The Effectiveness of Fruit Juices in Preventing Microbial Growth On Plain Bread

Chris Cai, Nikki Ebrahimi-Siaghi, Julie Wu

Abstract

Food waste has developed into a global issue that plays a significant role, not only for the economy but for the environment. The considerable amount of edible food lost as waste each year is mainly due to spoilage thus the aim of our study is to determine whether acidic fruit juices may be used as preservatives in mould growth prevention of bread. We conducted our experiment by soaking homemade plain bread slices with 5 different treatments for 17 days. We placed each piece of bread in a separate seal with a 1 cm x 1cm grid across. During the experimental period, we determined the microbial growth amount by the number of squares that had developed mould and recorded results daily. Initial results showed that the number of squares covered increased overtime for all treatments with pH of 4.3 to 6.3 however, by the 10th day, the more acidic treatments of pH 2.5-3.3 surpassed others with a significant amount of microbial growth. Further analysis determined statistically significant results with a p-value less than 0.05 yet an incomplete rejection of our null hypothesis stating that a more acidic liquid with a low pH will inhibit mould growth when applied to the bread. These results are not entirely consistent with previous literature which suggests the fruit juices that we had chosen were not necessarily the most effective antimicrobial agents thus further experimentation may be done with other solutions to determine what may be the most effective natural preservative.

Introduction

Our experiment considers how the pH of various fruit juices may affect the amount of mould growth in bread. Our hypothesis predicts that a more acidic liquid with a low pH will inhibit mould growth when applied to the bread. These results have been observed through similar experiments in the past in which the effectiveness of various solutions was tested for their ability to act as antimicrobial agents. Such activity shows great potential for food preservation and storage which plays a significant role in the global waste issue as 1.3 billion tons of edible food is lost every year with a considerable portion of this being due to spoilage from farm to table. An effective preservative may be essential to decreasing this number in the future (Shwaiki et al., 2021). Previous literature shows such antimicrobial potential with a very limited pH range of 2.43-2.73 acid having the greatest inhibitory effect as it had the least mould growth over time (Amirthalingam et al., 2022). Such an effect is possible as acidity results in microbial stress. The undissociated molecules of acid can diffuse rapidly into a microbes plasma membrane and thus increase proton concentration. This effectively acidifies the cytoplasm and thus inhibits growth (Lambert & Stratford, 1999). We have taken this one step further by determining whether a greater range of acidic solutions may be responsible for antimicrobial activity. Fruit juices have also been suggested in the past as a possible natural preservative. Onion juice has been studied as a form of food preservation by pH manipulation for bread which determined a possible increase in shelf life (Lee et al., 2009). The possibility of other fruits having similar qualities and effects is determined through our experiment however, there are many factors to consider when working with mould growth. Silveira et al. (2019) determined that mould growth is influenced by environmental factors such as relative air humidity, indoor air temperature, and surface condensation. They conclude that the amount of sun and the presence of room insulation can affect mould growth, therefore, we have considered such factors in our experimental design to ensure all samples are in equal “living” conditions to limit data errors.

Methodology

Our experimental design was to record the growth of mould on bread that was soaked in different liquids with differing pH. We would take pictures of the bread every day and note how much of the bread was covered in mould every 1-2 days. We planned on using 5 liquids of differing pH. To obtain a more accurate measurement, we had three replicates for each liquid type, resulting in 15 pieces of bread needed for our experiment.

To achieve this, we decided to make home-baked bread to avoid encountering any issues with store-bought bread and any preservatives that may have been in them. It was homebaked using 4 cups of all-purpose flour, 1 tablespoon of yeast, 2 cups of warm water, and 2 teaspoons of salt. Using a Hamilton Beach branded Breadmaker, we kneaded the dough for 1 hour and baked it for 2 hours. After baking, they were cut into 13cm x 9cm slices of approximately 1cm thickness as demonstrated in Figure 1 below.

Figure (1): Our sliced pieces of bread

The different liquids used were lemon juice, orange juice, tomato juice, watermelon juice, and water. We juice the lemon and orange juices by hand while the watermelon and tomato juice was achieved by blending and then straining. The water was boiled and then left to room temperature before pouring it onto the bread. The liquid's pHs are as follows:

Lemon Juice	2.5
Orange Juice	3.3
Tomato Juice	4.3

Watermelon Juice	4.9
Water	6.3

Figure (2): pH Readings

The pHs were measured using pH indicator strips provided by our Lab.

