The Efficacy of Various Types of Mouthwash on Bacterial Growth on Preservative-Free Bread

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Abstract

The influence of mouthwash composition on bacterial growth is a crucial aspect of oral health, as the mouth serves as a primary gateway to the body's internal systems. This study aims to evaluate the effectiveness of various mouthwashes—Colgate Total, Tom's of Maine: Wicked Fresh, and Listerine—in inhibiting bacterial growth, particularly on a medium resembling the oral environment. Given the differences in their compositions, we hypothesized that natural mouthwashes, such as Tom's of Maine, might be more effective in reducing bacterial growth compared to their standard counterparts. Our methodology involved applying these mouthwashes to preservative-free bread slices, inoculated with bacteria, to simulate the oral environment. Over 12 days, bacterial growth was quantified using a grid system. The analysis, conducted via ANOVA, revealed a noticeable variation in the antimicrobial efficacy among the different mouthwashes. Notably, Colgate, which is alcohol-free, showed a higher average of mold coverage, while Tom's of Maine, known for its natural composition, demonstrated the least mold growth. The findings suggest that natural mouthwashes like Tom's of Maine might be more effective in controlling bacterial growth, contrasting with the performance of standard mouthwashes. These results have significant implications for both clinical and personal oral care, underscoring the importance of mouthwash composition in oral hygiene. Overall, our study contributes to a deeper understanding of how mouthwash ingredients can impact bacterial control in the oral environment.

Introduction

The use of various mouthwashes, especially those containing unique ingredients like biosurfactants mixed with chitosan, essential oils, and herbs, has been linked to a reduction in oral bacteria, plaque, and common oral diseases such as cavities and gingivitis (Farias et al., 2019). These findings highlight the importance of the specific composition of mouthwash formulas and how they interact with oral bacteria to improve overall oral health. Our research aims to investigate the effectiveness of different mouthwash treatments in controlling bacterial growth, focusing on comparing organic variants, which generally contain less potent chemicals, against standard mouthwashes. This study is crucial for enhancing our understanding of the antimicrobial properties of mouthwashes and their impact on oral health. Mouthwash, a fundamental component of oral hygiene routines, plays a vital role in controlling oral bacteria, the primary cause of dental and oral diseases, by breaking down their cell membranes (Alemany et. al, 2022).

This inquiry is underlined by the need to understand mouthwash treatments for oral hygiene. Our experimental procedure involves inoculating preservative-free bread with bacteria, followed by treating it with three different mouthwashes; Colgate Total, with its active ingredients being 0.075% Cetylpyridinium chloride (CPC) and 0.05 percent sodium fluoride, Tom's of Maine: Wicked Fresh Mouthwash, primarily containing Zinc Chloride, and Listerine, whose main ingredient is Ethyl Alcohol. Additionally, we utilized a grid system to measure the percent coverage and the extent of bacterial growth on the bread. To ensure the accuracy and reliability of our results, we will employ two distinct ANOVA tests: one assessing the percentage coverage of bacteria on the bread and the other evaluating the extent of the covered area. This dual-analysis approach is crucial for providing a comprehensive understanding of the antimicrobial efficacy of the mouthwashes tested. This research initiative sets the stage for a detailed and thorough investigation into the antimicrobial efficacy of different mouthwashes. Through this study, we aim to contribute valuable insights to the field of oral health research. The findings of this research could have significant implications for the selection of mouthwashes in both clinical and personal oral care, highlighting the importance of ingredient choice in the effectiveness of these products.

Methods

To conduct the experiment, under sterile conditions, 15 preservative-free bread slices were positioned on a surface. Subsequently, 15 resealable bags were prepared and labeled with distinct treatments (ethanol, water, Colgate, Toms, and Listerine), each undergoing three replicates. Application of treatments involved six sprays from a spray bottle onto the respective bread slices, ensuring uniformity and consistency by spraying horizontally at a fixed 25 cm distance. Maintaining constant spray settings was imperative for uniform volume distribution.

Following treatment, each bread slice was carefully sealed in its labeled Ziploc bag to prevent contamination. The Ziploc bags were then placed in an environment with consistent temperature and humidity. Bacterial growth was documented between Monday and Friday, excluding weekends. Data recording involved utilizing a grid with its left corner aligned to the bread's left corner, quantifying mold growth within an 8 x 8 square area and counting both individual squares and those entirely covered. Data, including observations and images of the bread, were recorded in an Excel spreadsheet and a Word document, respectively.

