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Statistical Analysis of Yeast Nutrient in Hard Cider Brewing

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ABSTRACT. In brewing, it is widely known that trace elements, particularly zinc, are required by yeast in order to grow and ferment. The most sensitive and time-consuming step of cider production is the fermentation process, and problems encountered during this process can lead to prolonged fermentation time and the deterioration of cider quality. 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, homebrewers more commonly supplement with yeast nutrient rather than zinc, as yeast nutrient is more widely available and easier to use. Given that the use of zinc in increasing fermentation rate has been so widely studied, it may be useful for homebrewers to know if zinc is as useful in increasing fermentation rate on a smaller scale. In this study, we explore different methods of increasing fermentation rate by supplementing with various levels of zinc and yeast nutrient, as well as a combination of zinc and yeast nutrient. We present statistical models that describe specific gravity as a function of time, identify underlying causes of variability in specific gravity, and determine if supplementing with zinc is an effective method of increasing fermentation rate on a smaller scale. The results of this study show that supplementing with the full recommended amount of yeast nutrient is the most effective method of increasing fermentation rate, while the other treatment levels were not significantly different from the control treatment.

1. Introduction

People began brewing in their homes over 6,000 years ago (Papazian, 2003). While homebrewing has been around for thousands of years, it has seen a spike in popularity in recent years, with 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

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available, many of these people want to make these products themselves or expand on what is currently available.

Making beer and 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 logical 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.

1.1. The homebrewing process

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). Before brewing begins and before ingredients are purchased, a homebrewer must decide whether they intend to use unpasteurized or pasteurized apple juice. If juice is unpasteurized, sulfite must be added to remove wild yeasts. Before any yeast or yeast supplements are added to the apple juice, 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 juice is measured, the yeast will be added. The addition of yeast to the unfermented cider is often referred to, among brewers, as pitching. Homebrewers often choose to supplement unfermented cider with yeast nutrient since apple juice can be deficient in nutrients needed for yeast to thrive. Homebrewers can also choose to supplement with other additives such as malic acid or sugar to achieve the optimal taste and alcohol level.

After the yeast is pitched and any supplements are added, 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 juice is now referred to as cider. This change in reference occurs due to the fact that the 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 cider being brewed. Generally, after two to three days of vigorous fermentation, activity will subside and yeast will die or become dormant due to food depletion and alcohol buildup and fermentation will begin to subside. After fermentation has ended, the cider will be bottled or kegged and then aged over a period of generally 7-14 days (Papazian, 2003).

1.2. 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 smaller the chances 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-Hegyes 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 that supplementing wort with zinc (Nicola and Walker, 2011).

Commercial brewers have an advantage over homebrewers in that they have the resources to test the composition of the unfermented beer or cider 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 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. Attenuation is a measure of how much the sugars were converted into alcohol and carbon dioxide by the fermentation process.

1.3. 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 (if a recipe calls for unfermented cider to be boiled prior to yeast pitching), 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

A pilot experiment was conducted prior to the experiment described in this paper; these batches were known as Cider1 and Cider2. For Cider2, Red Star pasteur champagne yeast, a yeast strain of Saccharomyces bayanus, was used. During sample preparation for Cider2, the attempt was 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.

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

3. Model descriptions

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 (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 $0.4 - 1.07$ are sufficient (Helin and Slaughter, 1977; 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 and 0.75. 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. Wyeast laboratories recommends using a half 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. We begin by graphically considering our data in Figure 3.1.

