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Reporting Assault: Salt's Negative Impact on Yeast Fermentation

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Yeast, a key ingredient of the baking of bread, cakes, and other baked goods, causes dough to rise by consuming sugar and other carbohydrates and producing carbon dioxide and alcohol. Another key ingredient in baked goods, however, has a tremendous impact on the functionality of yeast: salt. We examined the relationship between salt concentration and the rate of fermentation in a yeast solution, and whether or not too much salt could effectively cease the fermentation process. Our team measured the carbon dioxide production in five trials for three separate fermentation solutions containing different levels of salt concentration. We expect our experiment to provide scientific guidelines to the baking community in regard to the amount of salt that ought to be used in recipes to result in the fluffiest, tastiest treat.

Introduction

Yeast is a living, single-celled fungus that society relies on for the creation of one of its favorite, most cherished things: baked goods. As any Ina Garten fan knows, yeast is a paramount player in the baking of breads, light cakes, and homemade cinnamon rolls. Dry yeast is added to flour, sugar, salt, and warm water or milk to prepare an initial dough of any baked good. The warm water or milk activate the dry yeast in the batter, allowing the yeast to begin to ferment. The fermentation process consists of the yeast breaking down the sugar and producing both carbon dioxide and alcohol (Schultz and Weaver, 1982). As carbon dioxide gets trapped in air pockets as it attempts to escape, the thick dough begins to rise. The alcohol, a volatile substance, evaporates during the baking of the bread, as does the carbon dioxide, leaving the baked good airy, light, and fluffy. The more the yeast ferments, the more carbon dioxide is produced, resulting in a fluffier, lighter bread.

Since the beginning of time, bakers have searched for new and innovative ways to personalize the bread-baking process. One ingredient, however, seems to affect the batter more than any other: salt. What role does the salt play in this process of fermentation? Why does every recipe call for a dash of salt to be added to this initial batter? And why might adding too much or too little salt result in disastrous consequences for a novice baker, resulting in a very flat or explosive cake? Apart from adding a touch of flavor, salt also regulates the rate of fermentation in yeast samples (Casey et al., 2013). Our team of scientists investigated the following question: exactly how does the level of salt in a fermentation solution affect the rate of fermentation? We explored whether there was an inverse or direct relationship between salt concentration and the rate of

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fermentation, measured by the amount of carbon dioxide produced by a yeast, honey, water and salt solution.

Introducing a salt solution could have a wide array of effects on the yeast solution. Salt solutions have been found to dehydrate organic matter (Martinez et al, 1999). The presence of salt will draw water out of the yeast, effectively dehydrating it. As the yeast becomes increasingly dehydrated, it functions less efficiently. We hypothesized that the higher the concentration of salt is in a fermentation solution, the slower the fermentation will be, and the less carbon dioxide will be produced, because salt in larger concentrations will more easily dehydrate yeast, rendering the yeast ineffective for fermentation. If our hypothesis is supported, the trials in which the salt concentration is the highest will produce the smallest amount of carbon dioxide. If our hypothesis is not supported, then the trials in which the salt concentration was nonexistent will produce a smaller or equal amount of carbon dioxide.

Methods

We tested the effect of salt concentration on the rate of fermentation in a yeast solution. We manipulated the concentration of salt (our independent variable) in each trial in order to examine how the rates of fermentation are affected between the different concentrations. The rate of fermentation (our dependent variable) was observed through our measuring of the carbon dioxide produced by each solution over a span of five minutes with a carbon dioxide probe in parts per million (ppm). From this, the rate of fermentation was found by subtracting the initial amount of carbon dioxide from the final amount and then dividing by 300 seconds. The first group had no salt, and this was our control group, as it was not affected by our independent variable at all. It gave a baseline for the manipulated groups to be tested against and so that we could clearly see how the rate of fermentation was affected by the salt concentration in the solution. The manipulated groups were different in that they contained either 5 mL of 0.3 M salt solution and 5 mL of deionized water or just 10 mL of 0.3 M salt solution.

The variables that remained constant throughout the experiment include: the type of sugar solution, the type and amount of yeast, and the setup and time of each run. The sugar solution used in the experiment was a 0.3 M honey solution, which is a 50/50 mixture of monosaccharides glucose and fructose (French and Shaw, 2018). This sugar solution provided the yeast with fuel and food, which it needs in order to ferment. The yeast used was S. cerevisiae and was prepared the same way each time. Five minutes prior to the addition of the honey solution, the yeast was added to 10 mL of deionized water and stirred to make sure that it was completely dissolved before the fermentation process started to get the best results. This was done very carefully and consistently before each trial to eliminate any outlying factors that could impact the fermentation process. Another part of the lab that was kept consistent each trial was our adding of the salt solution before the honey solution. This was done to make sure that the salt solution was already in the fermentation chamber, the 250 mL beaker, and any effects the salt had would be instantaneous when the honey solution was added.