Figure (3): Lemon juice

The bread slices were stored in sealed sandwich bags that were marked with 1cm x 1cm grids. 10ml, measured out using a graduated cylinder, of each liquid was poured onto the bread in the bag before sealing them and placing them in the sandwich bags as seen in Figure 4 below. All pieces were then kept in a room with minimal sunlight and low heating. The experiment spanned 17 days starting on October 27th and ending on November 13th of 2022.

Figure (4): The bread slices in order of increasing pH: Lemon, Orange, Tomato, Watermelon, and Water

After the experiment time was over, we reviewed the photos and counted the number of squares that contained mould for each piece to use for data analysis.

Results

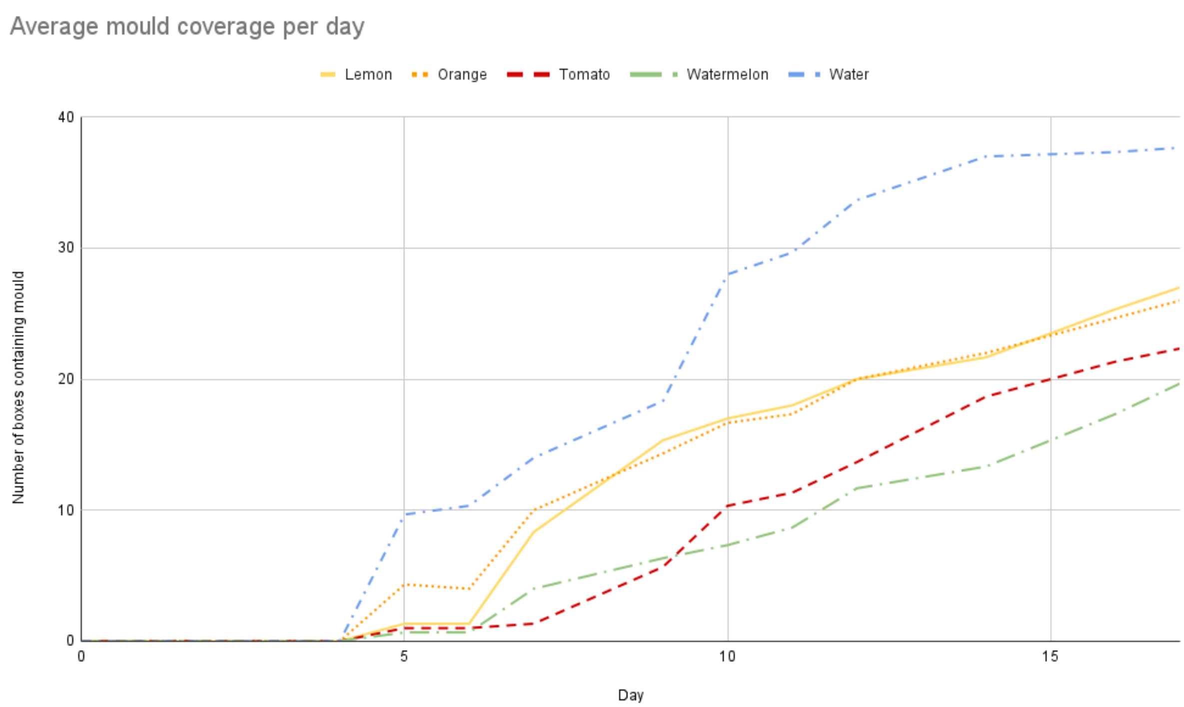

Figure (5): Recorded mould coverage for each liquid type represented by the change in the number of mould-covered sections over time.

Figure 5. shows the average mould coverage for each treatment over time. The number of blocks covered by mould was increasing over time for all of the treatments, while no mould growth was observed for the first four days. The control group, the bread with water, had the highest coverage of all time. From day five to day six, the mould coverage of bread with treatments of watermelon, lemon, and tomato was close to each other, while bread with the treatment of orange had a higher mould coverage. After day six, bread with treatments of orange

and lemon had a very similar mould coverage, which was higher than the one with watermelon and tomato. Before day ten, the mould coverage of bread was higher for the one with watermelon. After day ten, the opposite was observed.

The slope of each treatment for each replicate was calculated by using the trendline equation in google sheets. Then the slopes were used to calculate the p-value by using the one-way ANOVA with an alpha level of 0.05 in GraphPad. The p-value for the ANOVA test was 0.0068. In addition, Tukey's test was applied to determine which treatments had significant differences from each other. The adjusted p-value was 0.0348 for treatments of orange and water, 0.0403 for treatments of tomato and water, and 0.0039 for treatments of watermelon and water. The adjusted p-value for other comparisons was all greater than 0.05.

