Measuring the Activity of Saccharomyces Cerevisiae in Relation to Home-Based Additives by Measured Net Weight Loss

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This paper is dedicated to my father John Mainwaring, who not only taught me to bake bread, but thanks to his encouragement, love, and support throughout my life, made this paper possible.

Abstract

This research study is to measure the activity of saccharomyces cerevisiae through selected additives which were added in the hydration step of making bread dough. The saccharomyces cerevisiae is sensitive to sugars (Mazzoleni, S. et al.2015) and by using multiple additives that can be found at home, we can compare which ones give a healthier yeast and therefore a better rise to the dough. As the saccharomyces cerevisiae ferments, it consumes the sugars naturally in the dough, creates an acidic environment to maintain its growth, and produces CO_2 as a product of this reaction, which is the cause for the rising dough. This reaction can be tracked by how active the yeast is to its mean weight loss by measuring the weight loss of the three separate batches and comparing the results through a Multiple Comparisons of Means: Tukey Contrasts test to see if the significance to what is added to what was added to help the fermentation process of the yeast. We can see that easily soluble sugars are the best choices for promoting the health of the saccharomyces cerevisiae based on the additives used in each batch, with the test results, where $F(9,20)=14.49$, $p<0.0001$.

Introduction

Baking bread is a science, and every recipe calls for the same four ingredients for a yeasted bread. The key ingredients for a yeasted bread are flour, water, salt, and yeast. There can be other ingredients, but most recipes call for these ingredients (Fromartz, 2014). Bakers use these four ingredients to quickly communicate a certain ratio in recipes though a system called "Baker's Percent" (The Perfect Load, 2020). The yeast's activity is the marker for a good rise in baking bread. This commonly is done with a strain of yeast called saccharomyces cerevisiae, more commonly known by the name of baker's yeast. The purpose of this study was to see and measure the activity of the yeast through different additives in the hydration of the water that would be added to the flour to make the dough. The baker's percent recipe was 100%/75%/4%/2% while using 150 grams of All-Purpose white flour at the starting point. 75% is the hydration level of the dough, which in relation to 150 grams of flour, would be a weight of 112.5 grams of the hydration mixture. What I wanted for data was to see which additive would create the most activity in the yeast, which would then be measured after being combined and

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kneaded in containers which were sealed to see how much weight was lost due to the yeast's fermentation to the nearest tenth of a gram.

This research project was to see how each of the additives affected the rise of the bread by measuring the weight of the bread at initial time of mixing, sealing the container and taking the weights again every 20 minutes afterwards for a total of 120 minutes to detect how active the yeast was and how much weight change there was in the respective dough. The additives for this were selected both for the fact that they are commonly found in households, but also in order to see if the activity of the yeast was more related to easily digestible sugars or changing the pH levels of the dough itself to help the yeast thrive. The additives I selected for this were 2% milk, baking soda, brown sugar, honey, vinegar, white sugar, and lemon juice.

The additives were used in the hydration step as the response variable. My hypothesis is that the yeast activity will distinctively change based on the additive added to the hydration water. The goal of this experiment was to see what additive was best to increase the yeast's health and therefore its activity. Since the activity and health of the saccharomyces cerevisiae can be easily tracked by how much mass is lost from the dough by the activity of the yeast, the weight of the dough was the easiest and most accurate way to measure the health and activity of the yeast in a closed controlled environment.

The decision to include these additives to the experiment was to make sure there was some form of control for pH levels. To test this, I used baking soda as one of the additives since it makes the water more alkaline which would hinder the yeast activity, demonstrating that the pH is important to the yeast's health. My main comparison is the control of just water in the hydration of the dough. If the baking soda batch of bread still rose like the others, it could rule out that pH was important for the baker's yeast. The control for this experiment was just water. Room temperature tap water will indicate if any of the additives did contribute to the increase or decrease of the rate of reaction of the dough. If there was not a significant change in the control of water and those which were high in glucose, then those results could potentially rule out that just adding sugar would not stimulate the yeast. The expected results of this are that anything that would naturally increase the sugar content of the water without over saturating the solution would create a more favourable environment for the yeast and would perform better overall based on the weight loss measured. The significance of the results can be used later to help make the yeast perform better for industrial purposes or even to help home bakers who want to have a better loaf of yeasted bread.

