# Exploration of Sugar's Inhibitory Effect on Bread Mold Growth

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## Abstract

Preserving food has been challenging for many years; therefore, effective methods to restrain spoilage and bacterial growth are needed. While traditional preservation methods often alter the texture and taste of food, sugaring has been discovered as a viable option. This study explores the inhibitory effects of sugar, specifically sucrose, on visible molds commonly found on bread. Our hypothesis expects inhibited mold growth in sucrose concentrations ranging from 2.5% to 5%. Organic, preservative-free bread slices were treated with sugar solutions of varying concentrations, and mold growth was monitored over three weeks. The results showed insignificant evidence to show that sugar concentrations affect mold growth on bread. The identified mold species varied, suggesting potential external factors. Future research could focus on specific mold inoculation and lower sugar concentrations to explain optimal conditions for inhibiting mold growth. This study presents sugar as a promising household food preservation method, contributing to food safety and waste reduction.

## Introduction

Food preservation is an issue that has plagued civilization since the very beginning. Food preservation, or the treatment of food to greatly slow down spoilage and prevent the growth of bacteria, can be dated back thousands of years (Joardder & Masud, 2019). Preserving food is crucial to reducing food waste and preventing the spread of foodborne illnesses (Martindale & Schiebel, 2017). While there are many food preservation methods, some standard methods, such as refrigerating and heating, require energy and can affect the texture and taste of certain foods. One widely available method of preserving food is sugaring.

Sugaring is the method of food preservation where food items are packed in pure sugar to decrease microbial activity (Joardder & Masud, 2019). Sugar’s effects on bacterial growth are an area of interest because it has both pro-microbial and antimicrobial properties. Nutrients are essential for bacterial growth, and sugars can be a great source of nutrients (Reischke et al.,

2014). In contrast, it is observed that high concentrations of sugar can inhibit bacterial growth. A natural instance of this can be seen with honey, which, if properly stored and protected from contamination, will never spoil. Researchers have found that the high concentrations of sugars in honey have an osmotic effect, absorbing the necessary water needed for microbial growth away from the bacteria (Lusby et al., 2005). However, the specific sugar concentration required for antimicrobial effects for common bacteria and molds has not been thoroughly explored. Past research determines the threshold sugar concentration for antimicrobial effects to start on common food pathogens such as *Staphylococcus aureus, Escherichia coli* and *Salmonella enterica* (Mizzi et al., 2020). Unfortunately, these are all pathogens invisible to the naked eye, unlike common food molds such as *Aspergillus* and *Cladosporium*, which have different bacterial properties, indicating a different threshold sugar concentration needed for sugar to act as an antimicrobial agent.

Furthermore, the same study explores the effects of sugars, including sucrose, fructose and maltose. The most widely available sugar in the typical household is sucrose in the form of table sugar. Therefore, this study will observe the threshold sucrose concentration needed to have antimicrobial effects on common visible molds.

Based on these past studies observing other food pathogens (Mizzi et al., 2020), we hypothesize that we see inhibited bacterial growth from increased sugar concentrations. We predict inhibited bacterial growth is expected from the 2.5% to 5% sugar weight/water weight solution due to sugars' osmotic effect, which takes away water necessary for growth from existing bacteria. With this information, we hope to provide another method of preserving food in households everywhere to promote food safety and reduce food waste.

## Methodology

Organic preservative-free bread was obtained from Safeway and sliced into 18 one-centimetre-thick segments with a sterile knife, each standardized to 10cm x 10cm. Six experimental groups were established: one control and five treatment groups, each with three bread samples. The control group was sprayed with distilled water, while the treatment groups were subjected to sprays of sugar solutions of increasing concentrations: 2%, 4%, 6%, 8%, and 10%. The sugar solutions were diluted from a pre-prepared 60% sugar solution, using graduated cylinders for accurate measurement. All bread samples were exposed to an open environment for 30 minutes to initiate bacterial inoculation. Subsequently, each set of bread samples was individually enclosed in labelled Ziplock bags, and the designated sugar solutions were uniformly applied to the bread using spray bottles within the zip lock bags, aiming for approximately 2-3 mL of solution per bread sample. Ziplock bags were sealed securely, and no further openings were conducted throughout the experiment. Mold growth observations focused on percentage coverage, facilitated by a 10cm x 10cm square of clear plastic with a 10 x 10 grid representing 1%, placed over each bread sample during observations. Mold coverage was measured by the percentage of mold covered within the plastic squares. Measurements were recorded every day for three weeks or until the sample reached 100% coverage. At the end of the experiment, mold present on the bread will be identified using a dissecting microscope.