3.1. Gravity differences

We wished to determine if the amount of yeast supplement and/or zinc added to unfermented cider affected the rate of fermentation. We began by modeling the residuals $(y_{ij} - \bar{y}_i)$ rather than

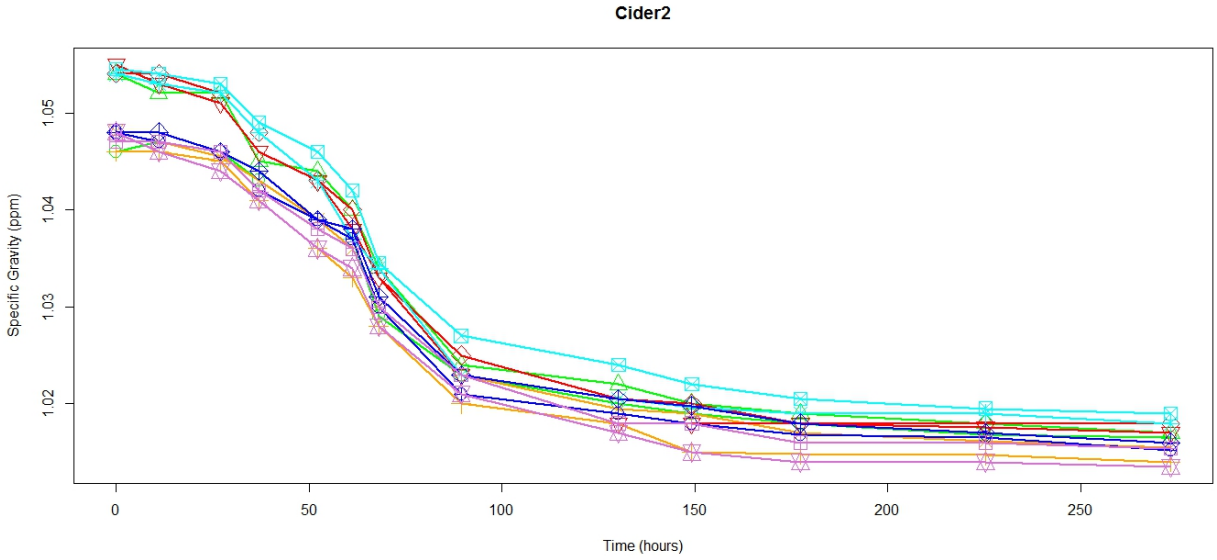

FIGURE 3.1. A plot of specific gravity against time (hours). Symbols: $\circ \equiv$ vessel 1 (green), $\triangle \equiv$ vessel 2 (green), both were in the control treatment, and as such, received no yeast nutrient or zinc. $+ \equiv$ vessel 3 (orange) and $\times \equiv$ vessel 4 (orange), both received half the recommended amount of yeast nutrient. $\diamond \equiv$ vessel 5 (red) and $\nabla \equiv$ vessel 6 (red), both received the full recommended amount of yeast nutrient. $\boxtimes \equiv$ vessel 7 (cyan) and $\equiv$ vessel 8 (cyan), both received the smaller amount of zinc. $\equiv$ vessel 9 (blue) and $\equiv$ vessel 10 (blue), both received the larger amount of zinc. $\equiv$ vessel 11 (purple) and $\equiv$ vessel 12 (purple), both received half the recommended amount of yeast nutrient and the smaller amount of zinc.

the response y_{ij} since we knew that our response variable, specific gravity (`refracsg`) did not follow a linear trend. Moreover, since we knew that time would have a significant effect on specific gravity, we modeled 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 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:

$$y_{ij} - \bar{y}_i = \beta_0 + \tau_k + b_j + \varepsilon_{ij} \tag{3.1}$$

where

- y_{ij} denotes the specific gravity of vessel j at time t_i for $i = 1, \ldots, 13$ and $j = 1, \ldots, 12$,
- $\bar{y}_i$ denotes the mean specific gravity at time t_i,
- β_0 denotes the intercept of the linear model,
- τ_k denotes the treatment effect due to the yeast nutrient and/or zinc in treatment level k, for $k = 1, \ldots, 6$, where $\tau_1 = 0$ for the control treatment,
- b_j denotes the random effect due to vessel j where it is assumed that $b_j \sim N(0, \sigma_b^2)$, and