Methods and Materials

A list of exact ingredients, brands, and types of containers used in this research project will be in the appendix, as well as a guide for terminology that is used in reference to the process of baking bread.

In this experiment, ten different additives were used to help the yeast proof and ferment prior to the hydration step of the dough making, and the weight of the dough was measured every twenty minutes for a total of two hours after the initial mixing to see how active the yeast was based on what was added to the water in the hydration step. The weight of the dough was then tracked to see how much weight the yeast consumed to the tenth of a gram in 3 batches of 10 doughs. The results were then compared through a Multiple Comparisons of Means: Tukey Contrasts test through RCmdr to compare the mean weight loss of the additives with each other and see if there is statistical evidence that is significantly different, and if conclusions could be drawn to what is the most effective additive to help the yeast start its fermentation process, as well as how active the yeast was based off the measured weight loss of the doughs. The data collected through the experiment was then run through RCmdr to get the statistical findings that were reported below.

The following was the process used to make each batch of dough:

1. The containers and lids were initially weighed for every batch, to track their weights and to determine what to subtract later to get the dough's mass without disturbing the sealed containers during the rising process. Then the flour was measured into each container to exactly 150.0 g.

2. Tap water was put into a bowl to use in measuring out the weight for the hydration of the dough. This was done at least 2 hours in advance so the water could come to a constant temperature.

3. All ingredients were weighed out into individual, clean containers.

4. Every additive was measured out to their respective weights, then room temperature water from the same bowl was added to bring up the weight of the mixture to 112.5 grams and left to sit for at least 10 minutes to bring the whole mixture to room temperature.

5. Salt was mixed with the flour, then the containers were sealed briefly and shaken side to side to mix the flour and salt together.

6. The yeast and the hydration mixture were added together, staggering the times between each of them by 2 minutes in order to allow the time to mix the doughs before the yeast was done proofing for 10 minutes in the mixture.

7. Mix the hydration mixture and yeast with the flour and salt and then knead the dough for 20-30 seconds on a clean surface. Put the dough in the container, and seal it with the lid, taking the initial weight measurement.

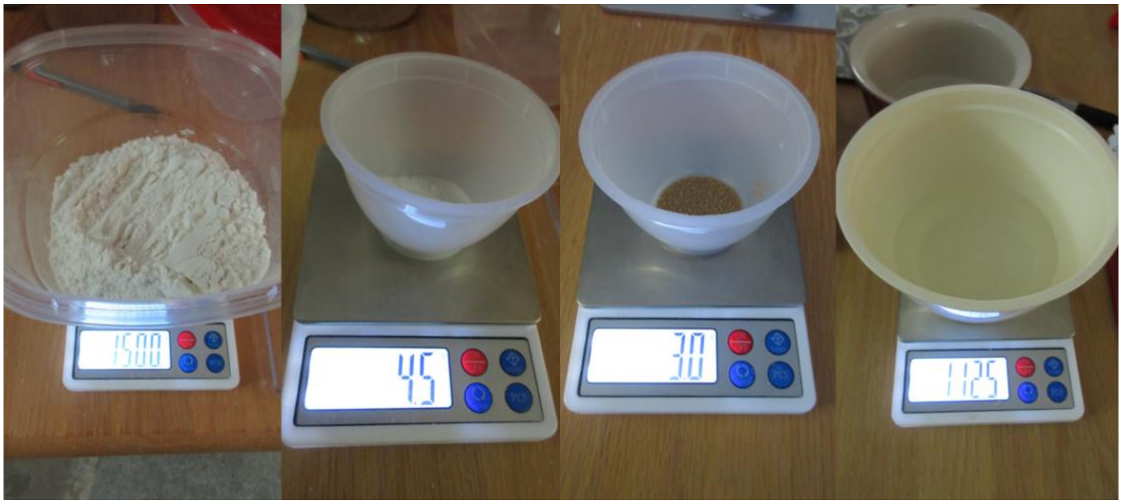
8. Every 20 minutes, take a new measurement of the weight in the container to track weight loss for a total of 120 minutes.

