Investigating the Effectiveness of Different Cleaners at Removing Hydrophobic Coatings on Fresh Produce

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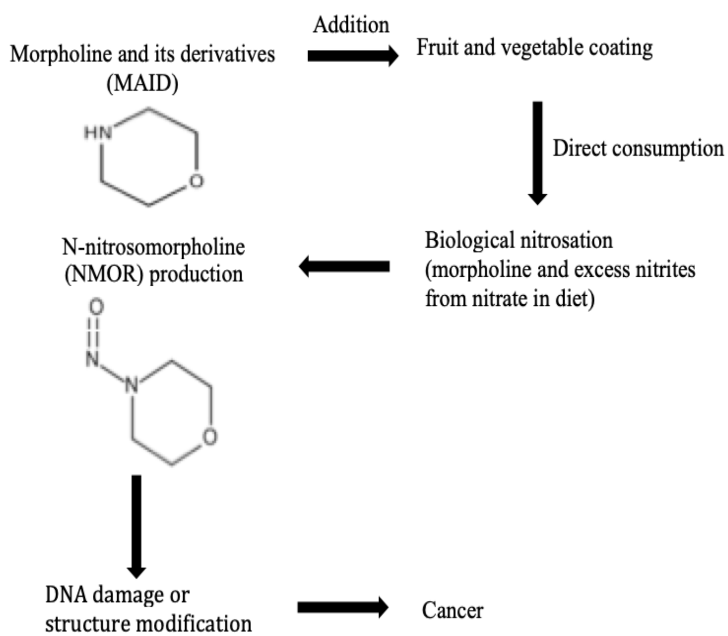
Abstract:

The presence of potentially harmful paraffin wax coatings on fresh produce is a source of growing concern in regards to exposure to allergens or carcinogens (Galus et al. 19; Kumar 33). Due to the health risks associated with edible wax coatings, it is important to thoroughly wash store-bought produce with suitable cleaners. The objective of this study was to investigate the effectiveness of four cleaners (tap water, dish soap, hand soap and white vinegar) at removing hydrophobic coatings (Vaseline) on mini cucumbers when viewed under a blacklight flashlight. The percent Vaseline removal was estimated for the four treatment groups and control, revealing that tap water samples removed the least Vaseline (7.7%), whereas those treated with dish soap, and vinegar removed the most (36.6% and 32.5%, respectively). A one-way ANOVA test was completed on GraphPad Prism 9, resulting in a P-value of < 0.0001. As the P-value is less than the significance level of 0.05, the null hypothesis that the four cleaners are equally effective at removing hydrophobic coatings can be rejected. It was determined that a significant difference did exist between the Vaseline removal efficiency of the cleaners. However, a Tukey Kramer test run on RStudio revealed no significant difference between dish soap and vinegar with a P-value of 0.725. Our research study concluded that dish soap and white vinegar are the best choices to effectively remove hydrophobic wax coatings to ensure the safe consumption of fresh produce.

Introduction:

Various techniques have been implemented in an effort to extend the shelf life of fresh produce. These may include controlled and modified atmosphere packaging, low temperature storage, or high relative humidity that is curated to given fruits and vegetables (Kore et al. 591). Recently, edible coatings have become increasingly utilized and determined advantageous compared to other techniques (Kore et al. 591). Edible coatings come in different forms including oil, lipid-based coatings, resins, and waxes (Kore et al. 592). In addition to shelf-life extension, the coatings also serve to cover fruit injuries, reduce water loss, and add a shiny gloss (Abdelfattah et al. 2).

Edible coatings alone do not control decay and are thus combined with morpholine (a colourless secondary amine ether (An et al. 1) to prevent mold and bacterial growth and to ensure an even application of the wax (Kumar 33). Despite these manufacturing benefits, there is concern regarding chemical reactions of morpholine during digestive processes that may result in a potential genotoxic carcinogen (Fig. 1). Another risk of consuming waxes on produce is that they often contain allergenic ingredients (Galus et al. 19). Unfortunately, because no labelling is required for fresh produce to declare coating, warnings of the potential dangers are not provided (“Undeclared Allergens”).



