Abstract

Background: Over the years, many reusable products have been invented to replace single-use disposable items to reduce waste. One of such products is the reusable beeswax food wrap, which aims to replace plastic film wraps to store food. According to manufacturer instructions, the beeswax wrap can only be washed with cold water and detergent. This presents the question whether the beeswax wrap can be effectively cleaned, as continuous reuse may present cross contamination issues. This study examines if manufacturer instructions is effective in cleaning the beeswax wrap.

Methods: ATP analysis was used to determine the level of cleanliness on the beeswax wrap between the pre-intervention and post-intervention treatments. Pre-intervention samples are the new beeswax wraps. Post-intervention samples are wraps that have been contaminated with avocado, washed, and dried. ATP counts (RLU) were measured with Hygiena SystemSURE Plus ATP monitoring system. Paired T-Test was done on NCSS to analyze the results.

Results: The mean of the pre-intervention group was measured at 8 RLU, which is considered clean under the Hygiena standard. The mean for the post-intervention group was measured at 67 RLU, which is considered a fail on cleanliness under the Hygiena standard. This shows that the manufacturer instructions on washing the beeswax wrap does not effectively clean the beeswax wrap. Statistical analysis show p-value is 0.000, therefore one can conclude there is a statistically significance difference in the mean ATP count between pre-intervention and post-intervention beeswax wrap samples.

Conclusion: Results show that some food residue remained on the wrap after washing. This means manufacturer instructions cannot effectively clean beeswax wrap. Therefore, it is recommended that manufactures should put a label on their packaging to let their customers know that the wrap can’t be thoroughly cleaned, and certain foods should be avoided for its use. During its use, the wraps should be labeled for the specific category of food it is used for. BCCDC can also use this result to add into the reusable container guideline.

Keywords: beeswax wrap, cross contamination, dishwashing, food safety, food wrap

Introduction

In recent years, sustainability has been an increasingly important topic. The City of Vancouver has developed a Greenest City Action Plan, with zero waste by 2040 as one of its top 3 focus (City of Vancouver, 2018). Hence, over the years, many reusable products have been invented to replace single-use disposable items to reduce waste. One of such products is the reusable beeswax food wrap, which aims to replace plastic film wraps to store food. It can be used from
covering a bowl of leftovers to wrapping a block of cheese or half an avocado for later use. Since this reusable food wrap is made of beeswax, it can only be washed in cold water with an alcohol-free soap to preserve the integrity of the wrap (Abeego Canada, 2018). Out of the author’s personal interest, this research will test the effectiveness of manual washing the reusable beeswax food wrap in domestic use. Although there are no guidelines for domestic dishwashing, the Food Retail and Food Services Code (Food Code) recommends manual dishwashing water temperature to be at least 45°C (113°F) for food service premises to ensure effective dishwashing (Food Code, 2016). Therefore, this research is of public health interest because the beeswax food wrap may not be effectively cleaned using cold water, which can result in cross contamination and accumulation of pathogens between uses. Results of this study can help educate consumers on proper usage.

**Literature Review**

The beeswax food wrap was invented in 2008, by the company Abeego founded in Victoria, Canada (Abeego Canada, 2018). Abeego beeswax wrap can easily be purchased online and at local retailers, so their product will be used for this experiment. The Abeego beeswax wrap is a hemp and organic cotton fabric infused with natural ingredients such as beeswax, tree resin and jojoba oil (Abeego Canada, 2018). Abeego manufacturer claims the product has a porous property that allows food to breath, keeping the quality fresh for a longer period compared to plastic film wraps. The company stated that at room temperature, it is soft, adhesive, and can be molded onto any food and container to store food. A disadvantage they mentioned was since it is made of beeswax and tree resin, it is soluble in alcohol and cannot be used with heat, therefore, can only be hand washed in cold water with an alcohol-free soap. Following Abeego, other beeswax wrap companies were created, like Beutiful in Australia and Bee’s Wrap in the United States. All wraps are made with similar ingredients, have the same properties and same wash instructions (Beutiful, 2018; Bee’s Wrap, 2017). Therefore, Abeego products is a good representative product for this research. This raises the research question on whether the beeswax food wrap can be effectively cleaned using the manufacturer prescribed method of only cold water with detergent. If it cannot be effectively cleaned, this is a potential concern for bacteria buildup and cross contamination.