The way the saccharomyces cerevisiae ferments, it consumes sugars in the dough and creates ethanol and CO2 as products of the reaction (Lahue, C. et al. 2020). This particular strain of yeast prefers a glucose rich environment (Mazzoleni, S. et al.2015). The additives that were selected for this project were based on many home ingredients since adding something to the yeast might yield a healthier yeast and therefore a healthier rise of the bread dough. Since the rise of the bread is the CO2 being created by the saccharomyces cerevisiae fermentation, we can see how active something is by how it consumes the natural sugars in the flour, and we can track this activity by the weight loss of the dough. For this experiment a total of 3 batches of 10 dough balls were made and then the average of the measured weight losses over a 2-hour period was recorded and graphed to show how much of the mean weight loss was based on the additive used in the water of each respective dough. The goal for this project was to get an accurate measurement of weight based on the additive solely of the additive in the hydration part of the bread making process. To make sure that everything was weighed out accurately, I checked yeast activity following explicit guidelines for each batch of doug.

Variables

To control any variables that may have arisen during the experiment, every batch of dough was made following a strict routine. The time of the experiments were done in the evening, from 7:00pm to about 9:30pm. All implements and containers were washed thoroughly by hand, and then put into a dishwasher to further clean and dry them. All ingredients for this experiment were from the same containers each time to make sure different batches of additives or the like could not be a variable. The ingredients for the dough should have added up to 270.0g. Though not 100% of the ingredients are perfectly transferred into the containers due to kneading on the surfaces or some moisture being left in the individual containers the initial weights of the doughs were slightly off that 270.0g. The exact measurements of the weights to the tenths of a gram were recorded and used to track the activity of the yeast throughout the 2-hour fermentation process in the experiment.

Figure 1. Measuring of the Flour, Salt, Yeast, and Water Being Recorded to Their Respective Weights Totaling 270.0g.



The lemon juice was measured in 3 different volumes to see if an increasing acidity level of the dough would encourage an easier fermentation for the baker's yeast, but the results show that a more sugar infused hydration is more beneficial than just simply increasing acidity levels. To consider possible cross contamination and despite my workstation being small, the kneading of the dough into balls happened on a work surface which was separated into 4 sections. My wife Sarah assisted by scraping, cleaning, and sanitizing the surfaces as the dough was kneaded, and kept things organized throughout the process. No dough ball was kneaded on any surface that was not initially clean or cleaned immediately after use. All mixing was done with clean, sanitized spoons. Everything was also weighed out in cleaned individual containers till they were the weight they were supposed to be. No water was added to the honey until the honey was measured to 5.0g, after water was added to bring it to 112.5g to make the dough 75% hydration.

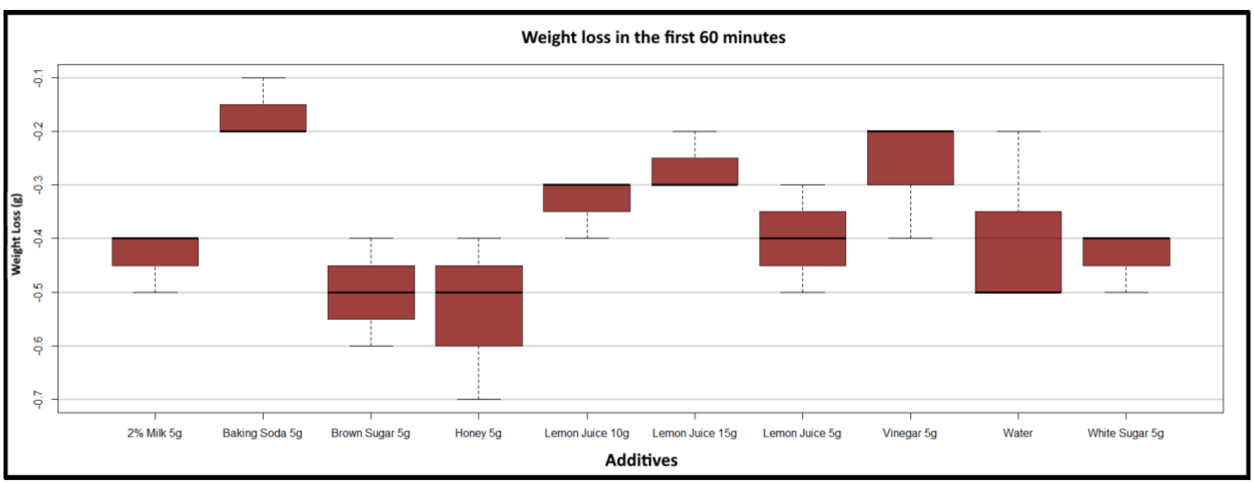
Figure 2. Measuring out the Additives and Adding the Water from the Same Source Afterwards to Bring Their Weight to 112.5 g.