First, we set up the carbon dioxide sensor by connecting it to the Logger-Pro system, switching it to its low setting, and allowing it five minutes to warm up. We measured out 0.5 g of dry yeast and poured it into a 250 mL beaker. Then, 10 mL of distilled water was also added to the 250 mL beaker and was placed atop a magnetic stir plate. The solution was mixed at a medium speed with a magnetic stir rod so that it did not splash on the sides of the beaker for five minutes. After five minutes, we added 10 mL of deionized water and then added 5 mL of the 0.3 M honey solution to the beaker. The Carbon Dioxide gas sensor was placed into the opening of the beaker, completely sealing the opening of the container. This was left for 5 minutes to record the amount of carbon dioxide produced in parts per million. While this was being recorded, the stir rod was still spinning. At the end of the five minutes, we collected data from the Logger-Pro that gave us the initial CO2 reading in the beaker and the final CO2 reading, allowing us to calculate a rate of CO2 production (our dependent variable) for each trial. This was repeated four more times for this ratio of yeast to sugar without salt.

After this trial was completed, we repeated the same steps with a different ratio. The 0.5 g of yeast and 10 mL of deionized water were allowed to mix for five minutes. Then instead of 10 mL of deionized water and 5 mL of 0.3 M honey solution; 5 mL of deionized water, 5 mL of 0.3 M salt solution, and 0.3 M honey solution were added. This was tested in the same manner as the initial group. Finally, a third group was tested, the difference being that instead of 5 mL of deionized water and 5 mL of 0.3 M salt solution, 10 mL of 0.3 M salt solution was used. These were all tested 5 times.

After the experiment, the data was presented in a box and whisker plot graph. This type of graph best represented the data, and it easily outlined the groups and how they relate to each other. After the graph was made, a One-Way ANOVA was used to test the significance of the results and the differences between the groups. This One-Way ANOVA test was done

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because in the experiment, there was no clear trend in the data. This test was used to see if there was any significance in the addition of salt on the rate of fermentation. After this, a t-Test was used to see if there was a significant correlation between the amount of salt added to the fermentation solution and the rate of carbon dioxide production.

Results

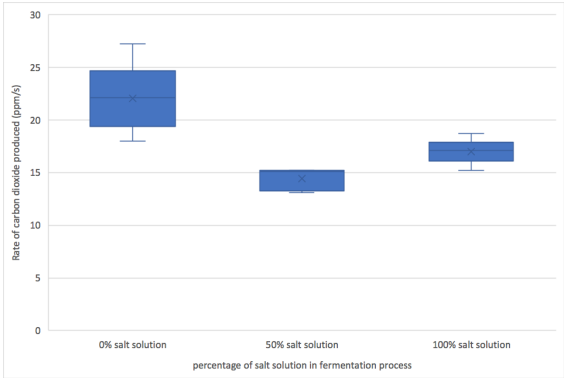

Figure 1| The graph shows the rate of carbon dioxide produced in ppm/s by how much salt solution was present during the fermentation process. The graph shows a trend that there is a drop in the rate of carbon dioxide produced when salt is added. However, from 50 percent salt solution to 100 percent salt solution, there was a slight increase in the rate. Both the One-Way ANOVA and the t-Test show that this data was statistically significant with p = 0.0002427 and 0.0030775, respectively.

The graph shows that as salt is added the rate of fermentation does decrease however it does increase when more salt is added. The average rate of the fermentation for the control group was 22.07 ppm/s and this decreased when the solution had 50 percent salt solution (i.e. 5 mL 0.3 M salt solution and 5 mL of deionized water) to 14.41 ppm/s. However, the rate actually increased when the solution added was 100 percent salt solution (10 mL of 0.3 M salt solution). There are a few outliers in the 0 percent salt solution as well as the 100 percent salt solution, but the 50 percent salt solution looks to be pretty precise. These outliers do affect the average rates; however, it seems to not be the main reason the rates are the way they are.

A One-Way ANOVA was conducted to compare the effect of the solution used on the rate of carbon dioxide produces in 0% salt solution, 50% salt solution, and 100% salt solution. There was a significant effect of the solution used on the rate of carbon dioxide between the three conditions; [F = 18.02; p = 0.0002427]. Since there was significance between the groups, a t-Test was used to test the effect between the 50% salt solution and the 100% salt solution.

An Unpaired t-Test was conducted to compare the effect of 50% salt solution and 100% salt solution on the rate of carbon dioxide produced. There was a significant difference between the two condition; t = 4.1805, df = 9, p = 0.0030775.

