The difference in microbial growth on organic bread when exposed to different surfaces

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ABSTRACT

Organic bread, which contains a limited amount of preservatives, provides an optimal growth medium for microbes. The purpose of this study was to determine the amount of microbial growth on organic bread exposed to different surfaces. To conduct this experiment, organic bread samples were dropped onto 3 different surfaces: cafeteria table, cafeteria floor and bathroom floor. The bread samples were immediately sealed in ziploc bags and stored in a warm, dark room to allow for microbial growth. After a 20-day period, microbial growth on each bread sample was measured and extrapolated to percent cover of microbial growth. We hypothesized that the different surfaces (cafeteria table, floor, and bathroom floor) contain varying amounts of microbes. We predicted that the bathroom floor would house the most microbes compared to the other treatment groups and the control. Therefore, the bread exposed to this surface would have the greatest percent cover of microbial growth. However, we found there was no statistically significant difference in percent cover of microbial growth between the different treatment groups (p-value > 0.2001). Overall, our study does not support our hypothesis that bread exposed to surfaces with varying levels of microbes will result in differences in percent cover of microbial growth between the treatment groups. Some sources of error that may have contributed to our results include increased indoor sanitization measures due to the COVID-19 pandemic, lack of moisture in food samples and on surfaces, and counting microbial growth with the naked eye.

INTRODUCTION

It is common practice to discard food that has been dropped onto unsanitary surfaces, as the food is no longer considered suitable for human consumption (Dawson et al., 2007;2006). In a study conducted by the Center for Disease Control and Prevention (CDC), due to the consumption of biologically-contaminated food, approximately 9 million cases of foodborne illnesses are reported each year in the U.S.A. (Scallan et al., 2011). Thus, cross-contamination, a process where bacteria and other pathogenic microorganisms are transferred from one object to another (Mylius et al., 2007), is an important factor that largely contributes to foodborne illnesses (Dawson et al., 2007;2006).

The bacterial composition of commonly-used indoor environments, such as public restrooms (Flores et al., 2011), has also been thoroughly investigated, as they are considered to be reservoirs for bacteria and pathogenic microbes (Miranda, 2016). In addition to validating the presence of pathogenic microorganisms in public restrooms, based on the number of microbes, as well as the high diversity of bacterial species found on high-touch surfaces, Flores et al. (2011) identified the most significant source of microbes as being human skin. With most of the bacterial species being on high-touch surfaces, the microbes may transfer to the skin and then further onto food if one decides to eat before washing their hands.

Food is exposed to a plethora of bacteria and other pathogenic microorganisms when it makes contact with surfaces that contain various types of microbes (Miranda, 2016). Contact surfaces allow bacteria to survive for a prolonged period of time (Miranda, 2016), and are thus widely thought to act as microbe reservoirs (Miranda, 2016). Surface types, such as wood, stainless steel, ceramic tile, and carpet, have thus been thoroughly examined in studies investigating the cross-contamination of food. Wooden surfaces, for instance, have been shown to allow for increased retention of microbial contaminants due to their high absorbency and high porosity (Miranda, 2016). On the other hand, ceramic tiles, which are coated with an epoxy resin (Acikbas & Yaman, 2018;2019), have been associated with a reduced amount of microbial contaminants, as they possess self-cleaning and anti-grease properties, as well as bactericidal, a substance capable of killing bacteria (Miranda, 2016). Similarly to ceramic tile, indoor cement flooring is also coated with an epoxy resin (Krzywiński & Sadowski, 2019), which allows one to assume that indoor cement flooring likely has a similar level of microbial contaminants as ceramic tile.

Due to its high water activity and moisture content (40%) (Czuchajowska et al., 1998), bread is often used to investigate the factors involved in the cross-contamination of food (Miranda, 2016). Although bread is commonly baked between the temperature of 190 and 260°C (Miranda, 2016), the center of the bread does not exceed temperatures of 97°C (Miranda, 2016). This makes bread an optimal environment for spore-forming bacteria, which can both survive and reproduce during the baking process (Miranda, 2016). This makes bread highly susceptible to microbial growth when exposed to suboptimal temperature and humidity conditions (Todd et al., 2007), or when exposed to an environment containing a large number of microbes (Todd et al., 2007).

