

# EXPLORING FERMENTATION RATE IN BEER AND HARD CIDER BREWING

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By

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# ABSTRACT

## EXPLORING FERMENTATION RATE IN BEER AND HARD CIDER BREWING

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In beer brewing, it is widely known that trace elements, particularly zinc, are required by yeast in order to grow and ferment. The general consensus among large-scale brewers and homebrewers alike is that the most sensitive and time-consuming step of beer production is the fermentation process. Problems encountered during fermentation can lead not only to prolonged fermentation time, but also to the deterioration of beer quality. These problems can often be contributed to a lack of necessary trace elements needed for the fermentation of yeast. While the wort provides some trace elements for the yeast, zinc may not be available in the required amount in the wort. To prevent such problems, brewers may supplement wort by adding additional zinc salts during wort boiling or by adding a yeast supplement that includes zinc to wort.

Several studies have been published on the optimal zinc concentration to increase the rate of fermentation, but few have studied this process in quantities that are practical to the average homebrewer. Furthermore, the specific gravity used in determining the rate of fermentation may not only be a function of time and wort composition, but also process characteristics outside of the fermenter. In this study, we explore different methods of increasing fermentation rate and present various statistical models that describe specific gravity as a function of time and additional variables. These additional variables include both pre-planned experimental factors and nuisance variables that may affect the final product.

# 1 INTRODUCTION

## A Brief Introduction to Homebrewing

People began brewing beer in their homes over 6,000 years ago [Papazian, 2003]. In the colonial period and up to the middle of the 19th century, homebrewed hard cider was one of the most popular beverages in America [Pittet, 2011]. While homebrewing has been around for thousands of years, it has seen a spike in popularity in recent years, which some credit to the growth of the microbrewing industry. But despite its recent spike in popularity, many people have never been involved in the homebrewing process. For people who have never participated in the homebrewing process, a common question directed to homebrewers is “Why homebrew?” Some common initial assumptions are that people homebrew in an effort to save money or because of a lack of variety in beers currently on the market. Even if people begin brewing their own beer for these reasons, they often find that these are not the reasons why they continue homebrewing.

It can, of course, be less expensive to brew beer at home rather than purchasing beer from commercial retailers. However, after people begin homebrewing they often find that their focus shifts from the lessened cost of homebrewing to the opportunity that homebrewing presents to be creative in producing quality, unique brews. With the wide range of brews currently on the market, it is harder to justify homebrewing on the basis that a desired product cannot be found on the market today. Though more and more people can enjoy the wide range of beer/cider currently available, many of these people want to make these products themselves or expand on what is currently available.

Making beer/cider at home has taken on new meaning in recent years. Homebrewers are becoming increasingly knowledgeable about brewing and are becoming more innovative and creative in their brewing practices. Homebrewing has become more than a hobby for many people. Many homebrewers now place a focus on the replicability of brews, which is indicative of the logi-

cal and developmental approach to brewing that is used by homebrewers today. Homebrewers and large-scale (commercial) brewers share a common goal of making the highest quality, most consistent brew in the shortest amount of time for a variety of reasons. One reason, the most obvious of reasons, is that brewers simply want their brew quickly. Commercial brewers want to get the product out to the customer as quickly as possible in an effort to achieve the shortest turnaround time possible, in order to maximize profits (i.e., move product through the fermenters quickly). Similarly, homebrewers want to have a usable product in the shortest amount of time possible. Another, more critical, reason that brewers desire a short turnaround time is that this shortens the amount of time that the brew remains in the fermentation stage, which is the most sensitive step of the brewing process. When brews remain in the fermentation stage for longer amounts of time, this increases the likelihood of encountering problems.

## The Homebrewing Process

Before brewing begins and before ingredients are purchased, a homebrewer must decide whether they intend to start from scratch and brew their beer exactly as commercial breweries do, or to choose the more simplistic route. Unlike commercial breweries, it may not be more cost-effective for homebrewers to grind or mash their own grains (i.e., all grain brewing). Instead, homebrewers can choose to avoid these time-consuming steps and use malt extract. Malt extract is malted barley that has been processed into a malt “soup,” where 70 to 80 percent of the water is evaporated, leaving a concentrated syrup or further dried to create a powder [Papazian, 2003]. Brewing with malt extract offers an advantage as it does not require as much time and equipment as all-grain brewing. While there are advantages and disadvantages to both all-grain brewing and malt extract brewing, the decision of which path to choose ultimately depends on the brewer’s personal preference. After deciding between the options of starting from scratch or using malt extract, the homebrewer must gather the required equipment and ingredients that correspond to the particular

beer or cider they wish to brew. A detailed outline of the required equipment and ingredients needed for a beginning homebrewer can be found in *The Complete Joy of Homebrewing* [Papazian, 2003].

Following the acquisition of all required equipment and ingredients, the malt extract (if used) and other ingredients, including hops (these are added to the brew pot after the boil starts), will be added to water and brought to a boil in a brew pot to prepare a wort. Wort is a term used by brewers to describe unfermented beer [Papazian, 2003]. After the wort has boiled for approximately one hour, it is transferred from the brew pot to the fermenter(s) and cooled. When the temperature of this mixture is below approximately 75 degrees Fahrenheit, a first measurement of specific gravity will be taken using a hydrometer or refractometer (this measurement will later be referred to as the original gravity). A hydrometer is an instrument that measures the density of a liquid relative to the density of water. This measure of density is known as specific gravity. The specific gravity of water is exactly 1.000, thus adding dissolvable solids such as sugar to the water causes the solution to become more dense and the specific gravity rises above 1.000 [Papazian, 2003]. After the original gravity of the wort is measured, the yeast will be added. The addition of yeast to the wort is often referred to, among brewers, as pitching. After the yeast is pitched, an airlock will be attached. The airlock is a device that allows carbon dioxide to be released from the fermenter without allowing air to enter the fermenter. The airlock is approximately half-filled with water, which will allow fermentation to become a visible process as the gases produced by fermentation bubble through the airlock.

After the yeast is pitched, the wort is now referred to as beer/cider. This change in reference occurs due to the fact that the beer/cider has now entered the fermentation stage. The fermentation stage is the most critical stage of the brewing process. The amount of time that a beer remains in the fermentation stage varies depending on the style of beer/cider being brewed. Generally, after two to three days of vigorous fermentation, activity will subside and yeast will become dormant due to food depletion and alcohol buildup, and fermentation will begin to subside. After fermentation has ended, the beer/cider will be bottled or kegged and then aged over a period of generally 7-14

days [Papazian, 2003].

Brewing cider is a decidedly more simple process in comparison to that of beer. Hard cider is made from apple juice or apple cider, and if the juice or cider is preservative free, there is no need to boil the juice or cider in a brew pot, although some recipes do call for boiling. Before brewing begins and before ingredients are purchased, a homebrewer must decide whether they intend to use unpasteurized or pasteurized apple juice/cider. If the juice/cider is unpasteurized, sulfite can be added to remove wild yeasts. Before any yeast or yeast supplements are added to the apple juice/cider, the original gravity will be measured using a hydrometer or refractometer, following which the yeast and yeast supplements will be added.

## The Importance of Fermentation

The general consensus among large-scale brewers and homebrewers alike is that the most sensitive and time-consuming step of beer production is the fermentation process. Depending on the style of beer, the fermentation process can last anywhere from ten days (e.g., ales) to several months (e.g., lagers) [Papazian, 1994]. During this fermentation process, yeast cells reproduce and disperse themselves throughout the fermenting beer, converting sugars to alcohols, carbon dioxide and various flavor compounds. After a few days the yeast will have consumed most of its sugar supply and fermentation will begin to subside.

Yeasts are living microbiological organisms, and as such, they are affected by environmental factors such as temperature or contamination by wild yeasts or other yeast strains. If the yeast in a homebrew does not have an environment in which it can thrive, this can lead to problems during fermentation. Additionally, if there is not an adequate amount of trace elements, particularly zinc, in the wort this can also potentially lead to problems during fermentation. Problems encountered during the fermentation process can lead to not only prolonged fermentation time, but also the deterioration of beer quality.

With the sensitivity of the fermentation stage comes a natural desire to increase the rate of

fermentation; the shorter amount of time a brew remains in the fermentation stage, the less chance you have of encountering problems. Given that homebrewers are becoming more innovative in their brewing, it is natural that they are seeking creative ways to increase the rate of fermentation. It is known, based on biochemical and microbiological experiments, that yeasts require certain trace elements in order to grow and ferment [Helin and Slaughter, 1977]. Trace elements that have been widely studied regarding their essential nature in fermentation are zinc, calcium, manganese, cobalt, and iron [Maddox and Hough, 1970], [Helin and Slaughter, 1977] [Papazian, 2003]. When these trace elements are not available in the required amounts, many problems can ensue. Potential problems include slow fermentation, mutation of yeasts, poor sedimentation, and off-flavors. It has been conjectured that problems encountered during fermentation can, most often, be contributed to low zinc concentration of the wort or the yeast [Vecseri-Hegyessy et al., 2005]. In an effort to promote yeast health and prevent off-flavors, many breweries add zinc sulfate, zinc chloride, or Servomyces to wort [White, 2013]. Servomyces are dead yeast that serve as a nutritional yeast supplement and work to cut down fermentation time and improve the health and viability of yeast. More recent findings suggest that preconditioning brewing yeast cells with zinc may be a more effective way to enhance fermentation performance rather than supplementing wort with zinc [De Nicola and Walker, 2011].

Commercial brewers have an advantage over homebrewers in that they have the resources to test wort composition and determine if it is necessary to supplement with additives such as zinc salts or Servomyces, which can be costly. For homebrewers, testing wort composition may not be a realistic option, and as a result, it simply may not seem cost-effective enough to supplement using these types of additives. Similarly, homebrewers may not have the equipment or resources necessary to precondition brewing yeast cells with zinc. However, one realistic, cost-efficient method of increasing the speed of fermentation that homebrewers commonly use is the addition of a commercially prepared yeast nutrient. Yeast nutrient provides a blend of vitamins, minerals, amino acids, nitrogen compounds, zinc, and trace elements, all of which are necessary for rapid and complete fermentation [Wyeast Laboratories, 2015]. Wyeast Laboratories Inc. is a worldwide

leader in the fermentation industry as a producer of yeast cultures and fermentation products. Wyeast claims that supplementing wort with yeast nutrient will reduce lag time, enhance yeast viability, and encourage consistent attenuation rates [Wyeast Laboratories, 2015]. Attenuation is a measure of how much of the sugars were converted into alcohol and carbon dioxide by the fermentation process.

Another factor that could potentially affect fermentation rate is trub. Trub is the sediment that collects at the bottom of the fermenter when brewing beer/cider. It is known that the presence of trub in fermenting wort can affect the fermentation and flavor [Papazian, 2003]. Commercial brewers are careful to remove this trub before even transferring the wort to its fermenter. Commercial brewers remove this trub by whirlpooling the wort as it is drawn from the brewpot. Homebrewers, however, may not have the ability to whirlpool the wort while transferring the wort into the fermenter. In this case, homebrewers must consider the effect that trub may have on the rate of fermentation. If a homebrewer transfers wort from the brewpot into multiple fermenters, the amount of trub in each of the fermenters will most likely not be equal. Given that we know that the presence of trub in the fermenting wort will affect the rate of fermentation, a natural question arises as to whether the order in which the fermenters are filled affect the rate of fermentation.

## Measuring Fermentation Rate

We know that when the fermentation process is near completion, the yeast will go dormant and either float to the top or sink to the bottom of the fermenter, depending on the strain of yeast. This process is commonly referred to as flocculation. While the flocculation of yeast is a good indication that fermentation is nearing completion, we can test the degree of attenuation to confirm that fermentation has ended. Recall that attenuation is a measure of how much of the sugars in beer/cider wort is converted by the fermentation process to alcohol and carbon dioxide, and is indicated by the difference between original gravity and final specific gravity [Papazian, 2003].

Recall that before the yeast has been pitched, the specific gravity is checked with a hydrometer. During fermentation, the specific gravity can be re-checked. The specific gravity will decrease over time since the sugars that were present in the wort will be consumed by yeast during fermentation. The consumption of sugar by yeast causes the density of the liquid to decrease, which as a result, lowers the specific gravity. It is common to consider fermentation finished when the hydrometer readings of specific gravity remain unchanged for 2-3 days [Papazian, 2003].

While hydrometers are the most common tool used to measure the specific gravity of cider or beer, there are other options. Another tool that can be used to measure specific gravity is called a refractometer. A refractometer is an instrument that measures the gravity of a solution by measuring the angle at which light passes through the sample. Refractometers are relatively inexpensive, but they do have one downfall: they are only accurate for original gravity. Refractometers are affected by the presence of alcohol in a solution, so they do not give accurate readings of specific gravity after the yeast has been pitched and fermentation begins. However, there are several advantages to using a refractometer in place of a hydrometer. Refractometers require very little volume of liquid, typically less than 1 mL, whereas hydrometers require a much larger volume of sample: the hydrometer used in these experiments requires between 75 to 95 mL of sample to read specific gravity. Taking several specific gravity measurements throughout the fermentation stage could lead to a waste of a substantial amount of beer/cider, since 355 mL are in a typical bottle of beer so one beer is used to obtain every 4-5 hydrometer readings. Most refractometers have an automatic temperature conversion, so samples can be taken directly from the kettle, whereas hydrometers are temperature dependent. Using a refractometer as opposed to a hydrometer also saves time when taking measurements of specific gravity. Taking several specific gravity measurements throughout the fermentation stage may take a substantial amount of time if using a hydrometer, whereas using a refractometer takes only a few seconds per reading.

## 2 METHODS AND MATERIALS

Multiple experiments were conducted over a period of several months, each with different materials and purposes. Below these materials and purposes are described, with the selected data analyses following in Chapter 3. Several of the earlier experiments served as vehicles for improving data collection techniques as the author had no experience with homebrewing prior to this work.

### Yeast Strains

Two yeast strains for four different brews have been employed in this study. The four different brews analyzed in this study are named Cider1, IPA38, IPA39, and Cider2. For Cider1 and Cider2, Red Star pasteur champagne yeast, a yeast strain of *Saccharomyces bayanus*, was used. For IPA38 and IPA39, Safale US05, a yeast strain of *Saccharomyces cerevisiae*, was used. The naming convention of the different brews is that of a local homebrewer. For instance, IPA38 and IPA39 are so named because they are 38th and 39th beers of India Pale Ale (IPA) style brewed by him.