**Efficacy of manual dishwashing**
First, the efficacy of manual dishwashing will be examined. A study was done by Lee et al. (2007) on the efficacy of manual dishwashing and microbial survival on different food contact surfaces. Their results show that at a low temperature (24°C) with a low sanitation concentration (150ppm for 5 seconds), a 5-log, or 99.999%, *E. coli* reduction was achieved for most samples. This meets Health Canada’s criteria that food contact surface sanitizers must reduce microbial contamination by 5-log (NCCEH, 2011). The only sample to yield a non-significant 5-log *E. coli* reduction was milk products on glass, which suggests that the type of food may have an impact on efficacy.

Another study done by Gkana et al. (2016) on manually washing cutting boards with tap water (15°C) and detergent also obtained a 5-log reduction in bacteria. Even though they used detergent instead of sanitizer, a 5-log reduction was still achieved, showing that detergents may be just as effective at reducing bacteria load. Detergents are mainly for cleaning, as they contain cleaning agents and surfactants that remove grease and soil (Soap and Detergent Association of Canada (SDAC), 2009). Sanitizers are different than detergents, as they contain disinfectant ingredients to reduce levels of microorganisms such as bacteria and viruses to acceptable levels (NCCEH, 2011).

Previous ENVH 8400 student research project show that there is a significant difference between washing cutting board with detergent only and washing with detergent followed by sanitizer (Man, 2018). Sanitizers will not be used in this research since in domestic dishwashing, there is usually no sanitizing step, only the wash and rinse step. This will allow the experimental results to relate more to what is seen in typical home dishwashing.

**Effect of surface material on dishwashing**

The type of surface also effects dishwashing, as Gkana et al. (2016) found that the 5-log reduction on the cutting boards mentioned earlier is only true for stainless steel and polyethylene cutting boards, but not wooden ones, which only achieved a 2-log reduction in bacteria. Similar findings were also seen in a study done by Soares et al. (2012), where they recovered small amounts of microorganisms from wooden cutting boards after washing them with cold water and detergent. No microorganisms were detected on the plastic and stainless-steel cutting boards (Soares et al., 2012). This may be because wood has tiny pores and crevices that allow bacteria to hide in, making it harder to wash. Therefore, the effectiveness of washing the beeswax wrap might be reduced.
since the wrap is porous, bacteria may hide inside the pores.

**Effect of temperature on dishwashing**

All the studies mentioned above used low temperature to wash the food contact surfaces and still achieved significant bacterial reduction, meaning that washing the beeswax wrap in cold water with detergent may be effective. However, the efficacy of manual dishwashing still increases with higher water temperature, and an increase in water temperature can bring a large increase in bacteria reduction (Lee et al., 2007; Mattick et al., 2003). At higher water temperature, grease and films are more easily removed with detergent (SDAC, 2009). Therefore, washing the beeswax wrap in cold water may still not be as effective as washing it in warm water of at least 45°C, the temperature recommended by Food Code.

**Antimicrobial activity of beeswax wrap**

Beeswax food wrap is interesting because not only does it help reduce plastic waste, it may also have antimicrobial properties. Crude beeswax by itself is known to have antimicrobial activity against several types of yeast and bacteria, including *E. coli* and *Staphylococcus aureus* (Fratini et al., 2016). Since the beeswax food wrap contains beeswax, it would be interesting to know if there are antimicrobial effects for these wraps. Recent research done by Pinto et al., 2017, investigated the antimicrobial activity of Abeego, to see if the tree resin, jojoba oil and the fabric influence the beeswax’s ability to inhibit bacteria growth. They found that Abeego wraps were able to significantly decrease viable cells of *Salmonella enteritidis* and *S. aureus*. There were some decrease in viruses and yeasts, but results were insignificant (Pinto et al., 2017). This implies that Abeego wraps can inhibit foodborne pathogens, preventing its spread. However, not a wide variety of bacterial pathogens were tested and there are viruses that can cause foodborne illnesses, therefore one cannot rely solely on the antimicrobial property of beeswax wrap to prevent spread of pathogens. Also, this was the only study of its kind, so there are no other literatures that can support or refute their study.