Due to the varying amount of microbial contaminants between wood and cement coated in an epoxy resin, we hypothesize that if bread is exposed to these different surface types (cafeteria table, cafeteria floor, and bathroom floor), then a difference in the percent cover of microbial growth will be observed in each of the treatment groups. Out of the four treatment groups, we predicted that the bathroom floor would contain the most microbes (Flores et al., 2011), so the bread exposed to this surface would contain the greatest percent cover of microbial growth.

METHODS

For our experiment, we tested and analyzed microbial growth on organic bread that was dropped on 3 different surfaces and compared them to the control (organic bread that was not dropped on any surface). We used organic bread as it has a built-in medium therefore it was safe to use and additionally, it does not have any preservatives in it that may inhibit microbial growth. On November 3, 2021, at 6:00 pm, we tested 3 treatment groups (shown in Figures 2-4): the wood cafeteria table in the LIFE building at the University of British Columbia, the cement floor

in the cafeteria of the LIFE building, and the cement floor in the bathroom of the LIFE building. The approximate area where we conducted the experiment in the cafeteria is shown in Figure 5. We chose these surfaces because they are high-traffic areas in which many individuals transmit bacteria from all different environments (e.g. from the bus, their homes, lecture halls, etc.). Therefore, we believed these areas would house a significant number of bacteria that could be transferred to the bread samples. To begin our experiment, while wearing sterile gloves we used sterile white plastic knives to cut one slice of bread into 4 squares of approximately equal size for each treatment group, as well as for the control. Materials used are shown in Figure 1. Each slice of bread was cut in a cross to make 4 squares. Each slice of bread was cut on a clean Ziploc bag to minimize the potential transfer of bacteria. Therefore, in total 16 pieces of bread were used, 4 replicates for each treatment group, and 4 for the control. Using sterile gloves, the 4 replicates for the control were placed directly into Ziploc bags without being dropped onto any surface, then labeled as "control" with the appropriate replicate number (1-4). As for the treatment groups, sterile gloves were used to drop a square of bread onto a treatment surface for 30 seconds timed with an iPhone timer. Then, the bread was immediately picked up with sterile tongs and sealed in a Ziploc bag in order to not further contaminate it. This step was repeated for each of the 4 replicates of the treatment in question. Each Ziploc bag was labeled with the corresponding treatment group (e.g. bathroom floor) and the replicate number (#1). After all the replicates were sealed into the Ziploc bags, they were placed in a dark warm room at ~24.5 degrees celsius for 20 days to allow for adequate microbial growth. Throughout these 3 weeks, the microbial growth was recorded using pictures every 2-3 days. On November 23, at 1:00 pm we measured the amount of microbial growth on all 16 replicates using a 7x7 cm grid. This grid was placed over the sealed Ziploc bags containing the bread samples and microbial growth was

then measured by counting the number of 1x1 cm squares that growth appeared in. Finally, this data was extrapolated to the percent cover of microbial growth. To analyze our data and determine the significance of our results, a one-way ANOVA test was conducted. The ANOVA test will allow us to determine whether there is a statistically significant difference between the mean microbial growth of the three treatment groups and the control.

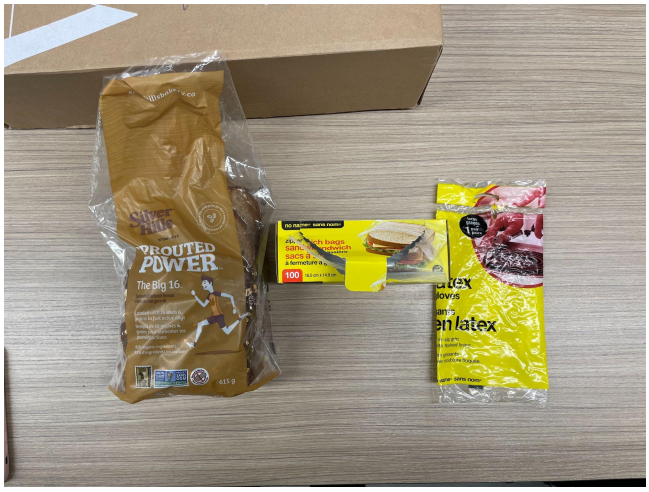

Figure 1. The organic bread, ziploc bags, and latex gloves used for the experiment.