### Sample Preparation

During sample preparation for Cider1, we were trying to determine how the amount of yeast nutrient added to cider would affect the fermentation rate. More specifically, we were trying to determine if adding yeast nutrient to cider could increase the rate of fermentation. This study used six one-gallon vessels of Earthfare Organic Apple Juice where each vessel was given an equivalent amount of yeast. Fermenting cider samples treated with varying amounts of yeast nutrient were examined. This experiment was carried out for 432 hours and specific gravity readings were obtained using a refractometer at 0, 45, 250, 288, 339, 387, and 432 hours.

During sample preparation for IPA38, we were trying to determine whether the order in which the vessels were filled from the kettle would affect the fermentation rate. In this study, approximately 12 gallons of wort was initially prepared and half (six gallons) went directly to the brewer in a 6.5-gallon carboy and the other half was emptied into six one-gallon vessels. The order of fill was such that one gallon was emptied into vessel 1 followed by one gallon into the carboy. Next, another gallon was emptied into vessel 2 followed by another (second) gallon into the carboy. The vessels were continually filled in this manner, so that vessel 1 contained wort near the bottom of the brew kettle and vessel 6 contained wort near the top of the brew kettle. This experiment was carried out for 290 hours and specific gravity readings were obtained using a refractometer at 0, 22, 55, 122, 147, 195, 240 and 290 hours.

During sample preparation for IPA39, we were attempting to quantify the error in refractometer readings in comparison to hydrometer readings over time. The vessels were filled in a manner similar to that of IPA38, so, in addition to the purpose of error quantification, we sought to determine whether order of fill was important for fermentation using a larger number of time observations. This experiment was carried out for 180.5 hours and specific gravity readings were obtained using both a refractometer and hydrometer at 19, 31, 46, 56, 68, 92, 104, 116, 128.5, 140, 152, 168.5 and 180.5 hours.

During sample preparation for Cider2, we were trying to determine how the amount of zinc and/or yeast nutrient added to cider would affect the fermentation rate. More specifically, we wanted to determine whether the addition of zinc sulfate, yeast nutrient, or a combination of zinc sulfate and yeast nutrient to cider could increase the fermentation rate, and if so, which treatment would have the greatest effect on fermentation rate. This study used twelve one-gallon vessels of Earthfare Organic Apple Juice where each vessel was given an equivalent amount of yeast. Fermenting cider samples treated with varying amounts of zinc sulfate and yeast nutrient were examined. This experiment was carried out for 369 hours and specific gravity readings were obtained

using a refractometer and/or hydrometer at 0, 11, 27, 37, 52.33, 61.33, 68, 89.67, 130.33, 149.33, 177.67, 225.67, 273.67, 320.17, and 369 hours.

## Sample Collection

Samples of fermenting beer and cider were obtained using an automatic temperature compensating refractometer and/or a hydrometer. Video recordings were also taken prior to the collection of beer/cider samples to monitor the air lock bubbling for each vessel.

### 3 DATA ANALYSIS

#### Cider1

Recall that we were using the Cider1 data to determine how the amount of yeast nutrient added to cider will affect the fermentation rate. More specifically, we were trying to determine if adding yeast nutrient to cider could increase the rate of fermentation. Wyeast laboratories recommends using half a teaspoon of yeast nutrient per five gallons of wort. With this in mind, we constructed three treatment levels for this experiment. In treatment level 1, vessels 1 and 2 received no yeast nutrient, in treatment level 2, vessels 3 and 4 received half the recommended amount of yeast nutrient, and in treatment level 3, vessels 5 and 6 received the full recommended amount of yeast nutrient. After the fermentation of Cider1 was complete, we began by graphically considering our data.

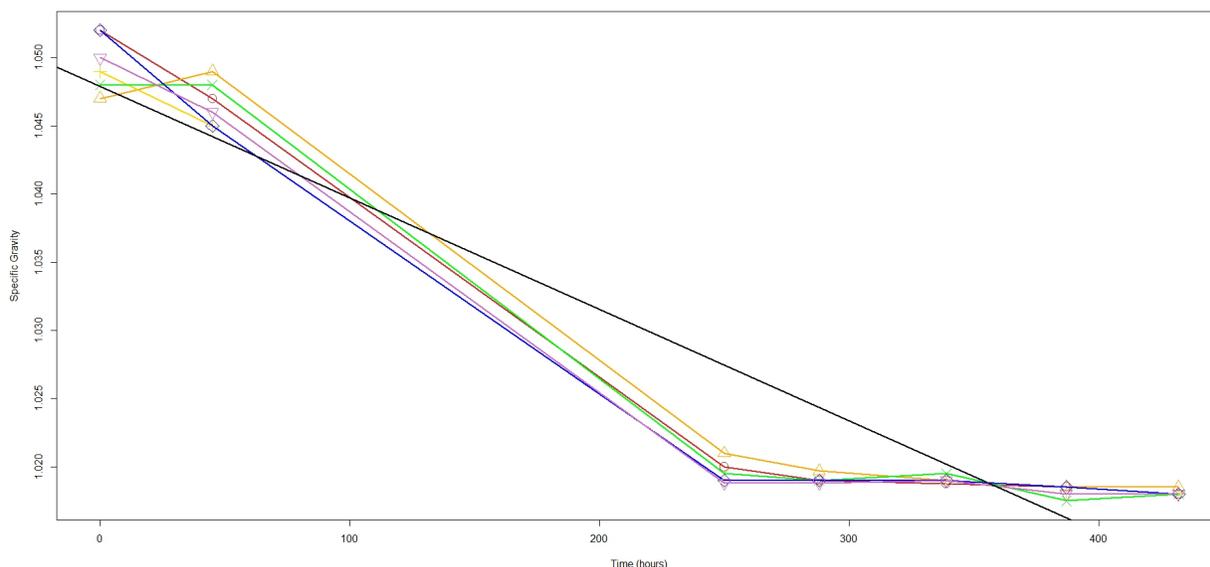


Figure 1: Cider1: A plot of specific gravity against time (hours), with least squares regression line (black). Symbols:  $\circ$   $\equiv$  vessel 1 (red) and  $\triangle$   $\equiv$  vessel 2 (orange), both of which received no yeast nutrient.  $+$   $\equiv$  vessel 3 (yellow) and  $\times$   $\equiv$  vessel 4 (green), both of which received half the recommended amount of yeast nutrient.  $\diamond$   $\equiv$  vessel 5 (blue) and  $\nabla$   $\equiv$  vessel 6 (purple), both of which received the full recommended amount of yeast nutrient.

From this plot of specific gravity versus time (hours), we see that the relationship between these two variables does not appear to be entirely linear. We also notice a large gap in specific gravity measurements between 50 and 250 hours. Cider1 was brewed immediately prior to a holiday week and measurements were not taken during this week. It should also be noted that vessels 2 and 4 experienced increases in specific gravity; generally this is not possible and is due to the limits of precision of the measurement instruments. Problems encountered with the limited precision of measurement instruments are not unique to this study and have been addressed in other studies [Defernez et al., 2007]. While the data may not suggest that the best model is a linear model, one starting model is a linear mixed-effects model (LME). Mixed-effects models have gained popularity over the last two decades, in part because of the development of reliable and efficient software for fitting and analyzing them [Pineiro and Bates, 2000]. A linear mixed-effects model is a good starting model for this data since we have repeated measurements on the

same subject over time (repeated specific gravity readings on each vessel over time).

We wish to determine if the amount of yeast supplement added to wort affects the rate of fermentation. Since we want to compare the three different treatment levels over time, we will use fixed effects for the treatment factor and the time factor. The six different vessels represent samples from the population about which we wish to make inferences, so we use random effects to model the vessel factor. We model the data as follows:

$$y_{ij} = \beta_0 + \beta_1 t_i + \tau_k + b_j + \varepsilon_{ij} \quad (1)$$

where

- $y_{ij}$  denotes the specific gravity of vessel  $j$  at time  $t_i$  for  $i = 1, \dots, 7$  and  $j = 1, \dots, 6$
- $\beta_0$  denotes the intercept of the linear model
- $\beta_1$  denotes the slope of the linear trend effect of specific gravity due to time
- $t_i$  denotes the time, in hours, at time point  $i$
- $\tau_k$  denotes the treatment effect due to the yeast nutrient in treatment level  $k$ , for  $k = 1, 2, 3$ , where  $\tau_1 = 0$  for the control treatment.
- $b_j$  denotes the random effect due to vessel  $j$  where it is assumed that  $b_j \sim N(0, \sigma_b^2)$
- $\varepsilon_{ij}$  denotes the random error of the observation at time  $t_i$  for vessel  $j$  where it is assumed that  $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ .

The estimates for the random-effects standard deviations are given by  $\hat{\sigma}_b = 1.614 \times 10^{-7}$  and  $\hat{\sigma}_\varepsilon = 0.005$ . Recall that  $\sigma_b$  represents the standard deviation between vessels and  $\sigma_\varepsilon$  represents the standard deviation within vessels. Thus we see that there is far less variability between vessels compared to within vessels. We note that while the p-value for time was 0, the p-values for treatment levels 2 and 3 were relatively high, 0.73 and 0.80, respectively. Rather than using

these results to reduce our model, we reconsider the fact that the relationship between specific gravity and time did not appear entirely linear, and this fact may contribute to poor model fit and compromised parameter estimates and p-values.

In an attempt to overcome problems due to nonlinearity, we first consider a transformation of the response variable, specific gravity ( $sg$ ), using a Box-Cox procedure [Sheather, 2009]. Box-Cox transformations are variable transformations that are commonly used to transform a strictly positive response variable. Two assumptions of linear models are that the response function is linear and the error terms come from a normal distribution; that is, the residuals come from a normal distribution with mean 0 and variance  $\sigma_{\epsilon}^2$ .

Box-Cox transformations use the method of maximum likelihood to identify the power to which to response variable should be raised, denoted by  $\lambda$ , in order to correct the skewness in the distribution of the error terms, as well as correct unequal error variances and nonlinearity of the regression function [Neter et al., 1996]. The unfortunate drawback of using variable transformations is that the interpretation of the response variable is not as originally measured. Variable transformations make the model more difficult to interpret and as a result, it is common practice to choose a value in the neighborhood of  $\lambda$  that makes the power transformation easy to understand [Neter et al., 1996]. Furthermore, due to the lack of interpretability of the response variable, we will only use the Box-Cox transformation if it is beneficial. We will attempt a Box-Cox transformation to transform the response variable,  $sg$ , to a new variable,  $(sg)^{\lambda}$ , where the value of  $\lambda$  is estimated with a Box-Cox procedure using the method of maximum likelihood. Note that Figure 2 provides plots of the log-likelihood against  $\lambda$  for the Cider1 data.

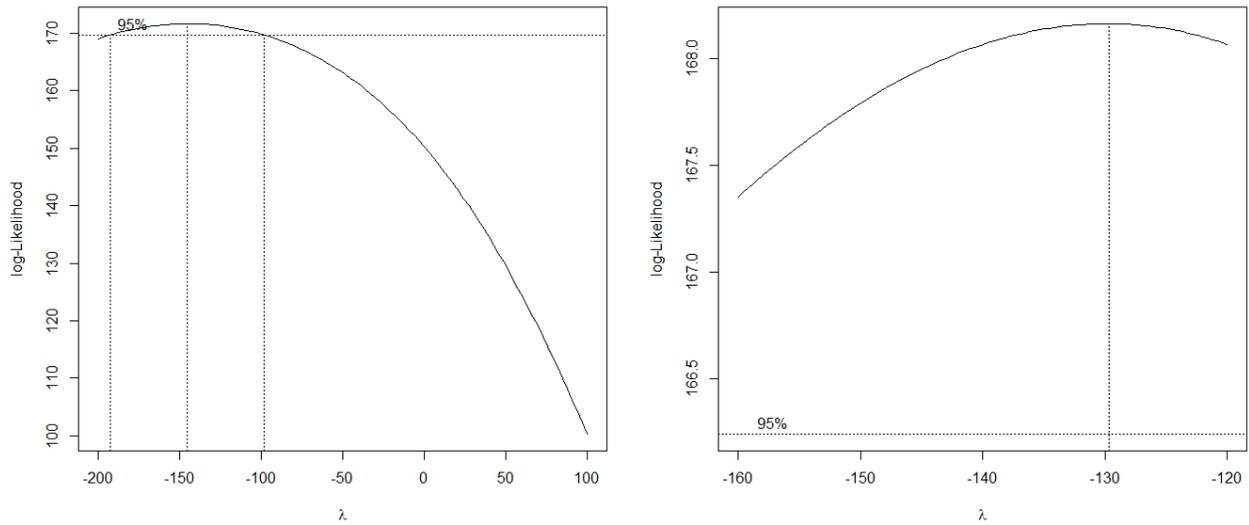


Figure 2: Cider1: Plots of log-likelihood against  $\lambda$  for proposed Box-Cox transformation

The value of  $\lambda$  that maximizes the log-likelihood and the 95% confidence limits are marked on each plot. The values on the horizontal axis of the right hand plot are restricted so that it is easier to identify the value of  $\lambda$  that maximizes the log-likelihood, and from Figure 2 we see that this value is  $\lambda \approx -130$ . That is, performing the Box-Cox transformation suggests that the appropriate transformation for this data results in the following model:

$$y_{ij}^{-130} = \beta_0 + \beta_1 t_i + \tau_k + b_j + \varepsilon_{ij}, \quad (2)$$

We note that  $\lambda = -130$  does not provide an easily interpretable transformation, but in order to assess whether performing the Box-Cox transformation was beneficial, we graphically explore and compare both the original model and the transformed model, in Figure 3.