The weights were taken after each dough was sealed in a container. Then the mixing was done, and weights were recorded on 20-minute intervals for a total of 2 hours. This strict process and the fact the containers were kept sealed through all 2 hours means that the yeast was undisturbed during its fermentation process and any weight loss recorded in those two hours would be from yeast's activity and based on what was the additive which was associated with it. No outside influences impacted the activity of the saccharomyces cerevisiae.

Results

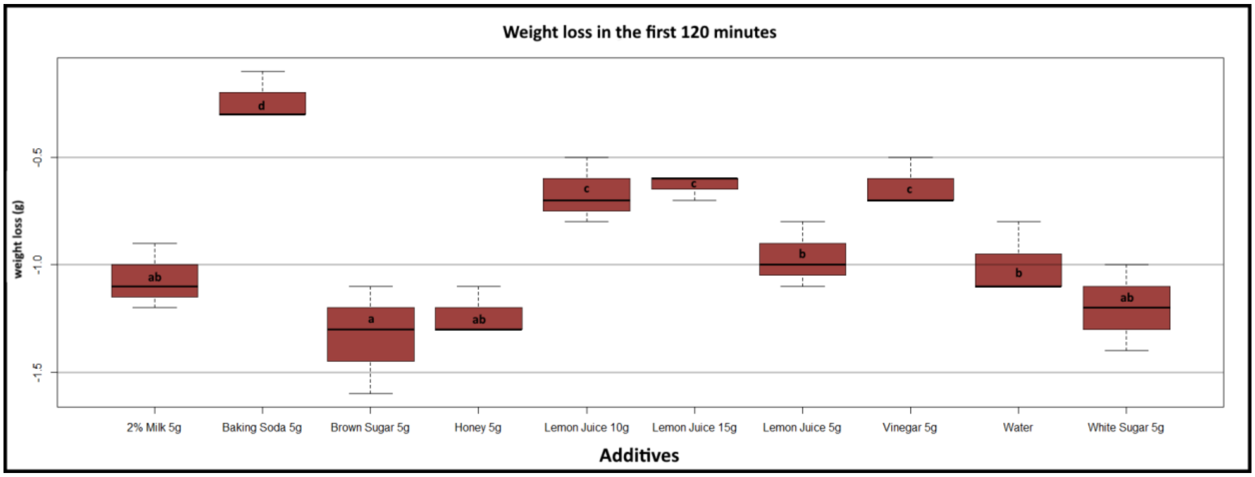
Since the additives were the independent variable, being able to adjust what was the additive gave a clear result in the yeast's health and activity. The results showed how certain additives that contain easily soluble glucose help stimulate the yeast and create a better environment for the yeast more than those which affect the pH of the dough by themselves. After collecting the data from the experiment, I ran an ANOVA test through Rcmdr to look for evidence that the additives and their weight loss were not equal. The results showed that the additives and their weight loss was not equal, where $F(9,20)=14.49$, $p<0.0001$. With these results from the ANOVA test I then was able to run the Multiple Comparisons of Means: Tukey Contrasts test to test which additive pairs and their means were different. The Tukey test showed that there was a statistical significance to the additives and how active the yeast was during the rising cycle for the dough. When looking at the results it showed that adding easily soluble and digestible sugars to saturate the hydration with the yeast rather than changing its pH environment is much more beneficial to the yeast for its activity to increase its rate of reaction (Mazzoleni, S. et al.2015). This would mean a better rise for dough because of the increased activity. We can see this by the weight loss and directly relate it to the activity of the yeast in the first 60 minutes and the following 60 minutes for a total of 120 minutes. The following figures show the 60-minute mark and 120-minute mark and their change in their net mean weights based on the additive. The data showed that the additives that have easily soluble glucose in them helped initiate the yeast for a quicker fermentation, which means the initial weight loss of the first 60 minutes tended to be more than for those additives which just made a better environment for the yeast by changing the pH levels of the dough, like the Lemon juice in all 3 volumes. But we can also see from both figures below that honey, white sugar and brown sugar all consistently experience higher weight loss, and the control of water, where the yeast is unimpeded, has a consistent net weight loss from the activity of the yeast than when comparing it with the other additives.