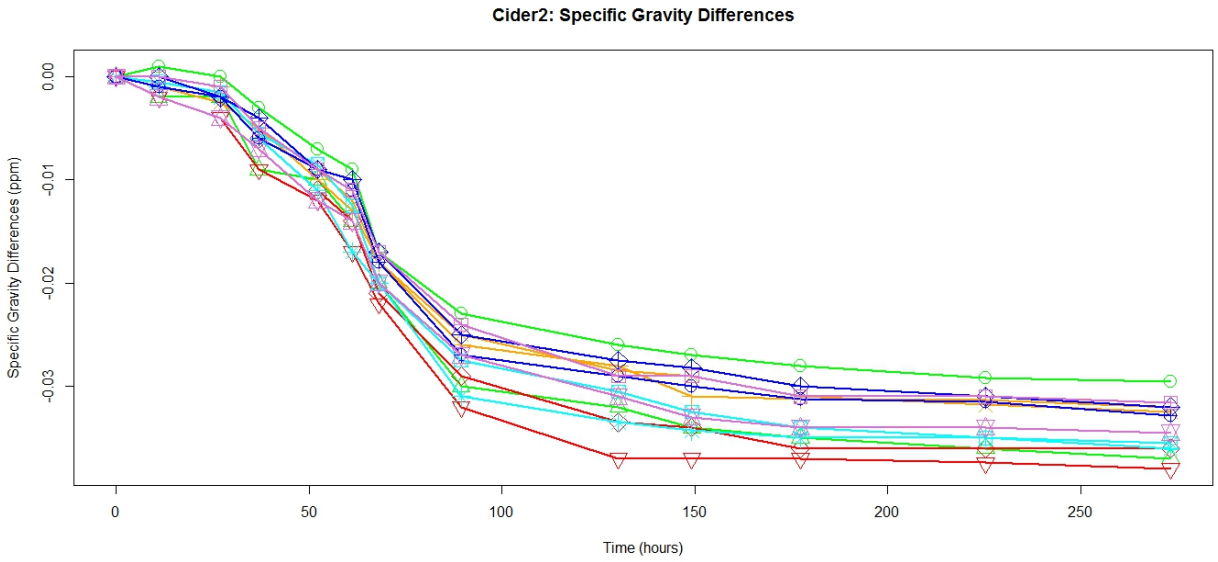

FIGURE 3.2. Cider2: Specific Gravity Differences versus Time.

- ε_{ij} denotes the random error of the observation at time t_i for vessel j where it is assumed that $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$.

3.2. Mean-corrected specific gravity differences

From the initial specific gravity measurements, we observed that the initial sugar contents of the apple juice vessels were unequal and tended to cluster into two groups. This difference in initial sugar content affected the results of the study, so we repeated the analysis through a correction for the initial sugar content in each vessel. Let y_{ij} denote the specific gravity of vessel j at time point i. In order 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 in Figure 3.2.

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. The twelve 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:

$$d_{ij} - \bar{d}_i = \beta_0 + \tau_k + b_j + \varepsilon_{ij} \tag{3.2}$$

where

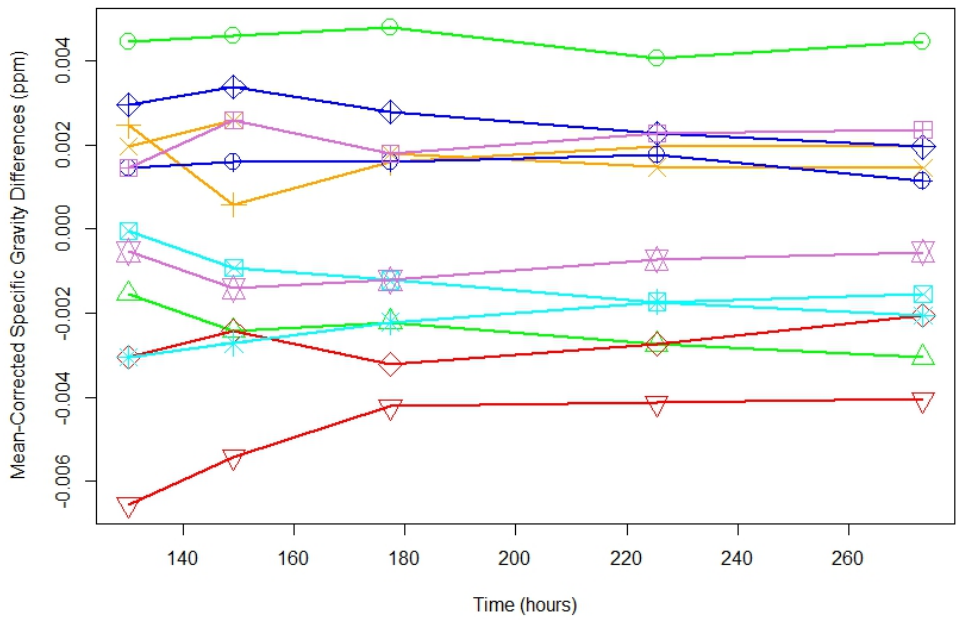

FIGURE 3.3. Cider2: Mean-Corrected Specific Gravity Differences vs Time (post hour 100).

- $d_{ij} = y_{ij} - y_{1j}$ denotes the specific gravity difference of vessel j at time t_i for $i = 1, \ldots, 13$ and $j = 1, \ldots, 12$,
- $\bar{d}_i$ denotes the mean specific gravity difference at time t_i,
- β_0 denotes the intercept of the linear model,
- τ_k denotes the treatment effect due to the yeast nutrient and/or zinc in treatment level k, for $k = 1, \ldots, 6$, where $\tau_1 = 0$ for the control treatment,
- b_j denotes the random effect due to vessel j where it is assumed that $b_j \sim N(0, \sigma_b^2)$, and
- ε_{ij} denotes the random error of the observation at time t_i for vessel j where it is assumed that $\varepsilon_{ij} \sim N(0, \sigma_\varepsilon^2)$.

Given a slight distinction in the specific gravity differences of our six vessels, we refit model (3.2) using the mean-corrected specific gravity differences after hour 100. We will, again, use fixed effects for the treatment factor and random effects to model the vessel factor. A model with intercept β_0, fixed effects τ_k for level k of the treatment factor, and random effects b_j for vessel j could be written as

$$d_{ij} - \bar{d}_i = \beta_0 + \tau_k + b_j + \varepsilon_{ij} \tag{3.3}$$

where $i = 9, \ldots, 13$, $j = 1, \ldots, 12$, and $k = 1, \ldots, 6$. Here restrict the timepoints (indexed by i) to those after time 100. These differences after time 100 are visible in Figure 3.3.

3.3. Variability within treatments

After we found that the variability between vessels was much greater than the variability within vessels, we will next test whether allowing the error variance to differ across treatment is necessary by comparing the maximized REML likelihoods for model (3.3), 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.

$$d_{ij} - \bar{d}_i = \beta_0 + \tau_k + b_j + \varepsilon_{ij}^{\text{treatment}} \tag{3.4}$$

where

- d_{ij} denotes the specific gravity difference of vessel j at time t_i for $i = 1, \ldots, 13$ and $j = 1, \ldots, 12$,
- $\bar{d}_i$ denotes the mean specific gravity difference at time t_i,
- β_0 denotes the intercept of the linear model,
- τ_k denotes the treatment effect due to the yeast nutrient and/or zinc in treatment level k, for $k = 1, \ldots, 6$, where $\tau_1 = 0$ for the control treatment,
- b_j denotes the random effect due to vessel j where it is assumed that $b_j \sim N(0, \sigma_b^2)$, and
- $\varepsilon_{ij}^{\text{treatment}}$ denotes the random error of the observation at time t_i for vessel j where the error variance may differ across treatment, and it is assumed that $\varepsilon_{ij}^{\text{treatment}} \sim N(0, \sigma_{\varepsilon_k}^2)$.