Upon completion of data collection, the mean of the data was calculated using an ANOVA test to elucidate variations in mold growth for each mouthwash treatment.

Results

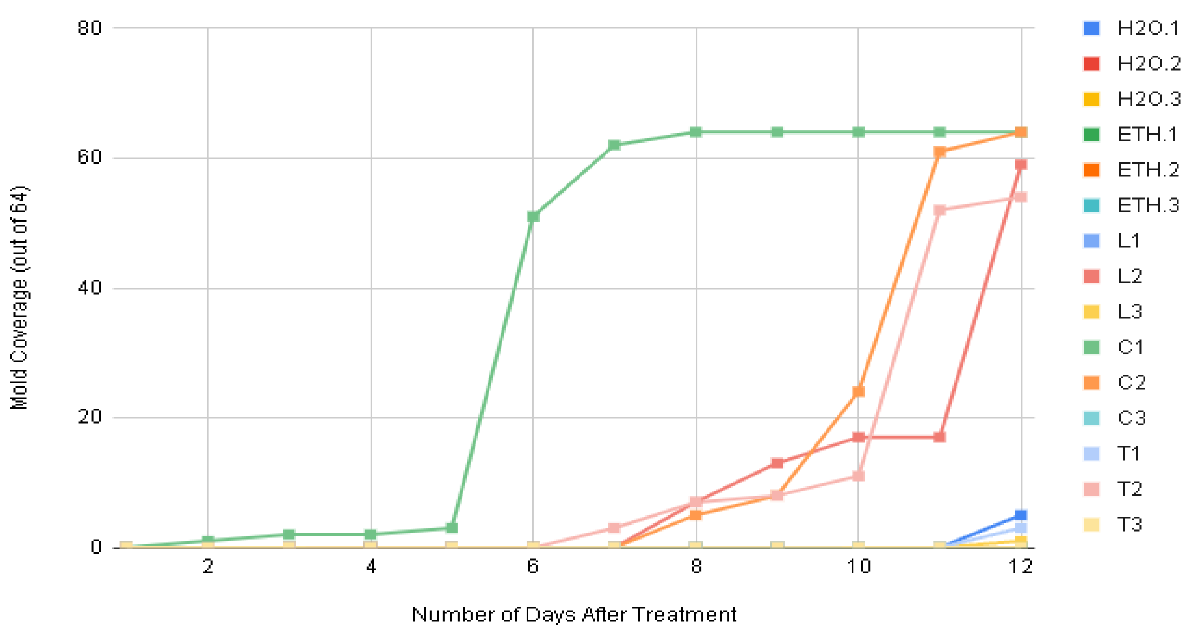

Figure 1. Number of grids with mold growth over time.

Figure 1 illustrates the relationship between the number of grids with mold growth over time. No mold growth was observed on the first day post-treatment. The first mold growth was found on Day 2 after treatment, specifically on a single square of Colgate 1 (C1). As is evident C1's increase (Fig 1). The second occurrence of mold growth took place on the seventh day following treatment, on Toms 2 (T2) bread. In a comparison of T2 and C1 in Figure 1 above, it can be seen that T2 steadily increases whereas C1 has an exponential increase of mold growth. The third instance of mold growth was found on Day 7 on Listerine 2 (L2). L2 showed a continuous increase in mold growth and surpassed the mold growth on T2 by Day 8. On the last day, Colgate 1 and 2 had maximum coverage of 64, followed by Listerine 2 and Toms 2. There is also a few mold growth <10 grids on H20 1, Toms 1, and Listerine 3.