Figure 3. Weight Loss After the First Hour of Fermentation in a Sealed Container of the Different Additives.



Here, we can see even with the standard deviation that brown sugar and honey overall had the largest initial uptick in fermentation reaction attributing to its higher weight loss.

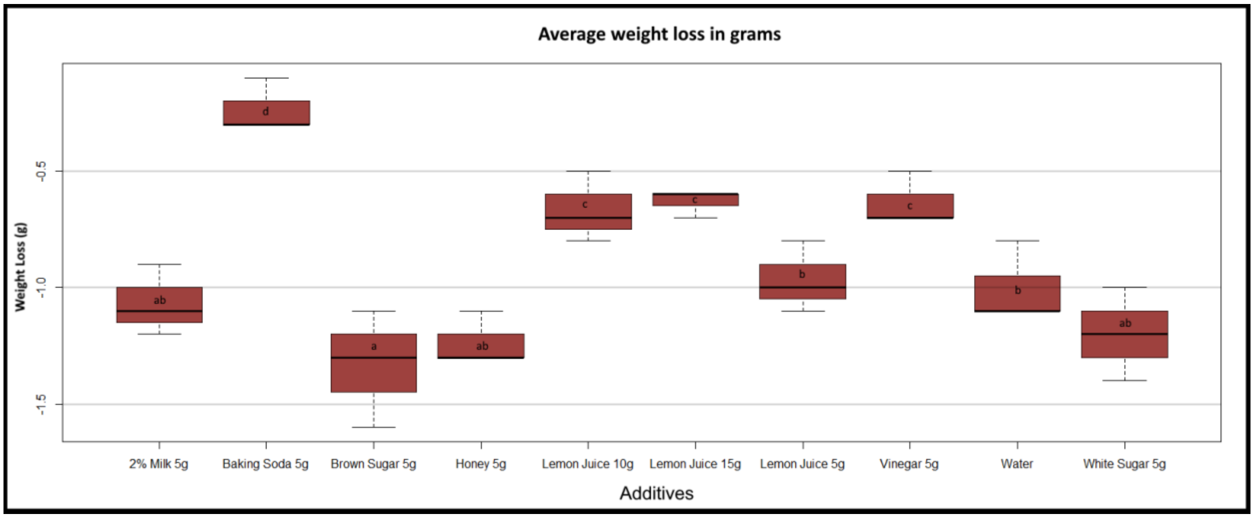
Figure 4. Weight Loss After the Second Hour of Fermentation in a Sealed Container.



Overall, we can see that increasing the pH level is not enough to keep the yeast happy as much as adding easily soluble sugars to the hydration, since the three which had the highest weight loss would be honey, brown sugar, and white sugar for the highest performing additives.

Those which are in this group are the ones in which are sugar which are easily water soluble. Mazzoleni's paper also points to this, that a water solution that is not over saturated in sugars helps the baker's yeast fermentation process since it is sensitive to sugar (Mazzoleni, S. et al.2015). The dough balls which had direct sugar added to the water did have a larger net weight loss during the 2-hour process overall compared to those which only raised the pH environment like lemon juice. We can see how they are grouped and the comparisons of the weight loss and with what they are grouped. We can see how sugar is indeed a factor to the health of the yeast and its rate of reaction when we compare the additives to each other. Since the net weight loss is tracking how active the yeast was during the rising process, we can say that either white or brown sugar or honey would be the best to add to water to promote a healthy growth of the yeast and promote its fermentation process. After averaging the weight losses, we can see the trends that form showing that the yeast was highly active in both sugars, and honey. Even the control of just water, where the yeast was unimpeded, was more active than those additives that directly affected the pH levels. When comparing the net average weight loss from the dough, we can see there is significance in the data to show that the weight loss can then be attributed to the health and activity of the saccharomyces cerevisiae and its additive.