As will be seen in Section 4, the error variances were not significantly different across treatments.

4. Results

In this section, we present the results from the models considered in Section 3.

4.1. Gravity differences

From the analysis of the model in (3.1), we find that we have the following estimates for random-effects standard deviations: $\hat{\sigma}_b = 0.001327$ and $\hat{\sigma}_\varepsilon = 0.00115$. Note that σ_b represents the standard deviation between vessels and σ_ε represents the standard deviation within vessels. Thus we see that there is more variability between vessels compared to within vessels.

From an analysis of this model, there were two p-values that are significant at the $\alpha = 0.1$ level: treatment level 2 ($b = -0.0027$, $t(6) = -2.00$, $p = 0.0920$) and treatment level 6 ($b = -0.0030$, $t(6) = -2.18$, $p = 0.0720$). 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 found in Figure 3.1.

Notice that 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 3.1 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. This was the motivation to remove the variability in specific gravity due to the amount of sugar initially found in each vessel and consider the model in (3.2).

4.2. Mean-corrected specific gravity differences

From the analysis of the model in (3.2), we find that we have the following estimates for random-effects standard deviations: $\hat{\sigma}_b = 0.0017$ and $\hat{\sigma}_\varepsilon = 0.0011$. From an analysis of this model, we find that while we no longer have any significant differences in the treatment effects, as the lowest p-value reported is for treatment level 3 ($b = -0.0032$, $t(6) = -1.83$, $p = 0.1164$). 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 ($b = -0.0018$, $t(6) = -1.01$, $p = 0.3494$). 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 running model (3.1). After observing the specific gravity differences, we 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 3.3.

From the model considered in (3.3), 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. From further analysis of this model, we find that we have a p-value that is significant at the $\alpha = 0.1$ level: treatment level 3 ($b = -0.0048$, $t(6) = -2.09$, $p = 0.0813$) . 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 as the coefficient is negative indicating a smaller specific gravity than the other treatments.

4.3. Variability within treatments

While the estimates of the fixed effects match that of model (3.3), 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. Upon consideration of the model in (3.4), 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 σ_b represents the standard deviation between vessels and σ_{ε_k} represents the standard deviation within vessels for treatment level k. Again we see that there is much more variability between vessels compared to within vessels. A likelihood ratio test of models (3.3) and (3.4) was not statistically significant ($X^2(5) = 6.11$, $p = 0.2958$). This indicates that model (3.4) does not provide a significantly better model in comparison to model (3.3).

5. Conclusions

The overarching goal of this experiment was to determine whether the addition of zinc sulfate, yeast nutrient, or a combination of zinc sulfate and yeast nutrient could increase the fermentation rate in hard cider, and if so, which treatment would have the greatest effect on fermentation rate. After conducting this experiment, we learned that the initial sugar content of the apple juice used to make hard cider can effect specific gravity measurements during fermentation. While apple juices with higher original gravities experience increased activity near the beginning of fermentation, apple juices with lower original gravities tend to have lower final gravities, which could

be misleading. Correcting for the initial differentiation in sugar content and performing a mean-correction to remove the variability due to time allows us to uncover the variability due to treatment level.

After removing these sources of variability, 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 level, we note that the power of our test is modest. To the average homebrewer, these results indicate that supplementing with the full recommended amount of yeast nutrient is a more effective method of increasing fermentation rate in hard cider compared to supplementing with zinc sulfate, yeast nutrient and zinc sulfate, and decreased amounts of yeast nutrient.

As was mentioned in an earlier section, video recordings were taken prior to the collection of cider samples to monitor the air lock bubbling in each vessel. Future work with this data set could attempt to use the air lock bubbling data as an indicator of fermentation rate. The use of smoothing-splines mixed-effects models has also been explored as an alternative method of modeling this data.

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