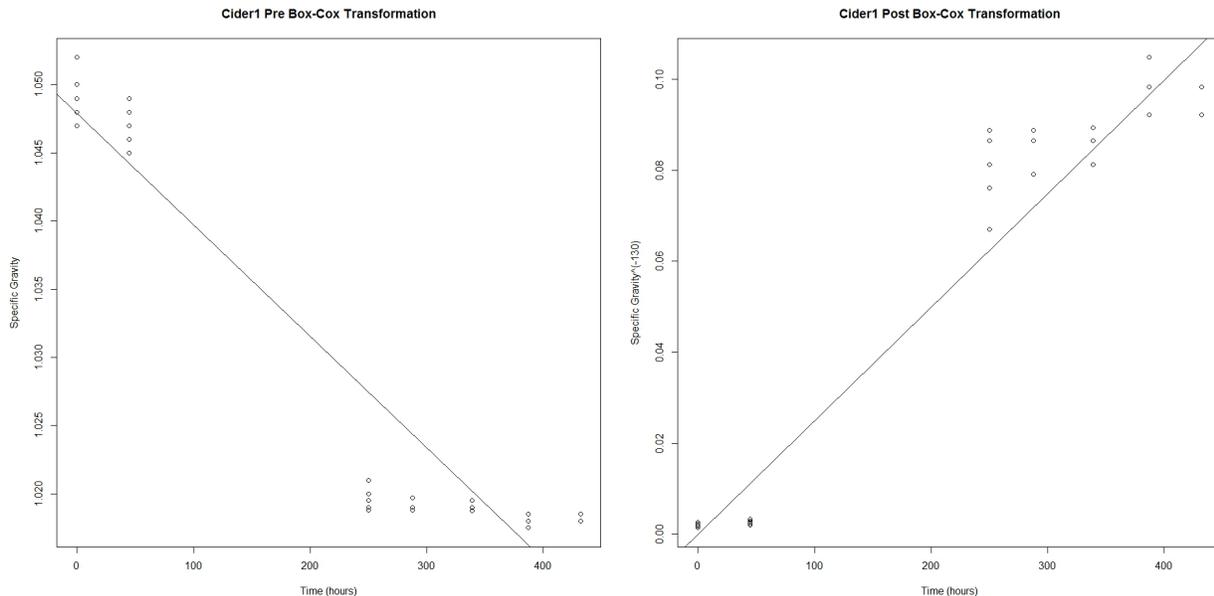


Figure 3: Cider1: Pre/Post Box-Cox Transformation

Comparing the plots of the original data and the transformed data, we observe that the Box-Cox transformation did not overcome our problems due to nonlinearity.

Given that our attempt at a variable transformation did not prove to be useful, we will proceed by further graphically exploring our original model. To better determine whether the treatment effects were significant, we group together the two replicates within each treatment so there are three panels, one for each of the three treatment levels, which can be seen in Figure 4

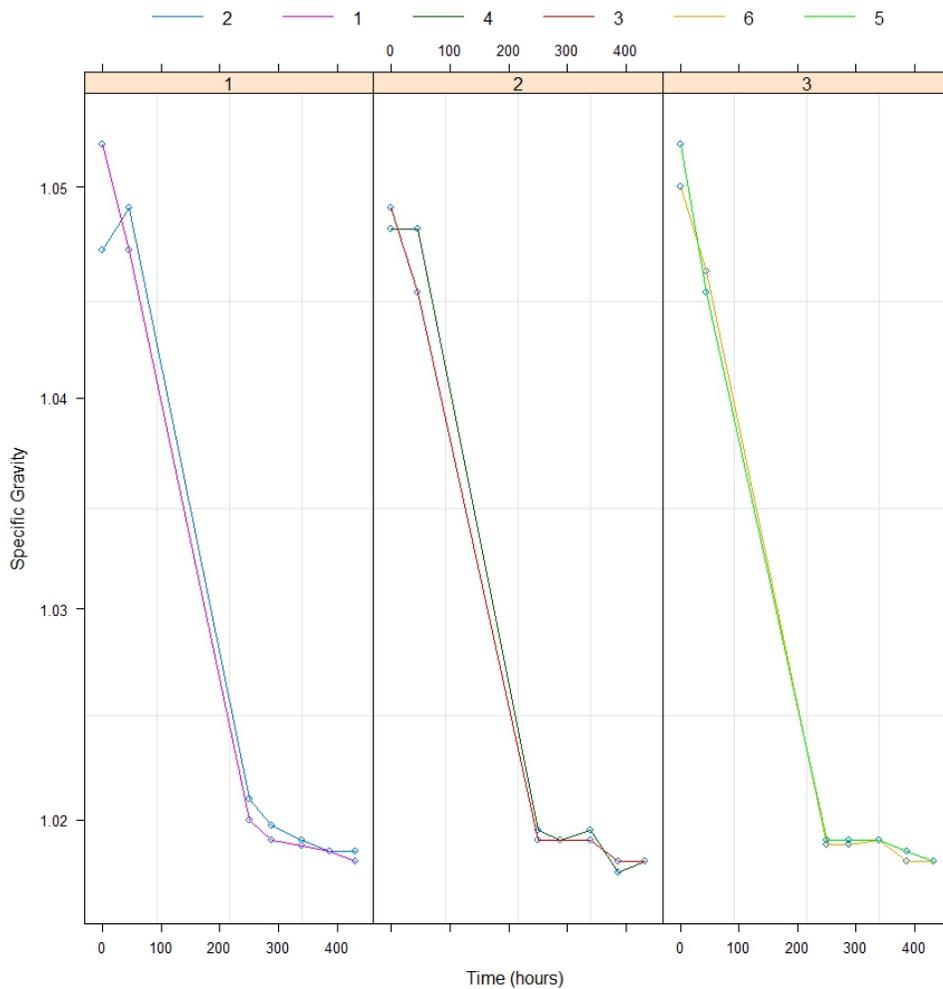


Figure 4: Cider1: Grouping replicates within each treatment level

Before we continue, we will consider a problem with our original model that we have not addressed thus far. The goal of model (1) was to determine whether there is a significant difference in the specific gravity of each vessel at each time point. But realistically, we know that the specific gravity of each vessel should not be very different at each time point, particularly at the beginning and end of fermentation. Since the cider in each of the six vessels should be approximately uniform, each of the vessels should have approximately the same alcohol content, with some variability due to the initial sugar content of the apple juice in each individual vessel. Similarly, when the fermentation of the cider nears completion, the specific gravity of each of the

vessels will begin to plateau to approximately the same final gravity. Thus, we hypothesize that model (1) may not be as useful in quantifying the effect of yeast nutrient since we are including specific gravity readings at each time point. More specifically, since the majority of the specific gravity readings taken for Cider1 are at the beginning and end of fermentation, and very few are in a range where the differences should be at a maximum, we acknowledge that model (1) may not be appropriate for answering our overarching question (with this data).

In order to determine whether the amount of yeast supplement added to each vessel has an effect on fermentation rate we may want to consider the time point at which the specific gravity of each individual vessel begins to plateau to this final gravity. In this case, the final gravity of each vessel was 1.018. Closer examination of the graph above will help us to determine the time point at which each vessels begins to plateau to the final gravity 1.018. Upon closer examination of Figure 4, we see that around hour 200, the specific gravity in vessels 3-6 appears to begin to plateau. Thus, we closely examine the difference in specific gravity after hour 200, which can be seen in the plot below. Note, again, that vessels 4 and 6 experience increases in specific gravity, which we know is not possible and is a result of the limit of precision of measurement instruments.

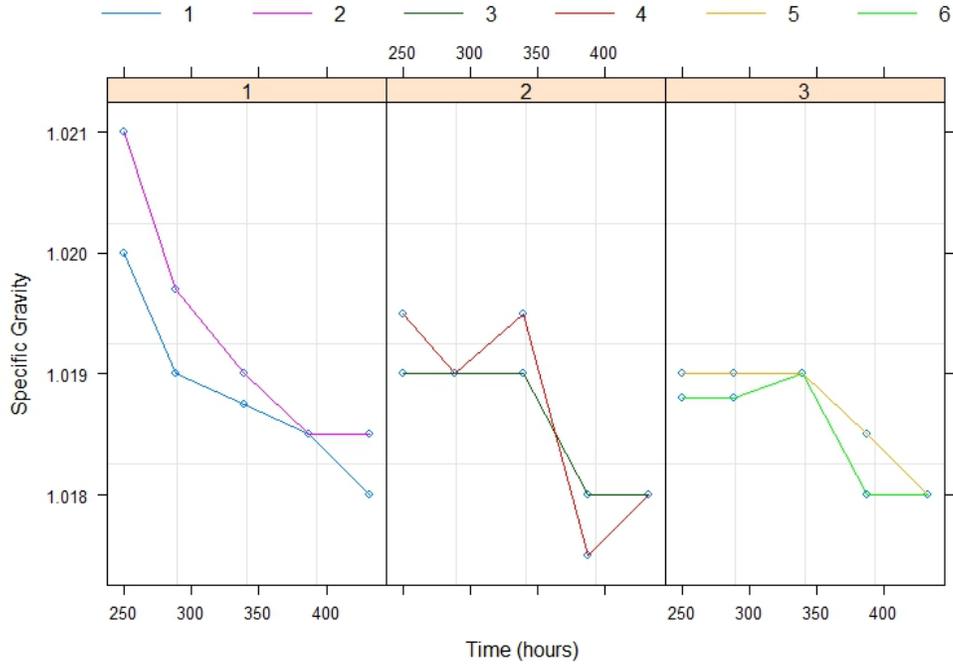


Figure 5: Cider1: Grouping replicates within each treatment level

Upon examination of Figure 5, we see that at hour 250, the specific gravity of vessels 3-6 is less than vessels 1-2. To verify this, we will construct a linear model that will test whether there is a significant difference in the specific gravity of each vessel at hour 250. We model the data using the one-way ANOVA model:

$$y_j = \beta_0 + \tau_k + \varepsilon_j, \quad (3)$$

where

- $y_j$  denotes the specific gravity of vessel  $j$ , where  $j = 1, \dots, 6$
- $\beta_0$  denotes the intercept of the linear model and the mean of the control treatment
- $\tau_k$  denotes the treatment effect due to the yeast nutrient in treatment level  $k$ , for  $k = 1, 2, 3$ , where  $\tau_1 = 0$  for the control treatment.

- $\varepsilon_j$  denotes the random error of the observation for vessel  $j$  where it is assumed that  $\varepsilon_j \sim N(0, \sigma_\varepsilon^2)$ .

The p-value for testing  $\beta_1 = 0$  is highly significant, but this test is not particularly useful as it tests whether the mean specific gravity of the control treatment is zero. The two other p-values test whether treatments 2 and 3 are equal to the control treatment, respectively. We find that treatment 2 is now significant at the  $\alpha = 0.10$  level and treatment level 3 is significant at the  $\alpha = 0.05$  level. Recall that in treatment level 2, vessels 3 and 4 received half the recommended amount of yeast nutrient and in treatment level 3, vessels 5 and 6 received the full recommended amount of yeast nutrient. Thus we have evidence to believe that at hour 250, treatment level 3 was most significantly different from the control treatment (treatment level 1).

We have had some success in determining which amount of yeast nutrient has the greatest effect on the rate of fermentation. We see that model (3) shows that treatment factors 2 and 3 are significantly different from the control treatment at hour 250. This gives us reason to believe that including time as a fixed effect may not be the best way to model this data. Since time has such a significant effect on specific gravity, it could mute the effect of the different treatment levels if time is not modeled accurately. Thus we will proceed by attempting to take a different approach to modeling our data.

Recall that we wish to determine if the amount of yeast nutrient added to unfermented cider affects the rate of fermentation. To accomplish this, we will begin by modeling the deviations at each time point ( $y_{ij} - \bar{y}_i$ ) rather than the response  $y_{ij}$ , since we know that our response function does not follow a linear trend. Modeling the residuals will allow us to remove the variability due to time in an attempt to identify other sources of variability in our experiment. We will begin this new approach by estimating the mean specific gravity at each time point. A plot showing the function that connects the mean specific gravities at each time point was generated in R and can be seen below in Figure 6 .



- $\bar{y}_i$  denotes the mean specific gravity at time  $t_i$
- $\beta_0$  denotes the intercept of the linear model
- $\tau_k$  denotes the treatment effect due to the yeast nutrient in treatment level  $k$ , for  $k = 1, 2, 3$ , where  $\tau_1 = 0$  for the control treatment.
- $b_j$  denotes the random effect due to vessel  $j$  where it is assumed that  $b_j \sim N(0, \sigma_b^2)$
- $\varepsilon_{ij}$  denotes the random error of the observation at time  $t_i$  for vessel  $j$  where it is assumed that  $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ .

Modeling the residuals proves to be useful for this data set. We obtain the following estimates for random-effects standard deviations:  $\hat{\sigma}_b = 5.54 \times 10^{-8}$  and  $\hat{\sigma}_\varepsilon = 0.00097$ . Recall that  $\sigma_b$  represents the standard deviation between vessels and  $\sigma_\varepsilon$  represents the standard deviation within vessels. Thus we see that there is much less variability between vessels compared to within vessels, but we note that these values are both relatively small. We also obtain better p-values for treatment factors. For treatment level 2, our p-value is now 0.1623 and for treatment level 3, our p-value is now 0.2736. In model (1) the p-value for treatment level 2 was 0.7291 and the p-value for treatment level 3 was 0.8007. We see that our p-values are much closer to significance in comparison to the original model.

Through the analysis of Cider1 data, we have determined that a majority of variability in specific gravity is explained by the time course. We also found yeast nutrient to be moderately significant in increasing fermentation rate at hour 250. We also determined that our estimate of the standard deviation between vessels,  $\hat{\sigma}_b$ , was close to zero because there is very little variability in specific gravity that is not explained by time and treatment effects.

## IPA38

Recall that we are using the data from IPA38 to address the following question: Given that we know that the presence of trub in the fermenting wort may affect the rate of fermentation, does the order in which the fermenters are filled affect the rate of fermentation? Note that the order of fill was such that one gallon was emptied into vessel 1 followed by one gallon into the carboy. Next, another gallon was emptied into vessel 2 followed by another (second) gallon into the carboy. The vessels were continually filled in this manner, so that vessel 1 contained wort near the bottom of the brew kettle and vessel 6 contained wort near the top of the brew kettle. After the fermentation of IPA38 was complete, we began the model building process by graphically considering our data.