**Research question**

The purpose of the study is to determine if hand washing beeswax food wrap using the manufacturer method of cold water and detergent can effectively clean the food wrap. Proper cleaning can reduce the risk of foodborne illnesses. Results from this study can be translated to a best practices procedure for using beeswax food wrap and perhaps for other similar reusable wraps as well to prevent cross contamination.
Materials and Methods

To test the effectiveness of cleaning, ATP analysis was used. Hygiena SystemSURE Plus ATP monitoring system and UltraSnap Surface ATP Test swabs were used to measure the ATP on the beeswax wrap (Hygiena LLC, 2012). Avocados were used as the contaminant. Measuring spoons and a knife were used to measure and spread the avocados onto the wrap. One box of medium Abeego beeswax wraps, 10” by 10” in size was purchased (Abeego, 2018). Other materials needed to wash the wraps are a one compartment sink, 15 sponges, and one bottle of Seventh Generation dish soap. A bimetal thermometer was used to measure the water temperature.

The experiment was done at BCIT, in room SW1 1260. First, all 15 Abeego beeswax wraps, each 2” by 5” in size, were swabbed to determine the initial ATP level present in clean samples. A 10cm by 5cm template was used to keep the area swabbed constant. This represents the pre-intervention ATP level. Standard procedure was used to measure ATP. To contaminate the beeswax wrap, an avocado was cut in half, and one teaspoon of avocado was scooped and spread evenly with a knife onto the wrap. Then, the tap was turned on to the cold side. The temperature of the water was measured at 13°C with a pre-calibrated bimetal thermometer. To wash the wraps, first, it was rinsed for 15 seconds under running water. Then, 2 drops of biodegradable detergent from Seventh Generation was squeezed onto a new sponge. The wrap was scrubbed for 15 seconds. After scrubbing, the wrap was rinsed under running water for another 20 seconds to wash off the detergent. Finally, it was placed onto a clean paper towel to dry. The wraps were placed in a temperature-controlled room at 23°C for two hours to dry. Once dried, a final ATP reading was taken as the post-intervention level. These steps were repeated for all the samples.

In between each sample reading, the ATP analyzer was tested against a blank sample. Although this was not necessary, it was done to check that the ATP analyzer gives consistent 0 reading for the blank samples to ensure that the equipment gives consistent measurements. A control was also done and found that the wrap can be brought down to 0 RLU when washed without contamination introduced. This helped verify that the pre-intervention ATP measured can be removed with washing and that the beeswax itself was not contributing to the ATP measured.

Hypothesis

Research question for this experiment is to see if manufacturer instructions is effective in
cleaning the beeswax wrap. This was done by seeing if the contaminated wraps after wash could bring the ATP level back down to the pre-wash (blank) level.

Null hypothesis (H0): There is no difference in the mean ATP count on the beeswax wrap between pre-intervention and post-intervention levels.

Alternative hypothesis (Ha): There is a difference in the mean ATP count on the beeswax wrap between pre-intervention and post-intervention levels.

**Statistical analysis**

This experiment compared the difference in ATP levels between the pre-intervention and post-intervention samples. Pre-intervention samples are the new beeswax wraps. Post-intervention samples are wraps that have been contaminated with avocado and went through the washing procedure. Since experiment is comparing the ATP levels of before and after treatment, Paired T-Test was used to see if there is a difference between the means (NCSS, 2018-a). The ATP counts (RLU) collected are whole numbers, numerical, a ratio and discrete. NCSS was used to perform the Paired T-Test statistical analysis.