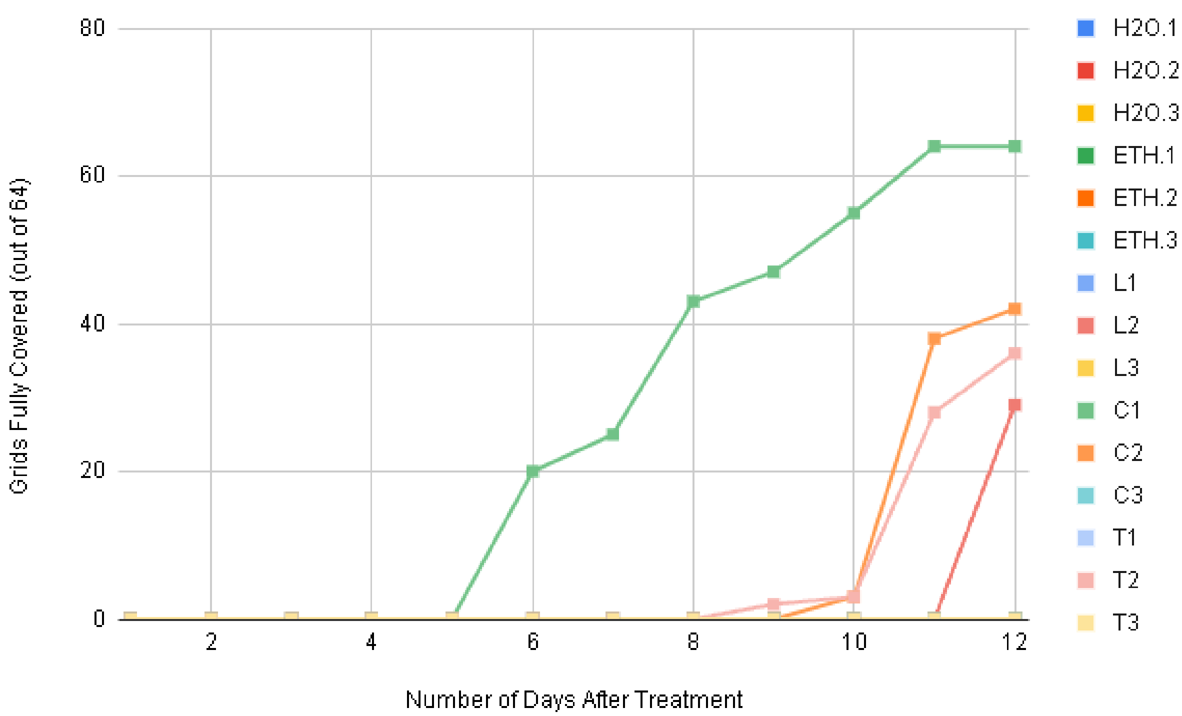

Figure 2. Number of grids fully covered with mold growth over time.

In Figure 2, the progression of grids fully covered in mold across the observed timeframe is presented. Notably, on Day 12 post-treatment, the most extensive mold coverage was observed on Colgate 1 (C1), encompassing all 64 grids. Colgate 2 (C2) exhibited significant coverage, with mold present on 42 grids. Following closely, Toms 2 displayed coverage on 36 grids, and Listerine 2 exhibited full coverage on 29 grids. These findings highlight distinct patterns in the extent of mold proliferation associated with different treatments.

Two ANOVA tests were conducted on grid coverage and grids fully covered. The p-value of each test was less than 0.05 (5.65E-13 and 9.14E-12 respectively). These findings show that both results are significant. The null hypothesis (H_0) proposes that there is no mean difference among mouthwash treatments, whereas the alternative hypothesis (H_a) suggests that at least one treatment group differs significantly from the overall mean of the dependent variable. Therefore, we can reject the null hypothesis.

Discussion

Our data indicates that there is a significant discrepancy between the different types of mouthwashes: Colgate (alcohol-free), Toms (natural), and Listerine. Specifically, Colgate displayed a higher average of mold coverage than the Toms and Listerine mouthwashes. Toms had the least mold coverage of all the mouthwashes. The control treatments of water and ethanol had no mold growth over the 12 days of data collection. Following an ANOVA test, the resulting p-value was determined to be 5.65E-13 for mold growth coverage and 9.14E-12 for mold growth filling squares (Figure 1). This indicates that we can reject the null hypothesis that there is no mean difference between the various mouthwash treatments. Therefore, the alternative hypothesis is favored, meaning at least one group differs significantly from the overall mean. These findings underscore the importance of assessing bacterial growth in various mouthwash formulas. The discrepancies observed among the three mouthwashes, coupled with the statistical evidence, emphasizes the need for oral care producers to consider the efficacy of their mouthwash products. This study contributes valuable insights that can inform both industry practices, providing information surrounding oral hygiene.