Data analysis involved comparing mold coverage percentages between the control and treatment groups, utilizing statistical tests such as t-tests or ANOVA to determine significance. A graphical representation was also generated to illustrate the progression of mold coverage percentages over time, providing a comprehensive visual overview of the experiment's outcomes.

**Results**

The analysis aimed to answer the research question: will increased sugar concentrations inhibit bacterial growth from the 2.5% to 5% sugar weight/water weight solution due to the sugars' osmotic effect, which removes water necessary for growth away from existing bacteria?" Our analysis produced 3 line graphs showing correlations between different sugar concentration levels and the percentage of mold growth throughout the 2-3 weeks.

We plotted time and mold growth from the 18 treatment groups (Fig. 1.) The growth curves of every sample are organized by colour. The sample with the fastest, most consistent growth was one of our control samples with 0 sugar. However, none of the other control samples had similar results, having prolonged, delayed growth, with one control sample not showing any mold.

Furthermore, every other sample showed much slower mold growth. The two exceptions are one sample of the 6% group and one sample of the 10% group. These two samples had significantly delayed mold appearance; however, once it appeared, both growth rates were significantly higher than any other species. We conducted a one-way ANOVA analysis and got $p<0.05$.

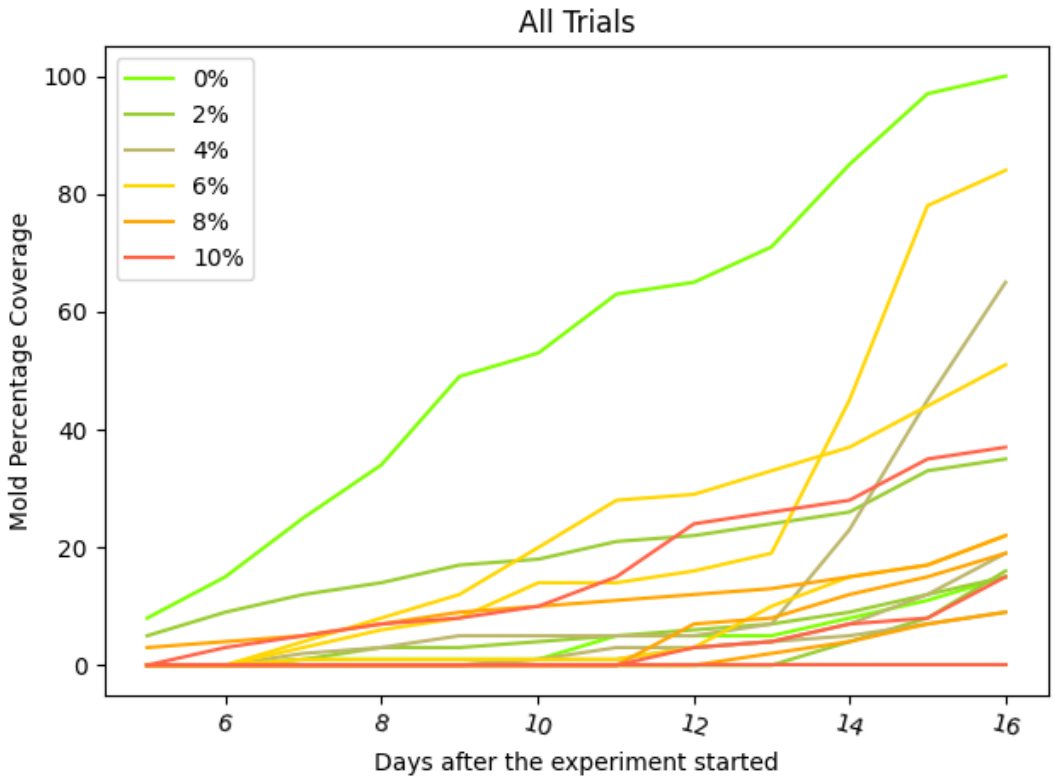


***Figure 1:*** *Mold percentage coverage on bread over time for all trials. Control group has water (0%). Rest of the groups have different levels of sugar concentration solution (2-10%) Sample size = 18*

The graph below illustrates the average growth curves of the three samples per treatment group (Fig 2). This shows the average of the three samples, and one of our controls did not show any mold; the control growth curve has decreased significantly. However, it remains the highest until the later half of our experiment, where mold from the 6% group showed massive growth. We conducted a one-way ANOVA analysis and got $p<0.05$. Therefore, the data is statistically significant.