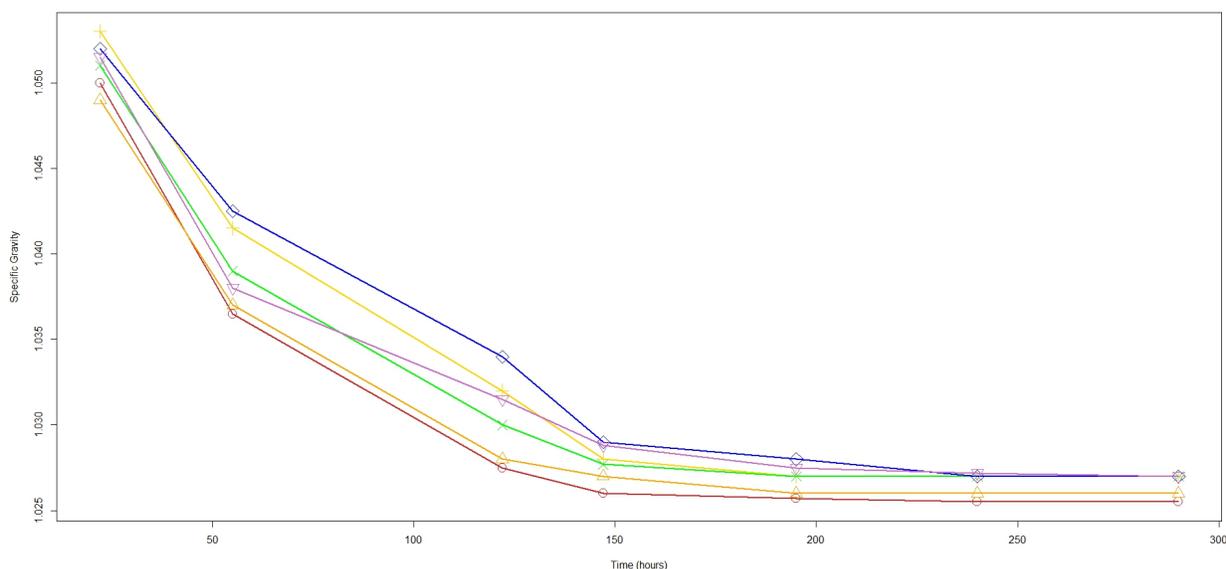


Figure 7: IPA38: A plot of specific gravity against time (hours). Symbols:  $\circ$   $\equiv$  vessel 1 (red),  $\triangle$   $\equiv$  vessel 2 (orange),  $+$   $\equiv$  vessel 3 (yellow) and  $\times$   $\equiv$  vessel 4 (green),  $\diamond$   $\equiv$  vessel 5 (blue) and  $\nabla$   $\equiv$  is vessel 6 (purple), where vessels 1-6 were filled in chronological order, with vessel 1 being filled first and vessel 6 being filled last.

Notice that the plot in Figure 7 looks similar to the plot in Figure 1. That is, this plot of specific gravity versus time (hours) shows that the relationship between these two variables does

not appear to be entirely linear. We also notice a gap in specific gravity measurements between 50 and approximately 125 hours. Given that we know that a linear mixed-effects (LME) model does not give a good fit to the data, but we did have some success with the Cider1 data after removing the variability due to time and modeling the residuals. Thus, we will proceed using this approach. We will begin by estimating the mean specific gravity at each time point and proceed to model the residuals. A plot showing the function that connects the mean specific gravities at each time point can be seen below in Figure 8.

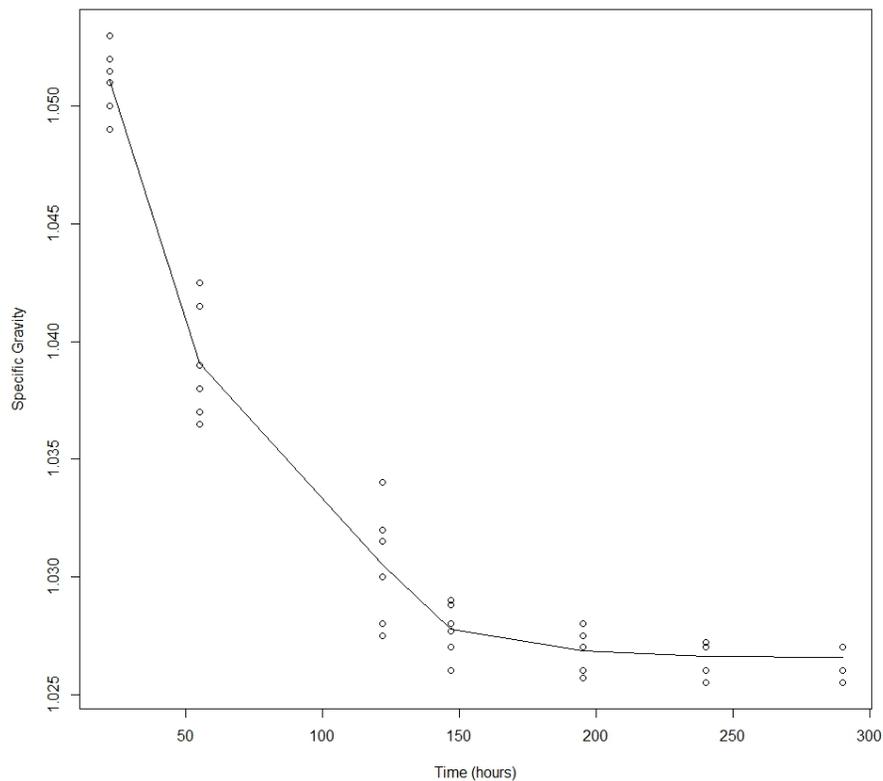


Figure 8: IPA38: Modeling Mean Specific Gravity

Now that we have estimated the mean specific gravity at each time point, we can proceed to model the residuals  $(y_{ij} - \bar{y}_i)$ , where  $y_{ij}$  is the specific gravity of vessel  $j$  at time  $t_i$  and  $\bar{y}_i$  is the mean specific gravity at time  $t_i$ . Recall that modeling the residuals allows us to remove the

variability due to time in an attempt to identify other sources of variability in specific gravity. We can model the residuals with a linear mixed-effects model using fixed effects for the treatment factor and random effects for the vessel factor. We model the data as follows:

$$y_{ij} - \bar{y}_i = \beta_0 + b_j + \varepsilon_{ij}, \quad (5)$$

where

- $y_{ij}$  denotes the specific gravity of vessel  $j$  at time  $t_i$  for  $i = 1, \dots, 7$  and  $j = 1, \dots, 6$
- $\bar{y}_i$  denotes the mean specific gravity at time  $t_i$
- $\beta_0$  denotes the intercept of the linear model
- $b_j$  denotes the random effect due to vessel  $j$  where it is assumed that  $b_j \sim N(0, \sigma_b^2)$
- $\varepsilon_{ij}$  denotes the random error of the observation at time  $t_i$  for vessel  $j$  where it is assumed that  $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ .

From R, we obtain the following estimates for random-effects standard deviations:  $\hat{\sigma}_b = 0.00125$  and  $\hat{\sigma}_\varepsilon = 0.00087$ . Recall that  $\sigma_b$  represents the standard deviation between vessels and  $\sigma_\varepsilon$  represents the standard deviation within vessels. Thus we see that there is more variability between vessels compared to within vessels.

We want to use the output to determine whether or not the order of fill affects specific gravity by comparing the mean specific gravities for each vessel. We will address this question by first extracting the random effects  $b_j$ ,  $j = 1, \dots, 6$ , from the model and determining the maximum distance between any two random effects. We found the maximum distance between any two random effects is between vessels 1 and 5, and we found the maximum distance to be  $|b_5 - b_1| = .003$ . The standard deviation of the random effects is given by  $\hat{\sigma}_b^2 = .00125$ . Note also that for any

vessels  $\alpha$  and  $\gamma$ , we have

$$\begin{aligned} \text{Var}(b_\alpha + b_\gamma) &= \text{Var}(b_\alpha) + \text{Var}(b_\gamma) + 2\text{Cov}(b_\alpha, b_\gamma) \\ &= \sigma_b^2 + \sigma_b^2 + 2(0), && \text{since Cov}(b_\alpha, b_\gamma) = 0 \\ &= 2\sigma_b^2 \end{aligned}$$

We can now find a standardized score for the maximum distance between any two random effects.

We have:

$$\begin{aligned} z_{\text{max.diff}} &= \frac{(b_5 - b_1) - E(b_5 - b_1)}{\sqrt{2\hat{\sigma}_b^2}} \\ &= \frac{(b_5 - b_1) - [E(b_5) - E(b_1)]}{\sqrt{2\hat{\sigma}_b^2}} \\ &= \frac{(b_5 - b_1) - 0}{\sqrt{2\hat{\sigma}_b^2}}, && \text{since } b_j \sim N(0, \sigma_b^2) \text{ for all } j \\ &= \frac{.003}{\sqrt{2 * .00125^2}} = 1.72454 \end{aligned}$$

We compare the above standardized score with a critical value from a Student's  $t$ -distribution.

We use the Bonferroni correction to adjust the critical value to reflect that multiple pairs of random effects could be compared. Thus, our critical value should be based on a Type I error rate of  $\frac{\alpha}{\binom{6}{2}}$ , where  $\binom{6}{2}$  is the number of possible comparisons. The critical value from a Student's  $t$ -distribution with a Type I error rate of  $\frac{.05}{\binom{6}{2}}$  is approximately -3.1439. Since our standardized score of 1.72454 is less than the magnitude of our critical value of 3.1439, we do not have evidence to support that time order of fill has a significant effect on fermentation rate.

After extracting the estimates of the random effects and obtaining a standardized score for the maximum distance between any two random effects, we determined that time order of fill has no significant effect on fermentation rate. However, we also note that the power of our test is modest given our small sample size. We have also found that a lack of specific gravity measurements made

the data more difficult to model, and thus for future data sets, our goal will be to obtain more data near the beginning of fermentation. The Cider1 and IPA38 data sets have shown us that having a larger number of time points would have given us a better idea of how the specific gravity is changing near the beginning of fermentation when yeast-activity is at a maximum.

## IPA39

Recall that we are using the data from IPA39 to attempt to quantify the error in refractometer readings in comparison to hydrometer readings over time. The data gathered from IPA39 is unique within this study in that it is the only data set for which refractometer and hydrometer measurements were taken at each time point. After the fermentation of IPA39 was complete, we began the model building process by graphically considering our data. Note that two separate plots are shown below, one for refractometer specific gravity versus time (Figure 9) and one for hydrometer specific gravity versus time (Figure 10).

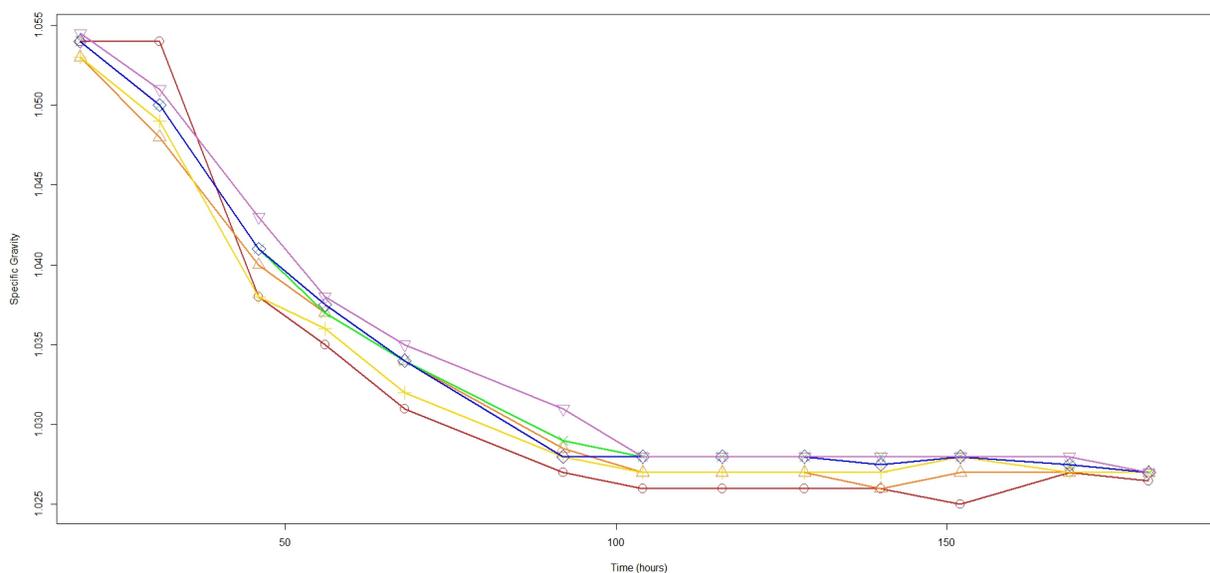


Figure 9: IPA39: A plot of specific gravity against time (hours), where a refractometer was used to measure specific gravity. Symbols:  $\circ$   $\equiv$  vessel 1 (red),  $\triangle$   $\equiv$  vessel 2 (orange),  $+$   $\equiv$  vessel 3 (yellow) and  $\times$   $\equiv$  vessel 4 (green),  $\diamond$   $\equiv$  vessel 5 (blue) and  $\nabla$   $\equiv$  vessel 6 (purple), where vessels 1-6 were filled in chronological order, with vessel 1 being filled first and vessel 6 being filled last.

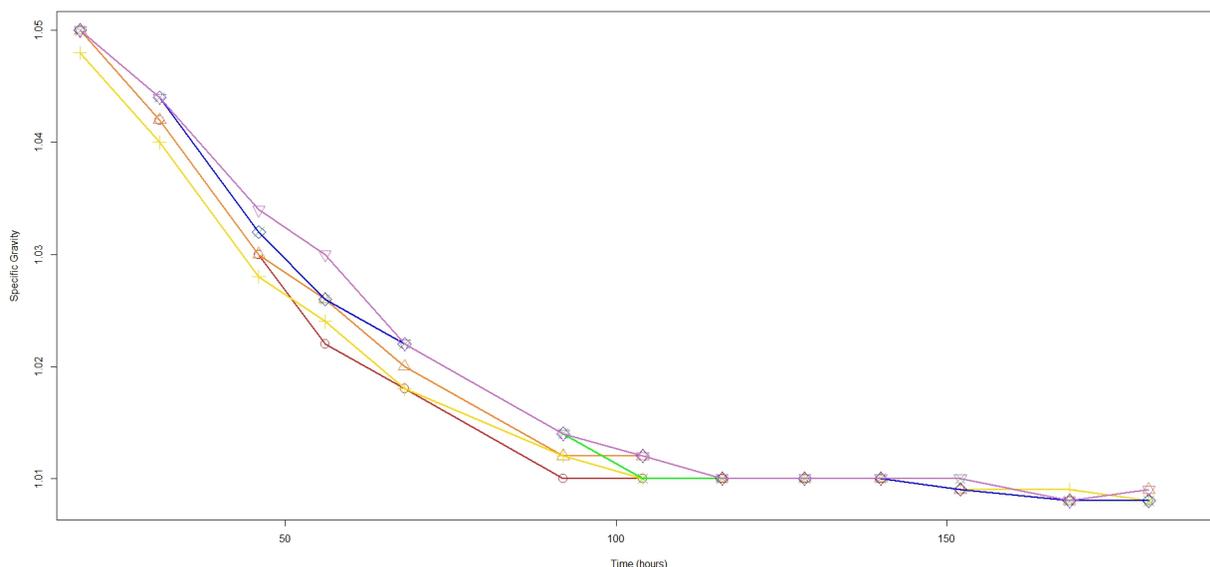


Figure 10: IPA39: A plot of specific gravity against time (hours), where a hydrometer was used to measure specific gravity. Symbols:  $\circ$   $\equiv$  vessel 1 (red),  $\triangle$   $\equiv$  vessel 2 (orange),  $+$   $\equiv$  vessel 3 (yellow) and  $\times$   $\equiv$  vessel 4 (green),  $\diamond$   $\equiv$  vessel 5 (blue) and  $\nabla$   $\equiv$  is vessel 6 (purple), where vessels 1-6 were filled in chronological order, with vessel 1 being filled first and vessel 6 being filled last.