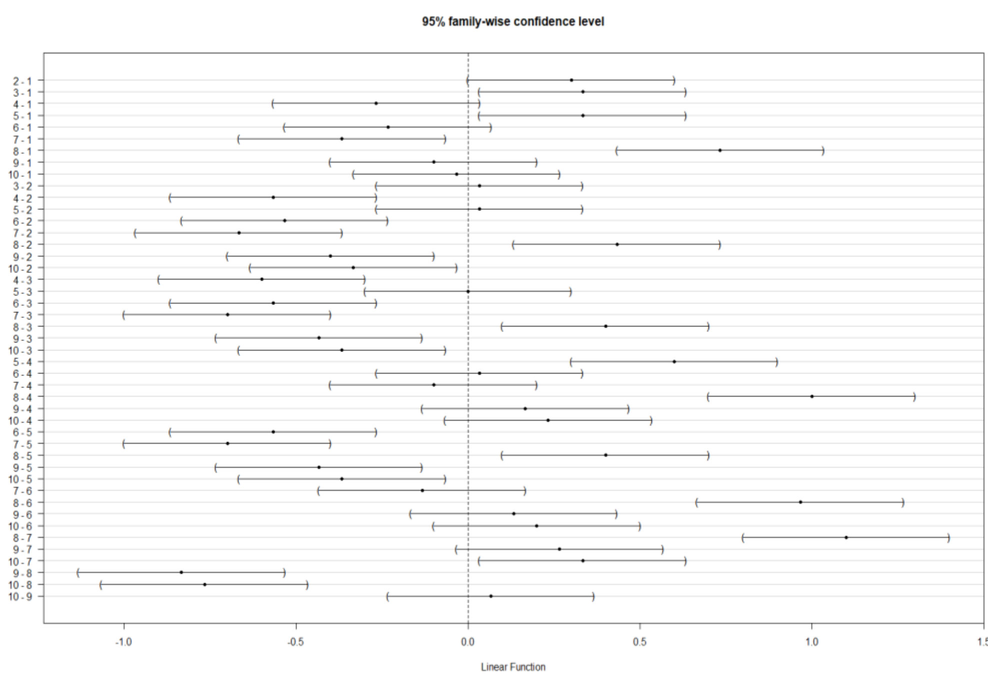
Figure 5. The Mean Weight Loss with Standard Deviation Showing Variation Between the Additives.



The additives have been marked to show the groups they can be associated with, relating their average weight loss based on yeast activity. It is interesting to note that 5g of lemon juice does increase activity of the yeast, but not as much as easily soluble sugar in water.

I compared all the means of the additives through simultaneous tests for general linear hypotheses to use for the Multiple Comparisons of Means: Tukey Contrasts. From that, we get this 95% family-wise confidence level graph. We see in the average net weight loss data how the CI procedure compares and how there is significance to the data gathered showing that the additives were the factor for the weight loss in the doughs.

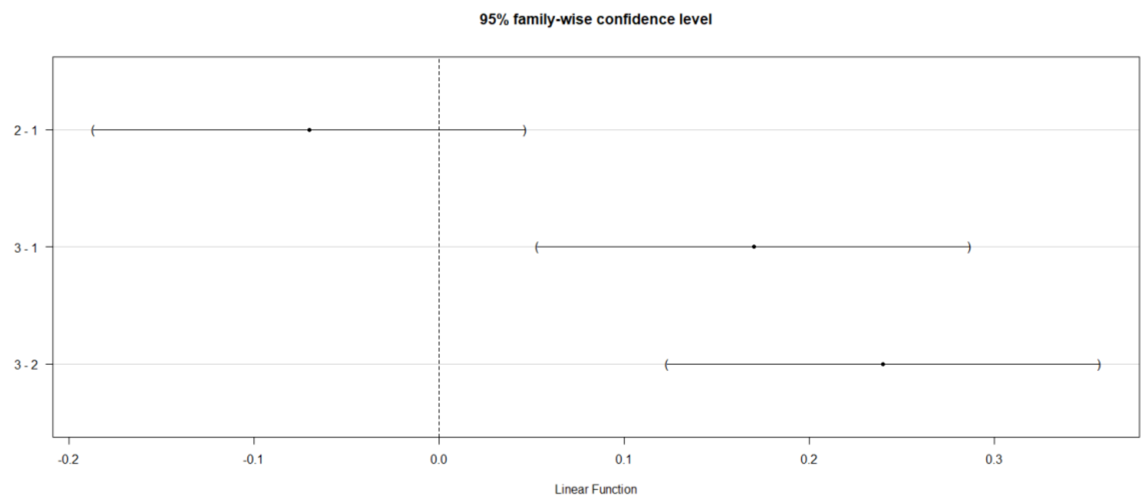
Figure 5. Running the Data through a Multiple Comparisons of Means.