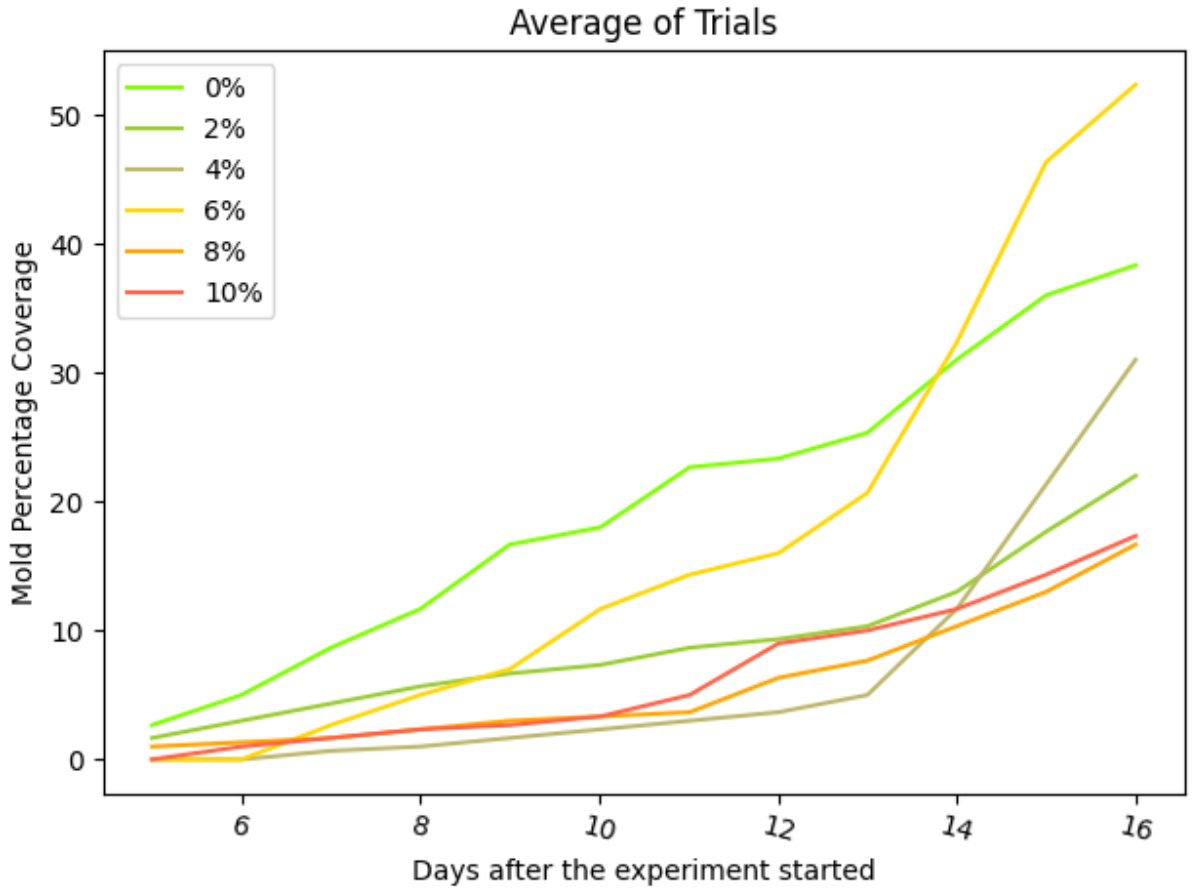


***Figure 2:*** *Average mold percentage coverage on bread over time for each group containing 3 trials. Control group has water (0%). Rest of the groups have different levels of sugar concentration solution (2-10%)*

Next is the average slope of the average growth curves (Fig 3). The sample weighed down the average slope of our control with zero molds on it. Every other experimental group has a significantly lower slope other than the 6%.