We will begin by addressing our question concerning the quantification of the error in refractometer readings. Recall that refractometer readings are only accurate for original gravity, as they are affected by the presence of alcohol. While refractometers are generally more costly than hydrometers, we noted that there are several advantages to using refractometers to measure specific gravity. Refractometers require a very small amount of sample, are independent of temperature, and give quicker readings. We have noted that refractometers have several advantages, but recall that the overarching disadvantage of refractometers is that they do not give accurate readings of specific gravity after fermentation begins.

For a homebrewer, it could be advantageous to have a simple formula that would allow them to convert a refractometer reading of specific gravity to a predicted hydrometer reading of specific gravity. This could potentially save the homebrewer both time and a substantial volume of beer/cider. When considering the relationship between refractometer and hydrometer readings,

a natural starting model would be the simple linear regression model relating the refractometer readings and hydrometer readings. This model can be written as

$$y_i = \beta_0 + \beta_1 x_i + \varepsilon_i, \quad (6)$$

where

- $y_i$  denotes the hydrometer reading of specific gravity for observation  $i$
- $x_i$  denotes the refractometer reading of specific gravity for observation  $i$
- $\varepsilon_i$  denotes the random error of the observation, where it is assumed that  $\varepsilon_i \sim N(0, \sigma_\varepsilon^2)$
- $\beta_0, \beta_1$  denote the intercept and slope of the regression line, respectively

The plot corresponding to the linear regression model (6) can be seen below in Figure 11.

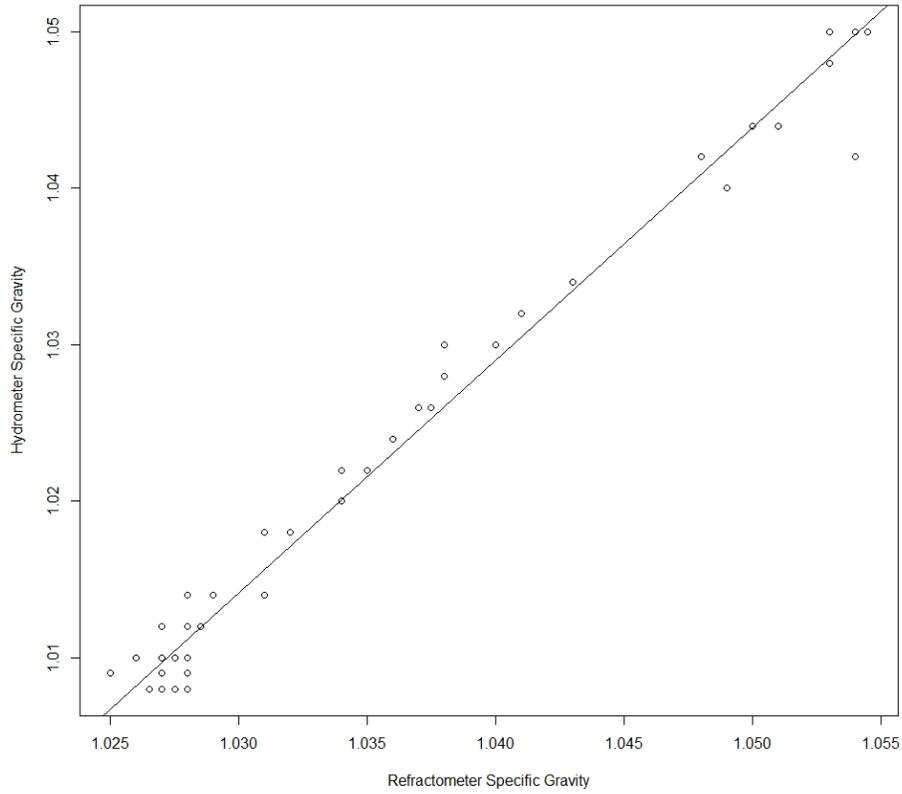


Figure 11: IPA39: Hydrometer Specific Gravity vs. Refractometer Specific Gravity

We find that the p-values of both of our coefficient estimates are significant at the  $\alpha = 0.001$  level. We also find that the residual standard error is estimated at 0.001775. This tells us that our model predicts hydrometer specific gravity to within  $2\sigma_\varepsilon = 2(0.001775) = 0.0036$  approximately 95% of the time.

Note that model (6) assumes that the observations are independent. However, since the observations were obtained through re-sampling the six vessels multiple times, we consider

$$y_{ij} = \beta_0 + \beta_1 x_i + b_j + \varepsilon_{ij} \quad (7)$$

where

- $y_{ij}$  denotes the hydrometer reading of specific gravity for vessel  $j$  at time  $t_i$  for  $i = 1, \dots, 13$  and  $j = 1, \dots, 6$
- $x_{ij}$  denotes the refractometer reading of specific gravity for vessel  $j$  at time  $t_i$  for  $i = 1, \dots, 13$  and  $j = 1, \dots, 6$
- $\beta_0$  denotes the intercept of the linear model
- $\beta_1$  denotes the slope of the linear trend effect of specific gravity due to time
- $b_j$  denotes the random effect due to vessel  $j$  where it is assumed that  $b_j \sim N(0, \sigma_b^2)$
- $\varepsilon_{ij}$  denotes the random error of the observation at time  $t_i$  for vessel  $j$  where it is assumed that  $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ .

We find that we have the following estimates for random-effects standard deviations:  $\hat{\sigma}_b = 9.31 \times 10^{-8}$  and  $\hat{\sigma}_\varepsilon = 0.00178$ . Recall that  $\sigma_b$  represents the standard deviation between vessels and  $\sigma_\varepsilon$  represents the standard deviation within vessels. Thus we see that there is much more variability within vessels compared to between vessels. It should be noted that since our estimate  $\hat{\sigma}_b$  is so close to zero, the fitted regression equation for the linear mixed-effects model matches the simple linear regression model. The linear mixed-effects model allows for random intercepts for each vessel, but these random intercepts turn out to be no different from the intercept  $\beta_0$  of the simple linear regression model in (6).

We have found and presented a simple linear regression model that can be used to predict the hydrometer reading of specific gravity using the refractometer reading of specific gravity for IPA39 and other beers made using a similar recipe.

## Cider2

The role of zinc in brewing and the effect of zinc on yeast viability has been studied extensively over the past four decades [Helin and Slaughter, 1977]. Yeast growth and metabolism are influenced by several trace elements, particularly zinc, as it is an essential metal ion for several enzymes, including alcohol dehydrogenase [De Nicola and Walker, 2011, Skands et al., 1997]. It is well known that zinc levels in unfermented beer and cider have an influence on fermentation, and the general consensus among several authors is that zinc levels of 0.4 – 1.07 ppm (parts per million) are sufficient [Helin and Slaughter, 1977, De Nicola and Walker, 2011, Skands et al., 1997]. In this study, we employed the use of a zinc sulfate solution, with two levels of zinc concentration: 0.47 ppm 0.75 ppm. We will refer to these concentrations as the smaller and larger amounts of zinc, respectively. Brewers add zinc to unfermented beer and cider in several different forms, a few of which include zinc acetate, zinc sulfate, and zinc chloride. However, homebrewers may currently be less likely to supplement their unfermented beer and cider with zinc as it is more difficult to obtain (even in specialty homebrewing stores) and is more difficult to work with in comparison to yeast nutrient.

It is a common practice for homebrewers to supplement their unfermented beer or cider with some sort of yeast nutrient. Yeast nutrient is a blend of vitamins, minerals, inorganic nitrogen, organic nitrogen, zinc, phosphates and other trace elements that claim to benefit yeast growth and complete fermentation. Homebrewers view it as a quick and easy way to promote yeast health and prevent sluggish fermentation. Recall from Cider1, Wyeast laboratories recommends using half a teaspoon of yeast nutrient per five gallons of wort. Wyeast claims that supplementing wort with yeast nutrient will reduce lag time and enhance yeast viability. Given that previous research has suggested that zinc has the greatest effect on yeast health and reduction of fermentation problems, it may be in the best interest of homebrewers to further explore other options for zinc supplementation.

We constructed six treatment levels for this experiment. In treatment level 1, vessels 1 and

2 received no zinc or yeast nutrient; treatment level 1 is our control treatment. In treatment level 2, vessels 3 and 4 received half the recommended amount of yeast nutrient, and in treatment level 3, vessels 5 and 6 received the full recommended amount of yeast nutrient. In treatment level 4, vessels 7 and 8 received the smaller amount of zinc, and in treatment level 5, vessels 9 and 10 received the larger amount of zinc. Finally, in treatment level 6, vessels 11 and 12 received half the recommended amount of yeast nutrient and the smaller amount of zinc. After the fermentation of Cider2 was complete, we began the model building process by graphically considering our data.

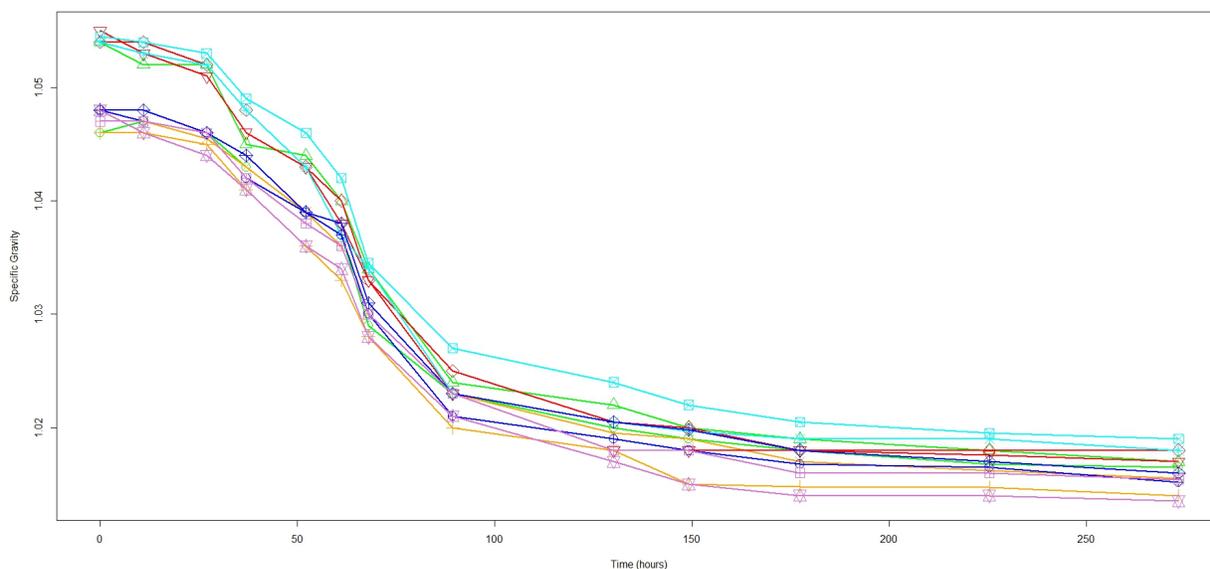


Figure 12: Cider2: A plot of specific gravity against time (hours).

Symbols:  $\circ$   $\equiv$  vessel 1 (green),  $\triangle$   $\equiv$  vessel 2 (green), both of which were in the control treatment, and as such, received no yeast nutrient or zinc.

$+$   $\equiv$  vessel 3 (orange) and  $\times$   $\equiv$  vessel 4 (orange), both of which received half the recommended amount of yeast nutrient.

$\diamond$   $\equiv$  vessel 5 (red) and  $\nabla$   $\equiv$  vessel 6 (red), both of which received the full recommended amount of yeast nutrient.

$\boxtimes$   $\equiv$  vessel 7 (cyan) and  $\ast$   $\equiv$  vessel 8 (cyan), both of which received the smaller amount of zinc.

$\diamond$   $\equiv$  vessel 9 (blue) and  $\oplus$   $\equiv$  vessel 10 (blue), both of which received the larger amount of zinc.

$\star$   $\equiv$  vessel 11 (purple) and  $\boxplus$   $\equiv$  vessel 12 (purple), both of which received half the recommended amount of yeast nutrient and the smaller amount of zinc.

We wish to determine if the amount of yeast supplement and/or zinc added to unfermented

cider affects the rate of fermentation. To accomplish this, we will make use of our past experience with Cider1, IPA38, and IPA39. Similar to the approach we took with the Cider1 and IPA38 data sets, we will begin by modeling the residuals  $(y_{ij} - \bar{y}_i)$  rather than the response  $y_{ij}$  since we know that our response variable, specific gravity (`refracsg`) does not follow a linear trend. Moreover, since we know that time has a significant effect on specific gravity, we model the residuals to remove the variability due to time in an attempt to identify further sources of variability in our experiment.

Since we want to compare the six different treatment levels over time, we will use fixed effects for the treatment factor (`trt.factor`). The six different vessels represent samples from the population about which we wish to make inferences, so we use random effects to model the vessel factor. We model the residuals as follows:

$$y_{ij} - \bar{y}_i = \beta_0 + \tau_k + b_j + \varepsilon_{ij} \quad (8)$$

where

- $y_{ij}$  denotes the specific gravity of vessel  $j$  at time  $t_i$  for  $i = 1, \dots, 13$  and  $j = 1, \dots, 12$
- $\bar{y}_i$  denotes the mean specific gravity at time  $t_i$
- $\beta_0$  denotes the intercept of the linear model
- $\tau_k$  denotes the treatment effect due to the yeast nutrient and/or zinc in treatment level  $k$ , for  $k = 1, \dots, 6$ , where  $\tau_1 = 0$  for the control treatment.
- $b_j$  denotes the random effect due to vessel  $j$  where it is assumed that  $b_j \sim N(0, \sigma_b^2)$
- $\varepsilon_{ij}$  denotes the random error of the observation at time  $t_i$  for vessel  $j$  where it is assumed that  $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ .

We find that we have the following estimates for random-effects standard deviations:  $\hat{\sigma}_b = 0.001327$  and  $\hat{\sigma}_\varepsilon = 0.00115$ . Recall that  $\sigma_b$  represents the standard deviation between vessels and

$\sigma_\varepsilon$  represents the standard deviation within vessels. Thus we see that there is more variability between vessels compared to within vessels.

Further inspecting the output, we find that we have two p-values that are significant at the  $\alpha = 0.1$  level: treatment levels 2 and 6. In treatment level 2, vessels 3 and 4 received half the recommended amount of yeast nutrient, and in treatment level 6, vessels 11 and 12 received half the recommended amount of yeast nutrient and the smaller amount of zinc. One may find these results surprising as one would expect treatment level 3 (full-recommended amount of yeast nutrient) to be significantly different from the control treatment if treatment level 2 (half-recommended amount of yeast nutrient) was found to be significantly different from the control treatment. Keeping in mind that treatment levels 2 and 6 were found to be significant, reconsider the graph of the Cider2 data.

Notice in Figure 12, before fermentation began (at hour 0), the vessels show a natural grouping into two groups. A close examination of the two groups of vessels in Figure 12 shows that treatment levels 2 and 6 fall into the lower group. This was not known at the beginning of the study, since the experimenter was “blinded” to the identity of the treatments assigned to each vessel. Note that in context, this means that the sugar content of the vessels in the lower group was less than the sugar content of the vessels in the higher group. Given that the vessels in treatment levels 2 and 6 went into the fermentation stage with a lower specific gravity, it is likely that this is what is causing them to be determined as significantly different from the control treatment. With this in mind, we wish to find a different approach, one that will allow us to remove the variability in specific gravity due to the amount of sugar initially found in each vessel.

Let  $y_{ij}$  denote the specific gravity of vessel  $j$  at time point  $i$ . In order for us to remove the variability in specific gravity due to the amount of sugar initially found in each vessel, we will consider the variable  $d_{ij} = y_{ij} - y_{1j}$ . The variable  $d_{ij}$  represents the specific gravity of vessel  $j$  at time point  $i$ , after subtracting off the original gravity of vessel  $j$ . We will refer to this new variable  $d_{ij}$  as the specific gravity difference of vessel  $j$  at time point  $i$ . A plot of specific gravity differences versus time is shown below in Figure 13

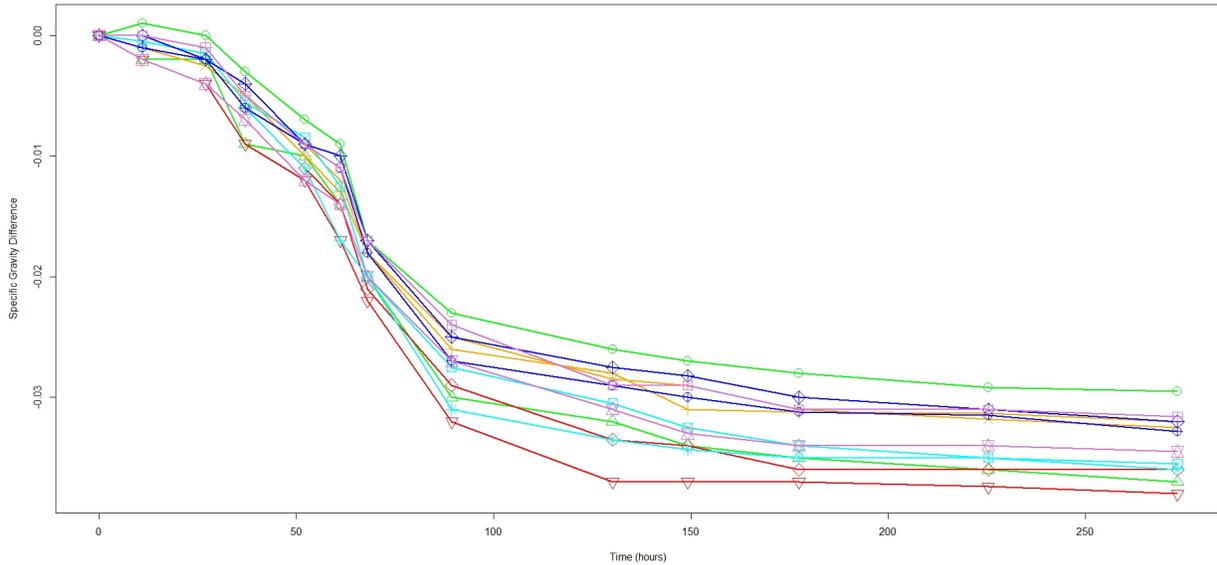


Figure 13: Cider2: Specific Gravity Differences versus Time

Notice that the specific gravity difference is initially equal to zero for all vessels. Now that we have removed the variability in specific gravity due to the amount of sugar initially found in each vessel, we will proceed by removing the variability due to time. We will approach this in a manner similar to that of modeling residuals, in that we will be modeling the mean corrected differences,  $(d_{ij} - \bar{d}_i)$  rather than modeling the specific gravity differences  $d_{ij}$ .

Since we want to compare the six different treatment levels over time we will, again, use fixed effects for the treatment factor (`trt.factor`). The six different vessels represent samples from the population about which we wish to make inferences, so we use random effects to model the vessel factor. We will model the mean-corrected specific gravity differences as follows:

$$d_{ij} - \bar{d}_i = \beta_0 + \tau_k + b_j + \epsilon_{ij} \quad (9)$$

where

- $d_{ij} = y_{ij} - y_{1j}$  denotes the specific gravity difference of vessel  $j$  at time  $t_i$  for  $i = 1, \dots, 13$

and  $j = 1, \dots, 12$

- $\bar{d}_i$  denotes the mean specific gravity difference at time  $t_i$
- $\beta_0$  denotes the intercept of the linear model
- $\tau_k$  denotes the treatment effect due to the yeast nutrient and/or zinc in treatment level  $k$ , for  $k = 1, \dots, 6$ , where  $\tau_1 = 0$  for the control treatment.
- $b_j$  denotes the random effect due to vessel  $j$  where it is assumed that  $b_j \sim N(0, \sigma_b^2)$
- $\varepsilon_{ij}$  denotes the random error of the observation at time  $t_i$  for vessel  $j$  where it is assumed that  $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$ .

We find that we have the following estimates for random-effects standard deviations:  $\hat{\sigma}_b = 0.0017$  and  $\hat{\sigma}_\varepsilon = 0.0011$ . Recall that  $\sigma_b$  represents the standard deviation between vessels and  $\sigma_\varepsilon$  represents the standard deviation within vessels. Again, we see that there is slightly more variability between vessels compared to within vessels. This is again a result of the fact that we now have a larger number of time points.

Further inspecting the output, we find that while we no longer have any significant p-values, the lowest p-value reported is for treatment level 3 with a p-value of 0.1164. Recall that in treatment level 3, vessels 5 and 6 received the full-recommended amount of yeast nutrient. As an aside, we also notice that the next lowest p-value is for treatment level 4 with a p-value of 0.3494. Recall that in treatment level 4, vessels 7 and 8 received the smaller amount of zinc. These results make more logical sense in comparison to the results we found after analyzing model (8). Observe the graph of the specific gravity differences, which is shown below. Notice that after hour 100, we begin to see a clear distinction in the specific gravity differences of our six vessels. A plot of the mean-corrected specific gravity differences after hour 100 is shown below in Figure 14.

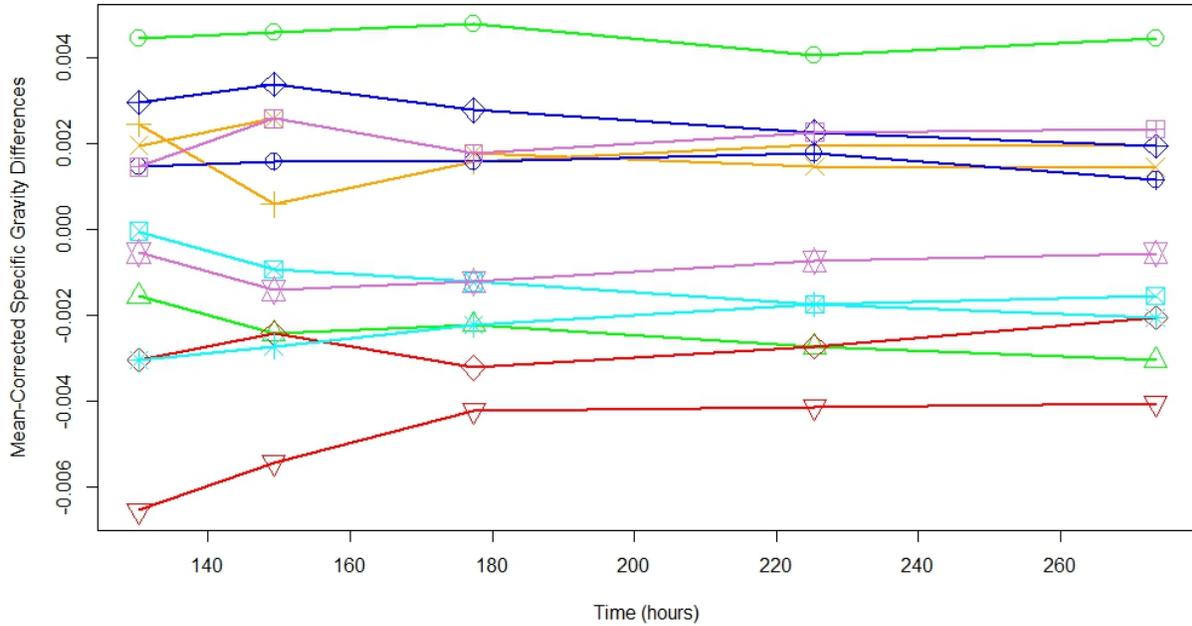


Figure 14: Cider2: Mean-Corrected Specific Gravity Differences vs Time (post hour 100)

Symbols:  $\circ$   $\equiv$  vessel 1 (green),  $\triangle$   $\equiv$  vessel 2 (green),  $+$   $\equiv$  vessel 3 (orange) and  $\times$   $\equiv$  vessel 4 (orange),  $\diamond$   $\equiv$  vessel 5 (red) and  $\nabla$   $\equiv$  vessel 6 (red),  $\boxtimes$   $\equiv$  vessel 7 (cyan) and  $\star$   $\equiv$  vessel 8 (cyan),  $\diamond$   $\equiv$  vessel 9 (blue) and  $\oplus$   $\equiv$  vessel 10 (blue),  $\star$   $\equiv$  vessel 11 (purple) and  $\boxplus$   $\equiv$  vessel 12 (purple).

Given a slight distinction in the specific gravity differences of our six vessels, we will refit model (9) and model the mean-corrected specific gravity differences after hour 100. We will, again, use fixed effects for the treatment factor (`trt.factor`) and random effects to model the vessel factor. A model with intercept  $\beta_0$ , fixed effects  $\tau_k$  for level  $k$  of the treatment factor, and random effects  $b_j$  for vessel  $j$  could be written as

$$d_{ij} - \bar{d}_i = \beta_0 + \tau_k + b_j + \varepsilon_{ij} \quad (10)$$

where  $i = 9, \dots, 13$ ,  $j = 1, \dots, 12$ , and  $k = 1, \dots, 6$ .

We have the following estimates for random-effects standard deviations:  $\hat{\sigma}_b = 0.002289$  and  $\hat{\sigma}_\varepsilon = 0.000575$ . We see that there is more variability between vessels compared to within

vessels. Notice, also, that the amount of variability between vessels has increased with each new model introduced for the Cider2 data. We can interpret this as an indication that by further specifying our models, we are uncovering more of the variability due to treatment level.

Further inspecting the output, we find that we have a p-value that is significant at the  $\alpha = 0.1$  level: treatment level 3. Recall that in treatment level 3, vessels 5 and 6 received the full-recommended amount of yeast nutrient. Thus, we have evidence to believe that supplementing the unfermented cider with the full-recommended amount of yeast nutrient can increase the rate of fermentation.

We will next test whether allowing the error variance to differ across treatment is necessary by comparing the maximized REML likelihoods for model (10), where error variance is constant across treatment and the following model with the same fixed and random effects, but in which the error variance is allowed to differ across treatment.

$$d_{ij} - \bar{d}_i = \beta_0 + \tau_k + b_j + \epsilon_{ij}^k \quad (11)$$

where

- $d_{ij}$  denotes the specific gravity difference of vessel  $j$  at time  $t_i$  for  $i = 1, \dots, 13$  and  $j = 1, \dots, 12$
- $\bar{d}_i$  denotes the mean specific gravity difference at time  $t_i$
- $\beta_0$  denotes the intercept of the linear model
- $\tau_k$  denotes the treatment effect due to the yeast nutrient and/or zinc in treatment level  $k$ , for  $k = 1, \dots, 6$ , where  $\tau_1 = 0$  for the control treatment.
- $b_j$  denotes the random effect due to vessel  $j$  where it is assumed that  $b_j \sim N(0, \sigma_b^2)$
- $\epsilon_{ij}^k$  denotes the random error of the observation at time  $t_i$  of vessel  $j$  in treatment  $k$ , where the error variance differs due to treatment, and is assumed that  $\epsilon_{ij}^k \sim N(0, \sigma_{\epsilon_k}^2)$

We see that while the estimates of the fixed effects match that of model (10), the standard errors of these estimates differ slightly across the two models. We also have different estimates for  $\hat{\sigma}_b$  and  $\hat{\sigma}_\varepsilon$ , the standard deviation between vessels and within vessels, respectively. We find that we now have the following estimates for random-effects standard deviations:  $\hat{\sigma}_b = 0.0023$ ,  $\hat{\sigma}_{\varepsilon_1} = 0.0004$ ,  $\hat{\sigma}_{\varepsilon_2} = 0.0006$ ,  $\hat{\sigma}_{\varepsilon_3} = 0.0008$ ,  $\hat{\sigma}_{\varepsilon_4} = 0.0006$ ,  $\hat{\sigma}_{\varepsilon_5} = 0.0004$ , and  $\hat{\sigma}_{\varepsilon_6} = 0.0004$ . Recall that  $\sigma_b$  represents the standard deviation between vessels and  $\sigma_{\varepsilon_k}$  represents the standard deviation within vessels for treatment  $k$ . Again, we see that there is much more variability between vessels compared to within vessels. Notice also that the estimate of  $\hat{\sigma}_b$  for model (11) is larger than the estimate of  $\hat{\sigma}_b$  for model (10). This is an intuitive result since we are now allowing treatments to have different variances. We will proceed by comparing the REML fits of models model (10) and (11) to determine if we can assume that error variance is relatively constant across treatment.

