
Thin Stillage

Settling Experiments

Whole stillage



Thin stillage



Syrup