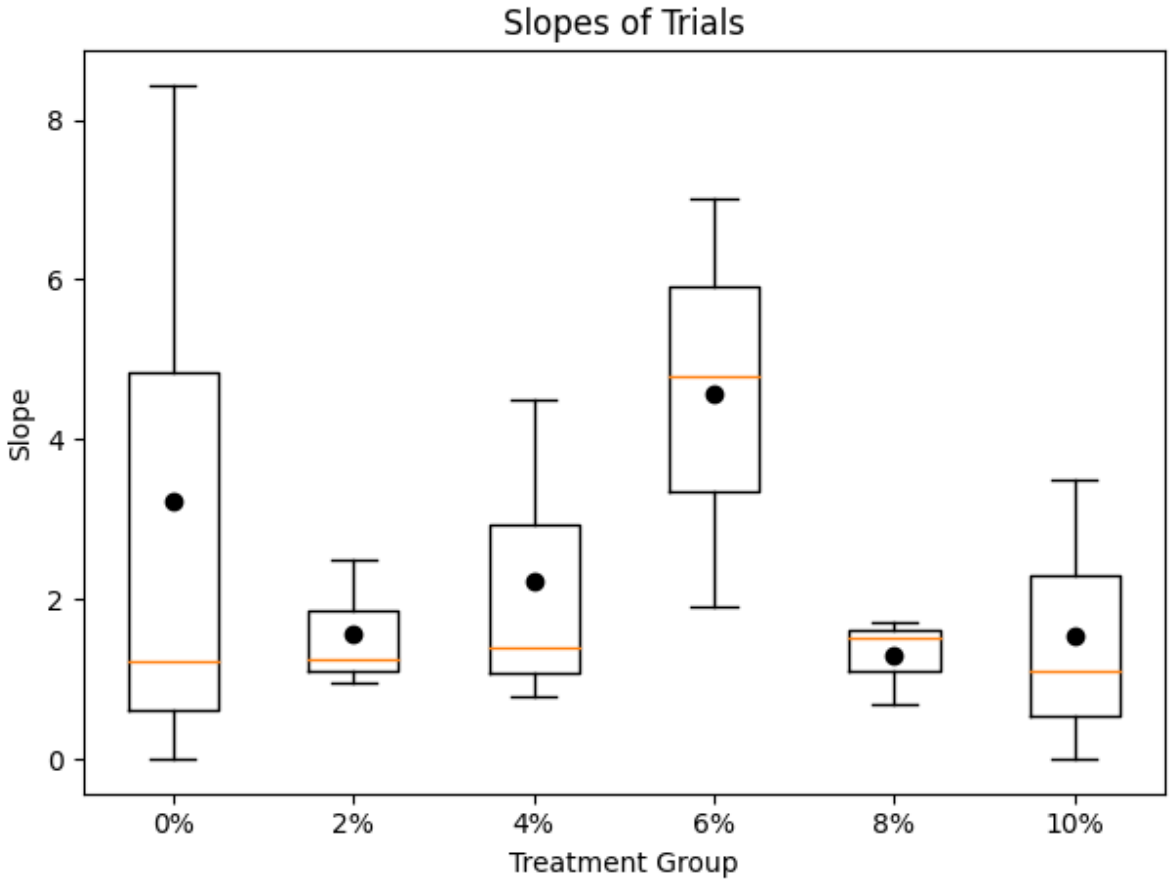


***Figure 3:*** *Average mold growth per day for each experimental group, containing different concentration sugar solutions. Orange = median growth rate. • = mean growth rate. p = 0.53*

At the end of the three weeks, a few different species of mold appeared on the bread. The most common one was Aspergillus, which appeared on 9 of 18 samples. There were also cases of penicillium, rhizopus, and neurospora. However, we could not identify some samples because only hypha growth was observed, and needed access to compound microscopes for more detailed analysis. Two samples, one from the control group and one in the 10% group, did not have any mold growth by the end of the 3 weeks.

**Discussion**

Individually, it seems that there may be significant differences in growth rates between the treatment and the control groups. However, when taking the average growth rate per day

overall three trials per group, it is clear that these differences are not significant. Due to a p-value of 0.53, there is too high of a probability that these differences are due to chance. While the control group had the most consistently fast growth, it also had no growth at all, with one of the trials not producing any mold. While it may be predicted that the 10% sample grew no mold due to the high sugar concentration, the absence of mold from the control group is an area of interest. It is highly unlikely that mold present on the bread didn't have the proper nutrients and environment for growth as the other control trials did grow mold, so it may be the fact that it had no mold that made it onto the sample during inoculation. While general trends do point to the fact that sugar concentrations as low as 2% inhibit mold growth, as the error bars within the control group span overlap the treatment groups that seemed to have inhibited mold growth as well as promoted mold growth, it is inconclusive to say whether different sugar concentrations had an impact in mold growth. General trends do seem to point to growth inhibition from as low as 2% sugar concentration. Nonetheless, more research must be completed due to a lack of statistical significance.

The reasoning for the sudden mold growth during the latter half of the experiment in the 6% and 10% groups is unknown. However, the mold species in the 6% sugar sample differed from the controls, and the one in the 10% group could not be identified because only hyphae growth was observed. Previous research (Mizzi et al., 2020) shows that different bacteria have different levels of sugar resilience, so it may be the case that different species of mold have different sugar resilience as well and that it is not viable to compare the growth rate of one species of mold to another. Mold is another term for fungi with different growth phases similar to bacteria. Research indicates that different fungi reach different growth phases during different times based on their environments (Meletiadis et al., 2001).

In future studies, to decrease the growth variability caused by the species, specific molds can be specifically inoculated onto the bread instead of testing general molds that may appear. We would also test lower sugar concentrations to find the threshold where sugar concentrations

promote mold growth. Furthermore, a larger sample size would provide increased precision, as random variations such as those encountered during this research would be less influential.

While food preservation using sugars may be an attractive option for those without consistent power, the inconclusive results of this paper propose further study before households risk their health, and promote food waste.

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