The likelihood ratio test is not statistically significant ( $X^2(5) = 6.11, p = 0.2958$ ), which indicates that model (11) does not provide a significantly better model and we have evidence to believe that error variance is constant across treatment.

Recall that using the IPA39 data, we developed a model that would allow us to predict the hydrometer specific gravity reading using the refractometer specific gravity reading. After examining the R output for model (6), we found that this model could be written as

$$\hat{y} = -0.515 + 1.485x, \quad (12)$$

where  $\hat{y}$  is the predicted hydrometer reading of specific gravity and  $x$  is the refractometer reading of specific gravity. For the Cider2 data, we took hydrometer readings of six vessels (one from each treatment level) at hours 89.4, 177.4, and 273.4. Using model (12), we will predict the hydrometer specific gravity reading of Cider2 using refractometer specific gravity readings and proceed to compare the predicted hydrometer readings to the actual hydrometer readings in the table below.

Notice that model (12) was not as effective as we may have hoped in predicting the hy-

| Vessel | Time (hours) | Refractometer Specific Gravity | Actual Hydrometer Specific Gravity | Predicted Hydrometer Specific Gravity |
|--------|--------------|--------------------------------|------------------------------------|---------------------------------------|
| 2      | 89.4         | 1.024                          | 1.012                              | 1.006                                 |
| 3      | 89.4         | 1.020                          | 1.009                              | 0.998                                 |
| 6      | 89.4         | 1.023                          | 1.010                              | 0.995                                 |
| 8      | 89.4         | 1.021                          | 1.01                               | 0.9997                                |
| 10     | 89.4         | 1.021                          | 1.01                               | 0.992                                 |
| 11     | 89.4         | 1.021                          | 1.014                              | 0.991                                 |
| 2      | 177.4        | 1.019                          | 1.004                              | 1.004                                 |
| 3      | 177.4        | 1.0148                         | 1.000                              | 0.997                                 |
| 6      | 177.4        | 1.018                          | 1.000                              | 0.991                                 |
| 8      | 177.4        | 1.019                          | 1.004                              | 1.001                                 |
| 10     | 177.4        | 1.0168                         | 1.002                              | 0.998                                 |
| 11     | 177.4        | 1.014                          | 1.002                              | 0.997                                 |
| 2      | 273.4        | 1.017                          | 1.000                              | 1.001                                 |
| 3      | 273.4        | 1.014                          | 0.990                              | 0.995                                 |
| 6      | 273.4        | 1.014                          | 0.990                              | 0.993                                 |
| 8      | 273.4        | 1.018                          | 1.000                              | 1.001                                 |
| 10     | 273.4        | 1.0152                         | 1.000                              | 0.991                                 |
| 11     | 273.4        | 1.0135                         | 0.980                              | 0.990                                 |

drometer readings of Cider2. But this is to be expected since beer and cider ferment differently. Instead, we will develop a different model for estimating the hydrometer specific gravity reading of Cider2 using our limited number of data points. We will, again consider the simple linear model relating the refractometer readings and hydrometer readings. Recall that this model can be written as

$$y_i = \beta_0 + \beta_1 x_i + \varepsilon_i, \quad (13)$$

where

- $y_i$  denotes the hydrometer reading of specific gravity for observation  $i$
- $x_i$  denotes the refractometer reading of specific gravity for observation  $i$
- $\varepsilon_i$  denotes the random error of observation  $i$ , where it is assumed that  $\varepsilon \sim N(0, \sigma_\varepsilon^2)$ .

The plot corresponding to model (13) can be seen below.

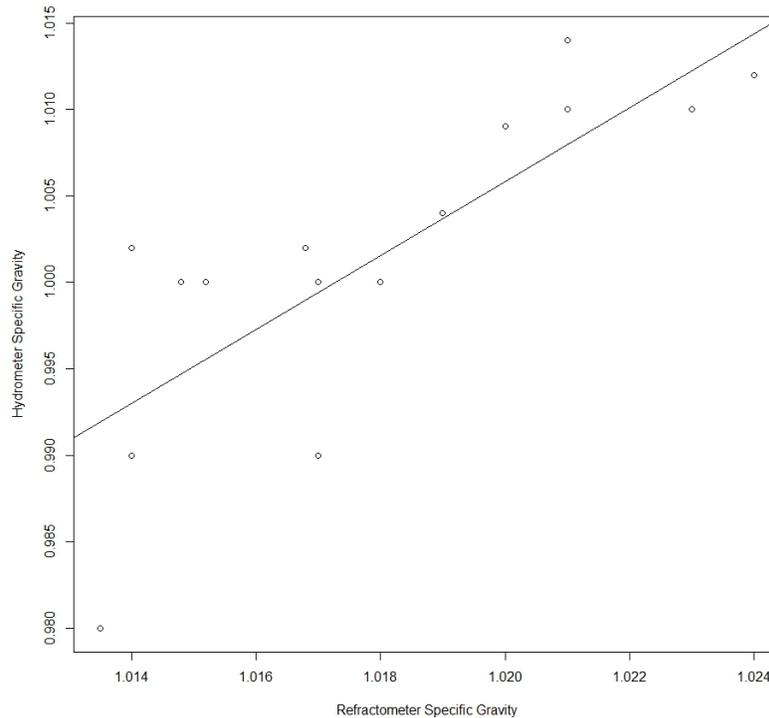


Figure 15: Cider2: Hydrometer versus Refractometer

We find that the p-values of both of our coefficient estimates are significant at the  $\alpha = 0.01$  level. We also find that the residual standard error is estimated at 0.0053. This tells us that our model predicts hydrometer specific gravity to within  $2\sigma_\epsilon = 2(0.0053) = 0.0106$  approximately 95% of the time. Recall that a linear mixed-effects model would be more appropriate, as it allows for random intercepts for each vessel, but as we saw with IPA39, these random intercepts turn out to be no different from the intercept  $\beta_0$  of the simple linear regression model in (13). Thus, since the estimate of the standard deviation between vessels,  $\hat{\sigma}_b$  is very close to zero, the fitted regression equation for the linear mixed-effects model matches the simple linear regression model in (13).

Using the Cider2 data, after removing the variability due to initial sugar content and removing the variability due to time, we found that supplementing with the full recommended amount of yeast nutrient works best at increasing the rate of fermentation, while the other treatment levels were not significantly different from the control treatment. However, since there were only two

replicates in each treatment, the power of our test is modest. We also found and presented a simple linear regression model that can be used to predict the hydrometer reading of specific gravity using the refractometer reading of specific gravity for ciders brewed using a recipe similar to that of Cider2.

## 4 CONCLUSIONS

The Cider1 data set was used to determine how the amount of yeast nutrient added to cider would affect the fermentation rate. More specifically, we were trying to determine if adding yeast nutrient to cider could increase the rate of fermentation. Through the analysis of Cider1 data, we determined that a majority of variability in specific gravity is explained by the time course. We also found yeast nutrient to be moderately significant in increasing fermentation rate at hour 250. However, we note that since there were only two replicates in each treatment, the power of our test is modest. We also determined that our estimate of the standard deviation between vessels,  $\hat{\sigma}_b$ , was close to zero because there is very little variability in specific gravity that is not explained by time and treatment effects.

The IPA38 data set was used to determine whether the order in which the vessels were filled from the kettle would affect the fermentation rate. In this study, the order of fill was such that one gallon was emptied into vessel 1 followed by one gallon into the carboy. Next, another gallon was emptied into vessel 2 followed by another (second) gallon into the carboy. The vessels were continually filled in this manner, so that vessel 1 contained wort near the bottom of the brew kettle and vessel 6 contained wort near the top of the brew kettle. After extracting the estimates of the random effects and obtaining a standardized score for the maximum distance between any two random effects, we determined that time order of fill had no significant effect on fermentation rate. However, we also note that the power of our test is modest given our small sample size. We have also found that a lack of specific gravity measurements made the data more difficult to model, and thus for future data sets, our goal will be to obtain more data near the beginning of fermentation. The Cider1 and IPA38 data sets have shown us that having a larger number of time points would have given us a better idea of how the specific gravity is changing near the beginning of fermentation when yeast activity is at a maximum.

The IPA39 data set was used to study the relationship between refractometer readings of

specific gravity in comparison to hydrometer readings of specific gravity over time. We presented a simple linear regression model that can be used to predict the hydrometer reading of specific gravity using the refractometer reading of specific gravity for IPA39 and other beers made using a similar recipe. We also presented a linear mixed-effects model for further exploration of the relationship between hydrometer and refractometer readings which allowed for random intercepts for each vessel. However, we found that our estimate of the standard deviation between vessels,  $\hat{\sigma}_b$ , was very small, and as a result, the fitted regression equation for the linear mixed-effects model matches the simple linear regression model.

The Cider2 data set was used to determine how the amount of zinc and/or yeast nutrient added to cider would affect the fermentation rate. More specifically, we wanted to determine whether the addition of zinc sulfate, yeast nutrient, or a combination of zinc sulfate and yeast nutrient to cider could increase the fermentation rate, and if so, which treatment would have the greatest effect on fermentation rate. After removing the variability due to initial sugar content and removing the variability due to time, we saw that supplementing with the full recommended amount of yeast nutrient works best at increasing the rate of fermentation, while the other treatment levels were not significantly different from the control treatment. However, since there were only two replicates in each treatment, the power of our test is modest. We also found and presented a simple linear regression model that can be used to predict the hydrometer reading of specific gravity using the refractometer reading of specific gravity for ciders brewed using a recipe similar to that of Cider2.

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## APPENDICES

### Appendix 1: R code for Cider1

```
##Appendix 1 Code (Cider1)

d =
  read.csv(file='C:/Users/Jamie/Documents/THESIS/UNCGconference/cider1.csv')
d$trt.factor = as.factor(d$trt)
d$vessel.factor = as.factor(d$vessel)

library(lme4)
library(nlme)

#Figure 1
plot(d$time,d$sg,type='n',xlab="Time (hours)", ylab="Specific Gravity")
col.vec = c('firebrick3','orange1','gold','green','blue','orchid3')

for(i in 1:length(unique(d$vessel1))){
  lines(d$time[d$vessel1==i],d$sg[d$vessel1==i],lty=1,lwd=2,col=col.vec[i])
  points(d$time[d$vessel1==i],d$sg[d$vessel1==i],pch=i,col=col.vec[i],cex=2)
}
abline(b=fixed.effects(summary(cider1.mod1))["time"]
,a=fixed.effects(summary(cider1.mod1))["(Intercept)"],lwd = 2)

}

#legend for Figure 1 (for Beamer)
legend(200,1.050,legend=c("Vessel 1", "Vessel 2", "Vessel 3",
"Vessel 4", "Vessel 5", "Vessel 6"),
col= c('firebrick3','orange1','gold','green','blue','orchid3'),
bty="n",cex =1.7,pch=1:6)

#plotting cider1 with regression line
plot(d$time,d$sg,type='n',main="Cider1",,xlab="Time (hours)",
ylab="Specific Gravity")
col.vec = c('firebrick3','orange1','gold','green','blue','orchid3')

for(i in 1:length(unique(d$vessel1))){
  lines(d$time[d$vessel1==i],d$sg[d$vessel1==i],lty=1,lwd=2,col=col.vec[i])
```

```

points(d$time[d$vessel==i],d$sg[d$vessel==i],pch=i,col=col.vec[i],cex=2)
}
abline(b=fixed.effects(summary(cider1.mod1))["time"]
,a=fixed.effects(summary(cider1.mod1))["(Intercept)"],lwd = 2)

#model (1)
cider1.mod1 = lme(sg~trt.factor+time,random=~1|vessel,method="REML",data=d);
summary(cider1.mod1)

#Figure 2 (Plots of log-likelihood against \lambda
#for proposed Box-Cox tranformation)

cidermod=lm(d$sg~d$time+d$trt.factor);summary(cidermod)
library(MASS)
par(mfrow=c(1,2))
boxcox(cidermod,lambda=seq(-200,100,length=20))
boxcox(mod,lambda=seq(-160,-120,length=20))

#running the linear boxcox transformed model

BCcidermod=lm(d$sg^(-130)~d$time+d$trt.factor); summary(BCcidermod)
par(mfrow=c(1,1))
plot(d$time,d$sg^(-130),main="Cider1 Post Box-Cox Transformation",
ylab="Specific Gravity^(-130)", xlab="Time (hours)")
abline(a=BCcidermod$coefficients[1],b=BCcidermod$coefficients[2])

#Figure 3 Cider1 Pre/Post Box-Cox Transformation
par(mfrow=c(1,2))
plot(d$time,d$sg,main="Cider1 Pre Box-Cox Transformation",
ylab="Specific Gravity", xlab="Time (hours)")
abline(a=cidermod$coefficients[1],b=cidermod$coefficients[2])

plot(d$time,d$sg^(-130),main="Cider1 Post Box-Cox Transformation",
ylab="Specific Gravity^(-130)", xlab="Time (hours)")
abline(a=BCcidermod$coefficients[1],b=BCcidermod$coefficients[2])

#Grouping Data
library(nlme)
library(lattice)

```

```

d1 = groupedData(sg~time|vessel1,outer=~trt.factor,d)

#Plotting time series for each of the individual vessels,
#ranked from left to right on the basis of mean sg

plot(d1)

#Figure 4: Grouping replicates within each treatment level
plot(d1,outer=T,xlab="Time (hours) ",ylab="Specific Gravity")

#zooming

dZOOM = d[-(1:12),]
dZOOM
dZOOM$trt.factorz = as.factor(dZOOM$trt)

#regrouping data and Figure 5
dlz = groupedData(sg~time|vessel1,outer=~trt.factorz,dZOOM)
plot(dlz,outer=T,xlab="Time (hours) ",ylab="Specific Gravity")

#model (3)
d2=d[13:18,];
m10 = lm(d2$sg~d2$trt.factor); summary(m10);

#Figure 6 and model(4) --- Residuals
d$mean.sg = d$sg
d
for(i in 1:nrow(d)){
d$mean.sg[i] = mean(d$sg[d$time==d$time[i]])
print(d$sg[d$time==d$time[i]])
}

plot(d$time,d$sg,ylab="Specific Gravity",xlab="Time (hours)",main="Cider1")
lines(d$time,d$mean.sg)

d$resid = d$sg-d$mean.sg;
d$trt.factor=as.factor(d$trt)
m1 = lme(resid~trt.factor,random=~1|vessel,method='REML',data=d); summary(m1)