The differences between means of the Colgate, Toms, and Listerine brand mouthwashes both conform to and deviate from our initial expectations. First, it was hypothesized that the natural Toms (T1, T2, and T3: Figure 1) mouthwash would have less coverage of mold growth on the bread than the Colgate and Listerine (L1, L2, and L3: Figure 1) mouthwashes. The bread sprayed with Colgate (C1 and C2: Figure 1) exhibited a larger mean value of mold growth than the two other types of mouthwash, which agrees with our assumptions. However, Colgate had more mold growth than Listerine which was unexpected. It was also predicted that all mouthwashes would have less mold coverage than water (H2O1, H2O2, and H2O3: Figure 1). However, our findings show that water had minimal mold growth compared to the mold growth on bread sprayed with mouthwashes. Lee & Choi (2006) conducted a study that investigated the effects of electrolyzed tap water (called Puri-water) on oral bacteria. It was found that Puri-water significantly reduced the amount of periodontopathogens, including oral bacteria, in culture and on toothbrushes (Lee & Choi, 2006). The results imply that water may be more effective compared to commercial mouthwashes, however, further research is necessary to validate these conclusions.

In Jeddy et al.'s (2018) research, they compared the efficacy of herbal mouth rinses with commercially available mouth rinses. Throughout the experiment, they gathered data about the comparative antimicrobial properties of an herbal mouthwash, otherwise classified as organic, against commonly recognized chemical mouthwashes (Jeddy et al., 2018). The findings indicate that the herbal mouthwash proved more effective in reducing oral bacterial counts than its chemical counterparts (Jeddy et al., 2018). This agrees with our findings, as the natural Toms mouthwash acquired the least amount of mold growth compared to the chemical mouthwashes, Colgate and Listerine.

A study conducted by Akande et al. (2004) investigated the effect of three different mouthwash brands, Colgate, Macleans, and Listerine, on the growth of oral bacteria. Specifically, the study examined the efficacy of these mouthwashes by comparing microbial counts in healthy adults' saliva (Akande et al., 2022). In comparison with our study, their use of Macleans was similar to our Colgate mouthwash as the primary ingredient was CPC (Akande et al., 2022). Therefore, it is marketed as an alcohol-free mouthwash commercially. It was found that the least microbial counts were associated with the Macleans brand, whereas our research found that the Colgate mouthwash had the most mold growth (Akande et al., 2022). Despite having identical ingredients, the two mouthwashes yielded contrasting results. This could be attributed to experimental design as our project consisted of mold growth on preservative-free bread and their research considered the growth of oral bacteria in human saliva. The different environments may contribute to variations in bacterial growth. Further, the only treatment we used was the mouthwashes on the bread. However, in the study described above, they made participants rinse with 10 mL of mouth rinse for 15 seconds twice daily, but did not take into account the possibility of these participants adding additional oral hygiene procedures that may have influenced the results.

One source of error could have occurred during the phase of applying treatments to the bread. The spray bottles could have been on different settings for each treatment. This means that the volume of mouthwash, water, or ethanol sprayed onto the bread may have been uneven, leading to incorrect results. For example, it could be that C1 had higher levels of mold growth due to an increased volume of mouthwash being sprayed onto it. To improve future experimental efforts, one must pay close attention to the settings of each spray bottle and must make it consistent amongst all applications.

It is critical to understand and consider the implications of various mouthwashes on oral bacterial growth. There is previous research in this area due to the importance of oral health care. In this project, it was hypothesized that there would be a significant difference in mold growth between the Toms, Listerine, and Colgate mouthwashes. We concluded that there was a statistical mean difference between the three mouthwashes. Future research could specifically investigate the types of bacteria that culminate and how this affects oral health care as a whole.

Conclusion

In this experiment, it was found that there was a correlation between the type of mouthwash and mold growth on bread. The Colgate (alcohol-free) mouthwash showed the greatest amount of mold coverage on bread, with Listerine and then Toms mouthwashes following behind. These results support the hypothesis that natural mouthwashes prevent the growth of bacteria and other microorganisms, thereby improving overall oral care more than its competitors.

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Christantyo Lukita (CL) made contributions to the data analysis and results. Ogechi Anumba (OA) enhanced our abstract and introduction and contributed to manuscript refinement. Kayla Oxland (KO) brought clarity to result interpretation and manuscript refinement. CL, OA, and KO implemented the study and participated in the data collection and interpretation.

Citations

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