**Results**

Results collected is shown in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Pre-intervention (RLU)</th>
<th>Post-intervention (RLU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>53</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>103</td>
</tr>
<tr>
<td>4</td>
<td>7</td>
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<tr>
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<tr>
<td>14</td>
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<td>3</td>
<td>457</td>
</tr>
<tr>
<td>16</td>
<td>11</td>
<td>168</td>
</tr>
</tbody>
</table>

*Outlier in the experiment. This was not used as part of the statistical analysis.*

Statistical analysis on NCSS show the data is normally distributed. P-value is 0.000, therefore the null hypothesis is rejected, and one can conclude there is a statistically significance difference in the mean ATP count between pre-intervention and post-intervention beeswax wrap samples.

A box plot is shown below as a visual on the ATP (RLU) measured in the samples.
Figure 1. Box plot on ATP levels (RLU) between the pre-intervention and post-intervention samples.

The mean of the pre-intervention and post-intervention groups are 8 RLU and 67 RLU, respectively. The standard deviation of the pre-intervention and post-intervention groups are 5 and 49, respectively.

Hygiena has its own standards on pass and fail RLU limits. RLU measurements under 10 are considered a pass, which also means it is clean (Hygiena, 2018-b). RLU between 11-30 are considered as a caution, and RLU above 30 are considered as a fail on cleanliness (Hygiena, 2018-b). Since the mean of the pre-intervention group is at 8 RLU, it is considered clean under the Hygiena standard. The mean for the post-intervention group is at 67 RLU, so it is considered a fail on cleanliness under the Hygiena standard. This shows that the manufacturer instructions on washing the beeswax wrap with cold water does not effectively clean the wrap.

Discussion

Results from the statistical analysis show that P-value is 0.000. Therefore, the null hypothesis is rejected, and one can conclude there is a statistically significance difference in the mean ATP count between pre-intervention and post-intervention beeswax wrap samples. This means that manufacture instructions are not sufficient in cleaning the beeswax food wrap.

As mentioned earlier, the mean RLU of the pre-intervention group is under 10 RLU, which is considered as clean (Hygiena, 2018-b). This means that the unused beeswax wraps are clean to start with and does not have much affect on the high ATP count of the post-intervention samples. The mean RLU of the post-intervention sample is higher than 30, which is a fail on cleanliness using Hygiena’s RLU limits scale (Hygiena, 2018-b). This means that when the wraps are washed with cold water after use, some food residue remained on the wrap and was not all washed off.

This finding matches the expected outcome from the literature review. Multiple studies show that the efficacy of manual dishwashing increases with higher water temperature, and
as water temperature increase, so does the bacteria reduction (Lee et al., 2007; Mattick et al., 2003). The Food Code recommends manual dishwashing water temperature to be at least 45°C (113°F) for food service premises to ensure effective dishwashing (Food Code, 2016). Since the beeswax food wraps were washed in cold temperature measured at 13°C, it is expected that not all the food residue will be washed off. In addition, higher water temperature removes grease, oils and fatty substances better. Avocados contain a high amount of fat, therefore it is expected that cold water would not be effective at removing it from the wrap compared to warmer water temperatures (California Avocado, 2019). Previous research also shows that the type of surface affects dishwashing, as a 5-log reduction in bacteria on cutting boards can only be achieved for stainless steel and polyethylene cutting boards, not wooden ones (Gkana et al., 2016). Study also show that for wooden cutting boards washed with cold water and detergent, small amounts of microorganisms were recovered (Soares et al., 2012). This is because wood has tiny pores and crevices that allow bacteria to hide in, making it harder to wash. Therefore, since the beeswax food wrap is porous like wood, it is expected that it can’t be cleaned as good as other types of surfaces like stainless steel and plastic. Therefore, results from this study agree with previous research findings.

**Knowledge Translation**

Since results show ATP levels are higher in beeswax wraps that have been contaminated and washed compared to new wraps, this means that there should be prevention measures for potential cross contamination. It is important to note that since ATP analysis capture both live and dead cells, results from this experiment cannot conclude if there is an actual health concern, but that there is a potential health concern. As beeswax wrap is mainly for domestic use, it is non regulated. However, manufactures should put a label on their packaging to let their customers know that the wrap can’t be thoroughly cleaned, and certain foods should be avoided for its use. This may be unrealistic as no company would put that because it will affect their sales. Caution tips can be posted on the packaging to list some examples of food to avoid wrapping with these wraps such as raw meat, deli meat and fatty foods.