In a Tukey test comparing all the additives to each other, we can see how they fall on the CI and relate to the groups reflected in Figure 3. Due to space on the graph the additives names have been changed to numbers, but the numbers here match the additives and their containers and additives, where 1 is lemon juice 5g, 2 is lemon juice 10g, 3 is lemon juice 15g, 4 is honey 5g, 5 is vinegar 5g, 6 is white sugar 5g, 7 is brown sugar 5g, 8 is baking soda 5g, 9 is 2% milk 5g, and 10 is the control of water.

For this experiment, factors had to be considered if the batches were a possible variable for the reason of an additive weight loss. Using Multiple Comparisons of Means: Tukey Test Contrasts and comparing the average net weight loss of the dough and the additives to show a 95% family-wise confidence level shows that batches 1 and 2 of the dough were found to be significantly similar. Batch 3 being different may have been due to the colder weather that happened when it was being made and batch 1 and 2 had the same relative city temperature in relation to each other, but the batches were not the reason for the difference in weight losses to the additives.

Figure 6. Comparing the batches to a 95% family-wise confidence level to see if they were significantly similar to each other.



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Batch 1 and 2 were similar, but 3 was different from the other two. This may be due to how the room temperature may have been different since it snowed on the day of making the third batch. The 95% family-wise confidence level shows that the batches were not the reason for the weight loss, comparing 2-1 $p<0.30161$, 3-1, $p<0.00447$ and 3-2, $p<0.001$.

Limitations

Even though the data itself does point to the trend that the additives are a factor in the activity of the yeast, there were some limitations with this study. First is the sample size. Due to time restrictions and limitations to how much could be done with the household supplies getting a sample size for this experiment. Time was also a factor, which limited the number of batches that could make during the time available to me. More time would have allowed me to make a few more batches to collect more data on the chosen additives. Everything was done in one and a half weeks and was not rushed, but more time would have helped in collecting and analyzing the data more thoroughly. Lastly, working within COVID restrictions also affected the experiment. If it was not for the pandemic, this experiment could have been done using a team of people to help with me in a proper climate-controlled kitchen setting over a longer period to get data to eliminate potential variables, but since it was just Sarah and me in the house, this slowed down the process at the start before measuring ingredients. That said, the limitations mentioned above did not affect the experiment at this small scale in an impactful way. If the limitations were addressed and more could be done, it would have only improved the data.

Discussion

Knowing how the yeast can thrive can affect numerous fields like biofuels, medicines, or even just how much someone can get out of the yeast for baking purposes (Gambacorta, F. et al. 2020). These results show that anything that either does not impede the yeast or increases the glucose concentration in the water increases its rate of reaction, and therefore how active the yeast is in the dough. As shown in Figure 3., using either honey, brown sugar or white sugar increases the activity of the yeast based on the weight loss attributed to it. This does mean that, like Mazzoleni’s findings, a glucose rich environment is better overall for the growth of yeast and thus increases its activity. The lemon juice 5g weight loss does show more weight loss than both other volumes of lemon juice, showing that there is a limit to the pH that the yeast can survive in. This means that if you want a more active yeast, you should activate the yeast in the proofing stage by adding some form of easily digestible glucose which is water soluble for the yeast to feed on and help kickstart the fermentation process of the saccharomyces cerevisiae rather than trying to create a better pH environment.

What this means from a home point of view is that when you are proofing your yeast, dissolving a touch of brown sugar, white sugar, or honey into the water, and then adding the baker's yeast during the proofing step will help the yeast ferment and activate for a healthier rise. This matches the results found with those additives (Figure 3) and tries to avoid things that would make the water either too acidic or too saturated for the yeast to function properly. In the case of industrial uses for S. cerevisiae, the yeast itself, as a single celled organism (Lahue, C. et al. 2020), has many industrial purposes, and having those uses means that we can help increase the production of its fermentation process, which means the yeast itself can be used to help break down waste to try to create ethanol (Lahue, C. et al. 2020). Additionally, having a proper environment for the yeast to thrive will increase its yields. This data can be beneficial to not only individuals but also groups and companies trying to get the most out of their yeast.