```

```

> summary(cider1.resid.model)
Linear mixed-effects model fit by REML
      AIC      BIC   logLik
-412.7202 -404.4024 211.3601

Random effects:
Formula: ~1 | vessel
      (Intercept)      Residual
StdDev: 5.535538e-08 0.0009683365

Fixed effects: resid ~ trt.factor
              Value      Std.Error DF   t-value p-value
(Intercept) 0.0003880952 0.0002587988 36  1.499602  0.1424
trt.factor2 -0.0006750000 0.0003659968  3 -1.844278  0.1623
trt.factor3 -0.0004892857 0.0003659968  3 -1.336858  0.2736
Correlation:
      (Intr) trt.f2
trt.factor2 -0.707
trt.factor3 -0.707  0.500

Standardized Within-Group Residuals:
      Min      Q1      Med      Q3      Max
-3.15464924 -0.43090591  0.01844105  0.22959109  2.51413004
Number of Observations: 42
Number of Groups: 6

```

## Appendix 2: R code for IPA38

```

dd =
read.csv(file='C:/Users/Jamie/Documents/THESIS/UNCGconference/ipa38.csv');
ddl = dd[-(1:6),] ;

plot(ddl$time,ddl$sg,type='n',xlab="Time (hours)", ylab="Specific Gravity")
col.vec = c('firebrick3','orange1','gold','green','blue','orchid3')

for(i in 1:length(unique(ddl$vessel))){
lines(ddl$time[ddl$vessel==i],ddl$sg[ddl$vessel==i],
lty=1,lwd=2,col=col.vec[i])
points(ddl$time[ddl$vessel==i],ddl$sg[ddl$vessel==i],
pch=i,col=col.vec[i],cex=2)
}
legend(165,1.050,legend=c("Vessel 1", "Vessel 2", "Vessel 3",
"Vessel 4", "Vessel 5", "Vessel 6"),col= c('firebrick3','orange1',
'gold','green','blue','orchid3'),bty="n",cex =1.7,pch=1:6)

library(lme4)
library(nlme)

ddl$mean.sg = ddl$sg
ddl
for(i in 1:nrow(ddl)){
ddl$mean.sg[i] = mean(ddl$sg[ddl$time==ddl$time[i]])
print(ddl$sg[ddl$time==ddl$time[i]])
}
ddl

ddl$resid = ddl$sg-ddl$mean.sg;
ddl$vessel.factor=as.factor(ddl$vessel)

#Figure (8)
plot(ddl$time,ddl$sg,ylab="Specific Gravity",xlab="Time (hours)")
lines(ddl$time,ddl$mean.sg)

#model (5)
ipa38.resid.model = lme(resid~1,random=~1|vessel,method='REML',
data=ddl);
summary(ipa38.resid.model)

#extracting random effects from the model

```

```

coef(ipa38.resid.model)
b = as.matrix(coef(ipa38.resid.model))
qqnorm(b); qqline(b)
max(b)-min(b)
sqrt(.001248555^2*2)
(max(b)-min(b))/sqrt(.001248555^2*2)
#[1] 1.72454

#Comparing to critical t-value using Bonferroni

new.p.value = .05/(2*15)
critical.t.value = qt(.05/(2*15),36)

> summary(IPA38.resid.model)
Linear mixed-effects model fit by REML
Data: ddl
      AIC      BIC  logLik
-437.9383 -432.7976 221.9692

Random effects:
Formula: ~1 | vessel
      (Intercept)      Residual
StdDev: 0.001248555 0.0008717874

Fixed effects: resid ~ 1
              Value      Std.Error DF      t-value p-value
(Intercept) 1.57804e-17 0.0005271721 36 2.993406e-14      1

Standardized Within-Group Residuals:
      Min      Q1      Med      Q3      Max
-1.70480234 -0.63598718 0.01580069 0.56251479 2.32701898

Number of Observations: 42
Number of Groups: 6

```

### Appendix 3: R code for IPA39

```

#plot of IPA39 data

d.39 =
  read.csv(file='C:/Users/Jamie/Documents/THESIS/Data/Dec3Data/ipa39.csv')

#IPA39 refractometer plot
plot(d.39$time,d.39$refracsg,type='n',xlab="Time (hours)",
ylab="Specific Gravity",xlim=c(22,185))
col.vec = c('firebrick3','chocolat1','gold','green','blue','orchid3')
for(i in 1:length(unique(d.39$vessel))){
lines(d.39$time[d.39$vessel==i],d.39$refracsg[d.39$vessel==i],
lty=1, lwd=2,col=col.vec[i])
points(d.39$time[d.39$vessel==i],d.39$refracsg[d.39$vessel==i],
pch=i,col=col.vec[i],cex=2)
}
legend(120,1.055,legend=c("Vessel 1", "Vessel 2", "Vessel 3",
"Vessel 4", "Vessel 5", "Vessel 6"),col= c('firebrick3','chocolat1',
'gold','green','blue','orchid3'),bty="n",cex =1.7,pch=1:6,lwd=2)

#IPA39 hydrometer plot
plot(d.39$time,d.39$hydrosrg,type='n',xlab="Time (hours)",
ylab="Specific Gravity",xlim=c(22,185))
col.vec = c('firebrick3','chocolat1','gold','green','blue','orchid3')
for(i in 1:length(unique(d.39$vessel))){
lines(d.39$time[d.39$vessel==i],d.39$hydrosrg[d.39$vessel==i],
lty=1, lwd=2,col=col.vec[i])
points(d.39$time[d.39$vessel==i],d.39$hydrosrg[d.39$vessel==i],
pch=i,col=col.vec[i],cex=2)
}
legend(120,1.05,legend=c("Vessel 1", "Vessel 2", "Vessel 3",
"Vessel 4", "Vessel 5", "Vessel 6"),col= c('firebrick3','chocolat1',
'gold','green','blue','orchid3'),bty="n",cex =1.7,pch=1:6,lwd=2)

d1.39 = d.39[-(1:6),] ; d1.39
d1.39$vessel= as.factor(d1.39$vessel)

#model (6)

```

```

Hydro.Refrac.LM = lm(d1.39$hydrosq~d1.39$refracsg);
summary(Hydro.Refrac.LM)

#Figure 11
plot(d1.39$hydrosq~d1.39$refracsg,xlab="Refractometer Specific Gravity",
ylab="Hydrometer Specific Gravity" )
abline(Hydro.Refrac.LM)

#model (7)
ipa39HvsR = lme(hydrosq~refracsg,random=~1|vessel,method='REML',data=d1.39)
summary(ipa39HvsR)

#predicting hydrometer reading from refractometer reading

d1.39 = d.39[-(1:6),] ; d1.39
> Hydro.Refrac.LM = lm(d1.39$hydrosq~d1.39$refracsg);
summary(Hydro.Refrac.LM)

Call:
lm(formula = d1.39$hydrosq ~ d1.39$refracsg)

Residuals:
      Min       1Q   Median       3Q      Max
-0.007798 -0.001194  0.000202  0.001269  0.003959

Coefficients:
              Estimate Std. Error t value Pr(>|t|)
(Intercept)  -0.51517    0.02298  -22.42  <2e-16 ***
d1.39$refracsg  1.48479    0.02224   66.77  <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.001775 on 76 degrees of freedom
Multiple R-squared:  0.9832,    Adjusted R-squared:  0.983
F-statistic:  4459 on 1 and 76 DF,  p-value: < 2.2e-16

```

## Appendix 4: R code for Cider2

```

dC2 =
read.csv(file='C:/Users/Jamie/Documents/
THESIS/data/cider2-final-minus2.csv')

#plot of Cider2
col.vec = c('green','green','orange','orange','red','red',
'cyan','cyan','blue','blue','orchid','orchid')
plot(dC2$time,dC2$refracsg,type='n',xlab="Time (hours)",
ylab="Specific Gravity")

for(i in 1:length(unique(dC2$vessel))){
lines(dC2$time[dC2$vessel==i],dC2$refracsg[dC2$vessel==i],
lty=1, lwd=2,col=col.vec[i])
points(dC2$time[dC2$vessel==i],dC2$refracsg[dC2$vessel==i],
pch=i,col=col.vec[i],cex=2)
}
legend(125,1.055,legend=c("Trt1", "Trt1", "Trt2", "Trt2", "Trt3", "Trt3"),
col= c('green','green','orange','orange','red','red'),
bty="n",cex =2.5,pch=1:6,lwd=2)

legend(200,1.055,legend=c("Trt4", "Trt4", "Trt5", "Trt5", "Trt6", "Trt6"),
col=c('cyan','cyan','blue','blue','orchid','orchid'),
bty="n",cex =2.5,pch=7:12,lwd=2)

#model (8) -- modeling residuals
library(lme4)
library(nlme)

dC2$vessel.factor = as.factor(dC2$vessel)
dC2$trt.factor = as.factor(dC2$trt)

dC2$mean.sg = dC2$refracsg
for(i in 1:nrow(dC2)){
dC2$mean.sg[i] = mean(dC2$refracsg[dC2$time==dC2$time[i]])
print(dC2$refracsg[dC2$time==dC2$time[i]])
}

```

```
dc2$resid = dc2$refracsg-dc2$mean.sg;
```

```
Cider2.mod2 <- lme(resid~trt.factor,data=dc2,
random=~1|vessel,method="REML")
summary(Cider2.mod2)
```

```
#Figure 13 -- Specific Gravity Differences
```

```
#plot of specific gravity differences
col.vec = c('green','green','orange','orange','red','red',
'cyan','cyan','blue','blue','orchid','orchid')
plot(dc2$time,dc2$sgdiff,type='n',xlab="Time (hours)",
ylab="Specific Gravity Differences")

for(i in 1:length(unique(dc2$vessel))){
lines(dc2$time[dc2$vessel==i],dc2$sgdiff[dc2$vessel==i],
lty=1, lwd=2,col=col.vec[i])
points(dc2$time[dc2$vessel==i],dc2$sgdiff[dc2$vessel==i],
pch=i,col=col.vec[i],cex=2)
}
legend(125,0,legend=c("Trt.1", "Trt.1", "Trt.2", "Trt.2", "Trt.3", "Trt.3"),
col= c('green','green','orange','orange','red','red'),
bty="n",cex =2.5,pch=1:6,lwd=2)

legend(200,0,legend=c("Trt.4", "Trt.4", "Trt.5", "Trt.5", "Trt.6", "Trt.6"),
col=c('cyan','cyan','blue','blue','orchid','orchid'),
bty="n",cex =2.5,pch=7:12,lwd=2)
```

```
#model (9)--- Modeling Mean-Corrected Specific Gravity Differences
```

```
dc2$mean.sg = dc2$sgdiff
for(i in 1:nrow(dc2)){
dc2$mean.sg[i] = mean(dc2$sgdiff[dc2$time==dc2$time[i]])
print(dc2$sgdiff[dc2$time==dc2$time[i]])
}
```

```
dc2$MCsgdiff = dc2$sgdiff-dc2$mean.sg;
```

```
Cider2.diff.mod1<- lme(MCsgdiff~trt.factor,data=dc2,random=~1|vessel,
```

```

method="REML")
summary(Cider2.diff.mod1)

#plot of mean-corrected specific gravity differences
col.vec = c('green','green','orange','orange','red','red',
'cyan','cyan','blue','blue','orchid','orchid')
plot(dC2$time,dC2$MCsgdiff,type='n',xlab="Time (hours)",
ylab="Specific Gravity")

for(i in 1:length(unique(dC2$vessel))){
lines(dC2$time[dC2$vessel==i],dC2$MCsgdiff[dC2$vessel==i],
lty=1, lwd=2,col=col.vec[i])
points(dC2$time[dC2$vessel==i],dC2$MCsgdiff[dC2$vessel==i],
pch=i,col=col.vec[i],cex=2)
}

# investigating mean-corrected SGD post 100 hours

dC2 =read.csv(file='C:/Users/Jamie/Documents/
THESIS/data/cider2-final-minus2-100.csv')
dC2$vessel.factor = as.factor(dC2$vessel)
dC2$trt.factor = as.factor(dC2$trt)

dC2$mean.sgdiff = dC2$sgdiff
for(i in 1:nrow(dC2)){
dC2$mean.sgdiff[i] = mean(dC2$sgdiff[dC2$time==dC2$time[i]])
print(dC2$sgdiff[dC2$time==dC2$time[i]])
}

dC2$MCsgdiff = dC2$sgdiff-dC2$mean.sgdiff;

#Figure 14 --- mean-corrected specific gravity differences post 100 hours

col.vec = c('green','green','orange','orange',
'red','red','cyan','cyan','blue','blue','orchid','orchid')
plot(dC2$time,dC2$MCsgdiff,type='n',
xlab="Time (hours)", ylab="Mean-Corrected Specific Gravity Differences")

for(i in 1:length(unique(dC2$vessel))){
lines(dC2$time[dC2$vessel==i],dC2$MCsgdiff[dC2$vessel==i],
lty=1, lwd=2,col=col.vec[i])
points(dC2$time[dC2$vessel==i],dC2$MCsgdiff[dC2$vessel==i],
pch=i,col=col.vec[i],cex=2)
}

```

```

#model (10) ---- modeling mean-corrected SGD post 100 hours

Cider2.Diff.mod2 <- lme(MCsgdiff ~trt.factor,data=dC2,
random=~1|vessel,method="REML")
summary(Cider2.Diff.mod2)

#model (11) ---modeling mean-corrected SGD post 100 hours
#while allowing error variance to differ across treatment

Cider2.Diff.mod1 <- lme(MCsgdiff ~trt.factor,data=dC2,
random=~1|vessel,method="REML",
weights=varIdent(form=~1|trt.factor))
summary(Cider2.Diff.mod1)

#Likelihood ratio test to see if model (11)
#is a significantly better model

anova(Cider2.Diff.mod1, Cider2.Diff.mod2)

#predicting hydrometer specific gravity
dC2 =
read.csv(file='C:/Users/Jamie/Documents/
THESIS/data/cider2-final-hydrorefrac.csv')

#model (13)
Cider2.Hydro.Refrac.LM = lm(dC2$hydrosg~dC2$refracsg);
summary(Cider2.Hydro.Refrac.LM)
plot(dC2$hydrosg~dC2$refracsg,xlab="Refractometer Specific Gravity",
ylab="Hydrometer Specific Gravity" )
abline(Cider2.Hydro.Refrac.LM )

#using a lme model to predict hydrometer specific gravity
cider1HvsR = lme(hydrosg~refracsg,
random=~1|vessel,method='REML', data=dC2)
summary(cider1HvsR)

```