It is unknown if restaurants out there use beeswax wrap, as it is mainly for domestic use. If beeswax food wrap is used in food premises, it is most likely in small local cafes and restaurants rather than larger restaurants. Therefore, a best practices guideline for using
beeswax wrap can be created for them. They should be labeled for the specific category of food it is used for. For example, wraps used to wrap cheese should not be used to wrap vegetables.

A lot of zero waste cafes and small local food premises out there have good intentions of reducing waste, they can unknowingly be growing pathogens and transferring it onto food with each reuse. BCCDC can also use this result to add into the reusable container guideline.

**Limitations**

Limitations around this experiment are mainly budget, time and lab constraints. Due to the limited funding, the sample size was only 15 samples instead of the recommended 30 samples. The public health interest of this experiment is to see if pathogens can remain on the wrap after use which can be a potential cross contamination issue. However, due to lab and budget constraints, live pathogens cannot be used. Therefore, avocados were used as a substitute to evaluate the effectiveness of cleaning the beeswax wrap. The limited time to complete this experiment makes ATP analysis a more feasible option compared to growing bacteria using 3M™ Petrifilm™ Aerobic Count Plates or contact plates. Since pathogens aren’t used as the contaminant, using the bacteria culture method was also unnecessary as ATP analyzes provides better results on the overall cleanliness. There are some limitations for using the ATP analysis. ATP reading captures both live and dead cells, and dead cells is not the interest from a public health perspective. Therefore, one cannot conclude from these experimental results whether this is actually a health concern or not.

As mentioned earlier, the beeswax food wrap is porous, so bacteria and food residue may hide inside the pores of the wrap (Abeego, 2018). This may mean the RLU count will be lower than the true value, since the ATP swab may not have picked up the ATP hidden in the pores. Several things can be done to improve this experiment. Greater sample size will help increase validity. More control samples can be done to verify that it was not just an outlier. Also, using the bacteria culture method would be a better choice than ATP as it would capture live cells which is the main interest.

**Future Research**

Further research is needed to fully examine the potential impact on transferring of pathogen and facilitating its growth on the beeswax food wrap. Research can be done to determine the highest water temperature the beeswax food wrap can withstand before the integrity of the wrap is compromised.
Washing it at different temperatures will give insight on whether it is the wrap material that is preventing the beeswax wrap from getting effectively cleaned or is solely due to the water temperature. Research can be done with a sanitization step after wash to see if the ATP level can be brought down to the original level. Third, an experiment can be done to compare the effectiveness of washing beeswax wrap to washing a different type of reusable wrap such as silicon wraps. This can allow consumers to have a better alternative if they want to wrap high risk foods. Finally, the public health concern is the spread of pathogens, therefore using live bacteria as a contaminant will be the best way to test the potential cross contamination issue from reusing the wraps.

**Conclusion**

The purpose of the study is to determine if washing beeswax food wrap using the manufacturer instructions of cold water and detergent can effectively clean the food wrap. Avocados were spread onto the beeswax wrap then washed. The pre-intervention and post-intervention ATP readings were taken. Results show a significant difference in the mean ATP count between pre-intervention and post-intervention beeswax wrap samples. This concludes that manufacture instructions are not sufficient in properly cleaning the beeswax food wrap.

Results from this study can be translated to a best practices procedure for using beeswax food wrap. Since it cannot be properly cleaned, it is recommended to use the beeswax food wrap for non-potentially hazardous foods or low risk foods only. Examples include using it to wrap food like fresh herbs and bread, using it to cover bowls, and covering rising dough. High risk food like raw meat, fish and deli meat are not recommended to be wrapped with beeswax food wraps. If used in food premises, proper labeling is required to prevent potential cross contamination. More research is needed to determine the health risks associated with using beeswax food wrap.

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**Competing interest**

The authors declare that there is no competing interest for this research.
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