Future Use for this Data

This data could be used for further growth in the fields of study that use yeasts and molds to harvest medicine, supplies, or even just help amateur bakers improve their bread at home, all of which can benefit from figuring out how to make the baker's yeast perform as best it can. This yeast has the potential to help build a better future for greener sources of biofuels. Understanding how to make the yeast work at its peak would be vital to getting a higher production from it that would benefit not only bread, but everything that can use saccharomyces cerevisiae, including industrial uses such as helping create biofuels or medicine (Gambacorta, F. et al. 2020).

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Appendix A: Tools and Ingredients

Containers Used

Rubbermaid Take-Alongs - Deep Square Containers - Assorted - 4 x 1.2L

Tools Used for Accuracy of Weight Measurements

Digital Gram Scale Toprime Mini Size Food Scale 2000g x 0.1g High Precision Pocket Scale with LCD Display and 1 Tray Stainless Steel PCS Convert Unit White

BD(tm) Becton Dickinson Disposable Syringes 3cc. (5)

Ingredients

Robin Hood All-Purpose Flour

Fleischmaan's Traditional Active Dry Yeast

Compliments Fine Grind Sea Salt

Compliments Lemon Juice

Gramma Bee's Honey

Compliments White Vinegar

Rogers White Sugar

Rogers Golden Yellow Sugar

ARM and HAMMER Baking Soda

Lucerne 2% Milk

Tap water

Appendix B: Glossary

Saccharomyces cerevisiae: This strain of yeast is the most common form of instant dry yeast which has many home and industrial purposes (Gambacorta, F. et al. 2020). In this paper it will be called by the following names. Saccharomyces cerevisiae, S. cerevisiae, Baker's yeast or just yeast. All of them in this report are interchangeable and refer to the same strain of yeast unless specified further.

Baker's percent: Baker's percent allows easy upscaling or downscaling of recipes. This is where you take the weight of the flour in grams, divide it by one hundred, and multiply it by the factor you need so the dough being made can be easily replicated by other bakers. Recipes by bakers are often discussed in terms of baker's percent. In it, you have the flour weight, hydration, salt content and yeast in percentages to the weight of the dough. In this study I used 150g of flour, 112.5g of hydration, 4.5g of sea salt and 3.0g of yeast. Or 100%/75%/4%/2% for the baker's percent.

Hydration: This is the second number in the baker's percent. This is the water and additive together for a total weight of 112.5g. All doughs had this much weight in the hydration step as a response variable I could control for this project

Proofing the yeast: This is to make sure the yeast is alive or active. Normally with the yeast you add your weighed out amount to the water and let it sit for about 10 minutes. If the yeast is good then it should bubble and rise to the surface, if the yeast is dormant or "killed" there will be little to no bubbles after the 10 minutes and should not be used in the bread dough. All the yeast used in this experiment came from the same bottle of dry active yeast and was proofed in room temperature water for 10 minutes in its hydration water mixture. The same batch of yeast was used throughout for all doughs.

Acknowledgements

I must acknowledge the help I got along the way with this research project. First, I need to thank my professor Dr. Karen Buro. Her guidance and enthusiasm for statistics in this research project helped me refine my topic, my focus, and helped me draw the conclusions I did from my data. I also have to say thank you to my wife Sarah, who helped me refine my ideas, kept surfaces clean during the experiment, and helped me stay organized to keep my variables to a minimum. It cannot be overstated how much she helped during this whole process.

Since this is a study that deals with food products, I need to acknowledge potential food waste. This is a problem in our society and performing research projects that create food waste is a problem as well. Before undertaking this research project I made sure that I knew what would happen to all the dough when I was finished with it. If anyone else were to replicate this project, I would ask that they plan to use the food either for charity, to feed wildlife, or for friends or family to help minimize food waste and potentially help those in need.

Figure 7. A happy bird who will be well fed and happy throughout autumn and winter.