Discussion

The purpose of this experiment was to answer the question of what effect salt has on yeast fermentation, as well as if the concentration of salt affects the fermentation rate as it rises. Our data suggests that while salt does have a negative effect on the rate of fermentation, this effect does not increase with an increased concentration of salt. The data collected both supports and does not support different elements of our hypothesis. While the addition of salt did affect the rate of fermentation, an unexpected outcome was observed when the amount of salt concentration in the salt solution rose from fifty percent to one-hundred percent. The rate of fermentation rose between the fifty percent and the one hundred percent salt solution trials from 14.01 ppm/second to 17.03 ppm/second, respectively. This jump does not support our hypothesis; however, both the fifty percent and the one hundred percent solution trials had slower rates of fermentation than the trials without salt did (22.07 ppm/second). Our data suggests that while the presence of salt slows the rate of fermentation, the amount of salt does not slow it further.

Statistically, the addition of salt to the fermentation did slow the rate of fermentation. Using a One-Way ANOVA, we found that there was a measurable difference between our control and experimental groups in a way that was statistically significant. Our t-Test showed a significant difference between our two experimental groups (the fifty percent and one hundred percent salt solutions). While they were statistically different in the t-Test, our experimental groups' data did not support our hypothesis, because the one hundred percent salt solution trials had a faster rate of fermentation than our fifty percent salt solution trials did.

We believe that there was a systematic error in our experiment that caused the rate of fermentation to increase between the fifty and one hundred percent solution trials. This error, we believe, was due to the provided salt solution's inaccurate molarity. Between the fifty percent trials and the one hundred percent trials, a

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new salt solution was provided to us, and there was a significant increase in the carbon dioxide production during the ensuing trials. We believe that this new salt solution had a lower concentration of salt than it was supposed to have, resulting in a faster rate of fermentation during those trials. Historically, scientific research has suggested that larger concentrations of salt slow the fermentation process, even causing it to cease completely, given a high enough concentration (Zahar et al., 2002). Our trials do not align with these findings, leading us to believe that something beyond our control altered our experimental results. The exact reason cannot be proved, however, because we ourselves could not test the molarity of our salt solution with the equipment available to us.

Fermentation rates could have also increased due to a change in temperature between our days of testing. Fermentation rates are faster in warmer solutions, so if the room or the solution itself were to be warmer between our trials (which occurred a week apart), the warmer solutions could have displayed faster fermentation rates (Kwon and Mheen, 1984). As we did not have access to a thermometer, this is purely speculation.

An alternate way of interpreting our data would be to accept our results at face value and agree that while the salt does slow down the rate of fermentation in a yeast solution, increasing the salt concentration in the solution will not have any further negative effect.

The practical applications of this experiment can be found in the kitchen. We found that salt had a negative effect on yeast fermentation. When preparing dough using yeast, not including any salt could be disastrous. The yeast would ferment at an exceedingly fast rate with nothing to impede it, resulting in a large amount of carbon dioxide being produced. This gas would get trapped in the dough, causing the bread to grow freakishly large.

As our results in regard to the effect of salt concentration on yeast fermentation were skewed, there exist many possibilities on how to expand upon this experiment in the future. For example, the experiment could be repeated while controlling for temperature in the room or manipulating the room temperature. Scientists could also use actual dough with different salt concentrations to measure the effects of salt on the rising of the pastries in a more physically obvious way. Also, scientists could experiment with the amounts of sugar in each solution in an attempt to observe whether or not the presence of a lot of sugar can counteract the effects of the salt. Yeast feeds on sugar to ferment, so perhaps an increase in the concentration of sugar in the yeast solution can raise the rate of fermentation regardless of the presence of salt (D'Amore, 1992). The amount of sugar and water in a yeast solution may be more important than the amount of salt present.

References

Casey, E., Mosier, N., Adamec, J.,Stockdale, Z., Ho, N., and Sedlak, M. (2013). Effect of salts on the Co-fermentation of glucose and xylose by a genetically engineered strain of *Saccharomyces cerevisiae. Biotechnology for biofuels. 6:83.*

D'Amore, T., (1992). Cambridge Prize Lecture Improving Yeast Fermentation Performance. 98: 375-382

French, D. and Shaw, T. (2018). Authentic Research in Introductory Biology, 2018 Ed. Fountainhead, Fort Worth.

Kwon, T. and Mheen, T. (1984). Effect of Temperature and Salt Concentration on Kimchi Fermentation. Korean J. Food Science Technology. 16(4): 443.

LoggerPro3 (Version 3) [Computer Software]. (2016). Beaverton, OR: Vernier Software & Technology.

Martinez, E., Moreira, R., and Sereno, A.M. (1999) Mass transfer coeficients during osmotic dehydration of apple in single and combined aqueous solutions of sugar and salt. Journal of Food Engineering. 47(2001): 43-49

Schultz, J. E. and Weaver, P. F. (1982) Fermentation and anaerobic respiration by Rhodospirillum rubrum and Rhodopseudomonas capsulate. Journal of Bacteriology. 149(1): 181-190

Zahar, M., Benkerroum, N., Guerouali, A., Laraki, Y., and El Yakoubi, K. (2002). Effect of temperature, anaerobiosis, stirring and salt addition on natural fermentation silage of sardine and sardine wastes in sugarcane molasses. Bioresource Technology. 82: 174

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