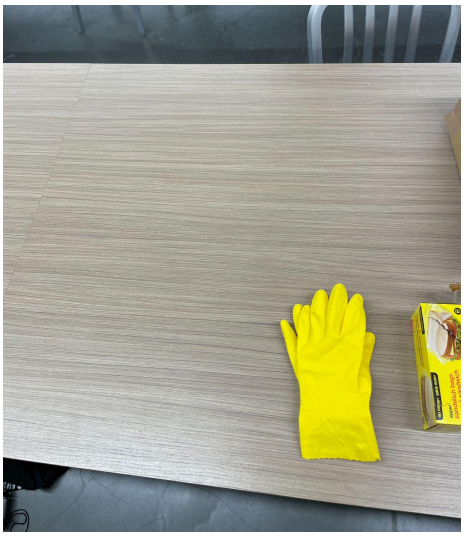

Figure 2. Wooden cafeteria table in the UBC LIFE building.

Figure 3. Cement floor in UBC LIFE building cafeteria.

Figure 4. Cement floor in UBC LIFE building bathroom.

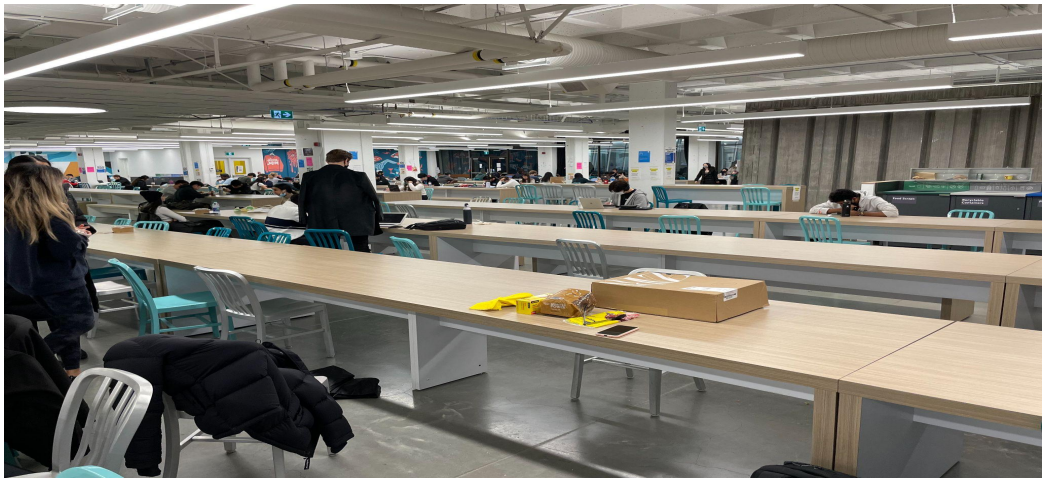

Figure 5. Area of UBC LIFE building cafeteria where experiment was done.

Figure 6. Schematic of bread samples kept in a dark room for growth. Top to bottom: control, cafeteria floor, cafeteria table, bathroom floor.

Figure 7. Grid used for measuring growth.

RESULTS

The graph shown below depicts the mean percent cover of microbial growth on bread that was exposed to the four different surfaces: Bathroom floor, Cafeteria Floor, Cafeteria Table, and Control (no treatment) in the LIFE building at the University of British Columbia. The mean

percent cover of microbial growth with their 95% confidence intervals are as follows: Bathroom floor is 6 +/-2 %, Cafeteria table is 7 +/- 4 %, Cafeteria Floor is 6 +/-2 %, and Control is 13 +/- 12 (Figure 8). As shown in Figure 8, the control appeared to have the highest mean for percent cover of microbial growth, followed by the cafeteria table, then the bathroom floor, and finally, the cafeteria floor had the lowest mean for percent cover of microbial growth. As seen in Figure 9, the control group bread samples displayed the most microbial growth, with replicate 4 being an outlier, which contributes to the large range of the 95% confidence interval for the control group. The p-value was obtained through a one-way ANOVA test. Based on the p-value of 0.2001, which is greater than 0.05, we can conclude that there is no statistical difference between the mean percent cover of microbial growth between the four treatment groups.