Discussion

Our experiment was conducted with the aim of observing the possible natural preventive effects of fruit juices as a method of decreasing bread spoilage by mould growth which is an essential step in dealing with the global food waste issue we currently face (Shwaiki et al., 2021). The p-value of the single-factor ANOVA test is less than 0.05. Therefore, the null hypothesis of the ANOVA test is rejected and it is concluded that there is a significant difference between some treatments. The result of Tukey's Test shows that the treatments of orange, tomato, and watermelon have a significant difference in mould coverage between the treatment of water as their adjusted p-value is less than 0.05. This means that solution acidity has some effects on inhibiting the development of microbial growth on plain bread compared to water. However, the results can not conclude that increasing acidity has greater effects on inhibiting the development of microbial growth as the adjusted p-value for comparison between treatments, such as orange and watermelon, is less than 0.05.

Based on our current findings, the results of our experiment reject our null hypothesis that an increase in solution acidity would result in a decrease in mould development, despite the significant research suggesting otherwise. Our results are not consistent with previous findings that solutions of a pH of 2.43-2.73 had the greatest inhibitory effect (Amirthalingam et al., 2022). Our results show a range of pH 4.3-4.9 to have the highest antimicrobial activity. Such a difference may be due to the fact that the previous experiment used various white vinegar and baking soda solutions of different concentrations when conducting their study, while we used fruit. The difference here can be seen in the methodology and requirements of the experimental design. While vinegar and baking soda solution can be properly measured to accurate concentrations in a lab, fruits must be juiced before use. We chose to use fresh fruit juice as opposed to pre-juiced samples from supermarkets as the preservative ingredients supplies chose to include in their samples would limit the accuracy of our results. We expected to see the effect of the type of juice itself, however, the lemon and orange juices were juiced differently than the tomato and watermelon juices and thus could be the reason our results deviate from previous literature. The lemon and orange juices might have contained more pulp than the tomato and watermelon juices, thus possibly resulting in more mould growth. Due to this distinction, it may be best to compare the results of lemon and orange separately to the other treatments as those two were juiced by hand while the others were blended mechanically. Such errors are what has resulted in our data supporting the alternate hypothesis of microbial growth increasing with pH.

We observed that the highest amount of mould growth was with our bread slices with water poured onto them. This is expected, as we predicted that the least acidic liquids should have the most mould growth. However, our data changes when it comes to our fruit juices. Our lemon and orange growths are quite similar, despite being different pHs, and have higher amounts of mould growth than the Tomato and Watermelon juice treated samples; this result is the opposite of what we predicted, as the more acidic samples were predicted to have less mould growth. Furthermore, the tomato samples seemed to have a higher mould growth than the watermelon samples, despite the tomato samples having a higher acidity.

There might be a few factors that can have an impact on our results. One of the factors is human errors. Some pieces of bread also had crevasses in them, so the shape of the bread was tried to be evened out between all types of liquid. This causes the bread to not be covered by juices entirely, as only the central parts of the bread are covered by juice. The coverage of mould may not be counted correctly as most of the bread is coloured by the juices. The pH values may also not be read accurately due to the ability to read indicators.

Environmental factors are likely to influence our results. Air humidity, temperature, and other factors could have an impact on mould growth (Silveira et al., 2019). Murdoch et al. (2013) conclude that violet light has a negative effect on the growth of fungi species they test on. Although sandwich bags are used to keep the bread and mould contained, and are kept in the same dark room, the results may still be influenced by environmental factors since the bread is placed inside the bags while they are still warm.

Total soluble solids are another factor that could have an impact on our results. Total soluble solids are solids that are soluble in water, and mainly contain sugar and other soluble substances (Li et al., 2016). Tremarin et al. (2017) found that for a given temperature, increasing the concentration of total soluble solids would decrease the maximum growth rate of Byssochlamys Fulva in apple juice. Furthermore, Reischke et al. (2014) found that increased glucose levels in soil were associated with increased fungal growth. These reasons may explain why the mould coverage with the treatment of watermelon juice is lower than the one with treatments of lemon juice and orange juice, as watermelon has a low total soluble solid level on average. Future studies could examine the effect of different pH on mould growth while maintaining a stable total soluble solids level for each treatment.

Conclusion

The aim of our study was to determine whether acidic fruit juice of different pH levels is able to successfully inhibit the development of microbial growth on plain bread. The results have shown no clear trend in the relationship between fruit juice acidity and mould growth for the chosen fruits contrary to our hypothesis that expected an inhibitory effect or stronger acidic fruit on microbial development. Our study thus rules out a handful of possibilities for food spoilage prevention and suggests a need to look further into other natural preservative solutions to the global waste problem.

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