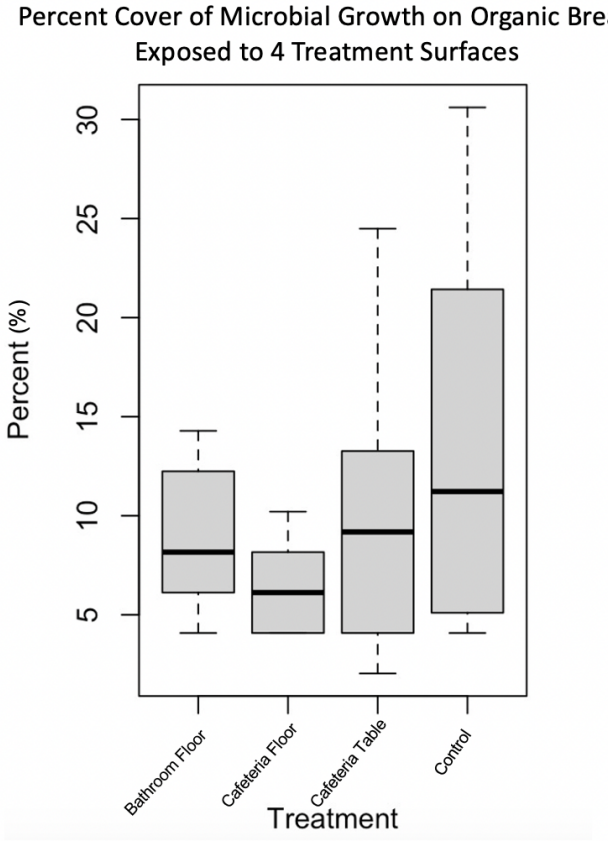

Figure 8. The mean percent cover of microbial growth on bread that was exposed to four different surfaces: bathroom floor, cafeteria floor, cafeteria table, and control (no treatment) in

the LIFE building at the University of British Columbia. Error bars represent 95% confidence intervals. p-value > 0.2001 (p-value > 0.05), indicates a non-significant difference between mean percent cover between the four treatment groups.

Figure 9. Control bread samples on final day (day 20) of growth. From left to right: Replicates 1-4.

Figure 10. Cafeteria floor bread samples on final day (day 20) of growth. From left to right: Replicates 1-4.

Figure 11. Cafeteria Table bread samples on final day (day 20) of growth. From left to right: Replicates 1-4.

Figure 12. Bathroom floor bread samples on final day (day 20) of growth. From left to right: Replicates 1-4.

Sample calculation for determining percent cover:

[(# of squares counted) / (49 cm^2)] * 100 = percent cover (%)

Ex. (2/49) * 100 = 4.08% cover for Control replicate #1

DISCUSSION

Our findings do not support our hypothesis that there is a difference in percent cover of microbial growth between the treatment groups. We had predicted that the treatment group with

bread being dropped on the bathroom floor would have the highest percent cover of microbial growth, as this is the surface that would house the most microbes. However, given that the difference in percent cover of microbial growth between our four treatment groups was not statistically significant, we fail to reject our null hypothesis that there is no difference in percent cover of microbial growth between the treatment groups.

Previous studies have shown contradicting results when comparing contamination of foods exposed to different surfaces. A study done by Miranda and Schaffner (2016) found differences in log percent transfer of bacteria to bread when exposed to surfaces such as tile, stainless steel, wood and carpet to bread, with the greatest transfer of bacteria to bread occurring with stainless steel. Similarly, in a study done by Dawson et al. (2006), differences in transfer of bacterial cells to bread were found with exposure to surfaces such as wood, tile and carpet.

A confluence of sources of error may have contributed to the conflicting findings in our study. For example, the surfaces used in our study were found in a common space accessed by hundreds of students, staff and visitors everyday at the University of British Columbia. These high touch surfaces are assumed to contain large numbers of microbes as human skin has been identified as the most significant source of microbes (Flores et al., 2011). However, these frequently contacted surfaces are also cleaned routinely everyday, hence bactericidal effects of disinfectants may have resulted in the insignificant difference in growth on bread between the different surfaces. The global COVID-19 pandemic has resulted in more vigorous and frequent cleaning and sanitation practices in public spaces, further increasing the chance of bactericidal disinfectants being a cause of limited differences of microbe growth between treatment groups in our study. This could also attribute to why the control group had more growth, as it was not exposed to any bactericidal contaminated surface. In contrast, previous studies have used more

rigorous methods of preparing surfaces. For example, Miranda et al.(2016) cultured a specific bacterial strain, *Enterobacter aerogenes,* and inoculated disinfected samples of the surfaces of interest. After exposing food samples to these surfaces, plates with the samples were incubated for a 24 hour time period and colonies were then counted. The differing surface preparation methods used in our study and previous studies, may have contributed to our findings of an insignificant difference in percent cover of microbial growth between the different surfaces.

Another reason that we may have seen no statistically significant difference among our treatment groups could be due to lack of moisture within our bread samples. Studies have shown that moisture levels of food samples are an important factor in transfer of microbes from surfaces to the food, where increased levels of moisture promote microbial transfer (Miranda et al., 2016; Moore et al., 2003). This is usually due to the presence of biofilms of bacteria on surfaces including those of our treatment groups and the bread. Upon drying of the surface, the numbers of bacteria in the biofilms are reduced, which may be the cause of low transfer from the surfaces to the food (Jensen et al., 2013; Moore et al., 2003).

In addition, while counting, we used our naked eye to determine where there was microbial growth on the bread samples, and extrapolated the amount of growth to percent cover. However, the bread was sprouted and contained many seeds, so it did not have a clear surface to start with. A source of error while counting may have came from not knowing which part of the bread is the seed, and which is growth.

It is unclear whether the lack of difference in microbial growth on bread between the different treatment groups is a result of no major difference in the amount of microbes existing on these surfaces. Further research could be conducted to ascertain whether or not the amount of microbes present on the cafeteria floor, cafeteria table and bathroom floor differ.

CONCLUSION

The microbial growth on organic bread after being exposed to different surfaces was examined. Regardless of the distinctive properties and varying amounts of microbial contaminants on the 3 different treatment surfaces, there was no statistically significant difference in microbial growth. These results are unable to determine if there is a correlation between the 3 surfaces tested and foodborne illnesses in humans.

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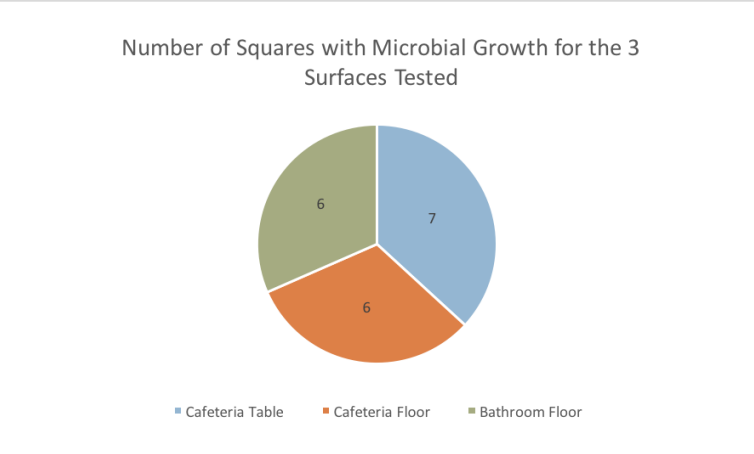
APPENDIX

	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Standard Deviation	Confidence Intervals	Mean
Control	4.08163265	12.244898	6.12244898	30.6122449	12.07363221	11.83194215	13.2653061
Cafeteria Table	4.08163265	2.04081633	8.16326531	12.244898	4.525215883	4.434630076	6.63265306
Cafeteria Floor	4.08163265	8.16326531	4.08163265	6.12244898	1.953932873	1.91481903	5.6122449
Bathroom Floor	4.08163265	4.08163265	8.16326531	8.16326531	2.356531711	2.309358641	6.12244898

Appendix A. Table showing percent cover of the 4 different treatment groups for each replicate that was done. Standard deviation, confidence intervals, and means using all four replicates for each treatment group were calculated.



Appendix B: Graph exemplifying the mean number of squares containing microbial growth out of the 49cm^2 grid used to measure the growth for the three surfaces tested. This shows that there is no significant difference in microbial growth across the 3 treatment groups tested.