This research study

Figure 1. The interlink between morpholine and potential carcinogenic risks. After direct consumption of wax coated produce, morpholine and its derivatives pass into the gut and the secondary amine functionality leads to nitrosation and formation of N-nitrosomorpholine (NMOR) (Kumar 35). NMOR is a genotoxic carcinogen and can cause cancer by directly altering genetic material (damage to DNA or modification of its structure) (Lee et al. 1). If DNA replication occurs prior to action of a repair mechanism, the mutations can cause tumors (Lee et al. 1). Specifically, liver and kidney cancer and impaired function of these organs were seen in models (Kumar 35).

focused on investigating the efficacy of different household cleaners (water, hand soap, dish soap, and white vinegar) on removing hydrophobic produce coating that may pose potential health risks. Petroleum jelly (Vaseline), a product of mineral oil and paraffin wax (Bekker et al. 99), was used to simulate edible coating which is often petroleum-based (Kore et al. 592). Petroleum jelly is also UV reactive which is an imperative characteristic as a UV blacklight was the instrument used to visualize the coatings. Cucumbers were chosen for the produce item as they are commonly wax coated and are not peeled prior to consumption. We hypothesized that if Vaseline covered cucumbers were cleaned with tap water, dish soap, hand soap, or white vinegar, then the percent of Vaseline removed will vary based on the respective properties of each cleaning solution. As dishwashing soap is a surfactant that can interact with and disperse the oil-based Vaseline, we predict it will be the most effective cleaner (Wasilewski et al. 1315). Hand soap is predicted to perform less robustly as it is formulated to be milder due to frequent use on the skin. Furthermore, while white vinegar is acidic and a regularly promoted cleaning agent, it does not have surfactant characteristics rendering it ineffective in this context (Flanery 1). Additionally, the vinegar used in this study is mostly water-based (hydrophilic) and will not interact with the coating (hydrophobic due to paraffin composition). Due to similar reasoning, water is expected to be minimally effective. The null hypothesis of this experiment was that there would be no significant difference between the ability of water, hand soap, dish soap, and vinegar to remove the petroleum coating.

Methods:

Twenty store-bought mini cucumbers were used, each cut lengthwise into four equal-sized slices, for a total of eighty cucumber slices. There were four trials, each completed by a different teammate. Each trial consisted of five cucumbers, which were all cut to produce four replicates for each of the five treatment groups. The skin of each cucumber slice was then coated with an unspecified measurement of white petroleum Vaseline until it appeared fully coated. To ensure that each slice was evenly and completely coated, the cucumber slices were viewed under black light using a LED 395 nm Vansky UV blacklight flashlight from Amazon. Under the blacklight, any sparse or uncovered areas appeared black while regions covered with Vaseline appeared bright blue. If any black was observed, more Vaseline was applied to that region and the skin was viewed under blacklight once again. This was repeated until all the slices uniformly appeared bright blue under the blacklight. Once fully coated in the Vaseline, four cucumber slices in each trial were assigned to five different groups: liquid Softsoap hand soap, liquid Dawn dish soap, white vinegar, tap water, and control. All cleaners were purchased from Walmart. The four trials in combination resulted in a total of 16 cucumber slices (n=16) for each treatment group. Next, medium-sized plastic cups were labelled with a specific treatment type (excluding the control group as they were not cleaned) and filled with 500 mL of lukewarm water as well as one tablespoon of the respective cleaner. The solutions were thoroughly mixed with a clean spoon and a cucumber slice was carefully submerged into each cup. The control group was not placed inside a cleaning solution and remained on paper towels with the skin side up instead. A timer was set for five minutes as soon as each slice was submerged in the cleaning solution. At the end of the 5 minutes, the slices were removed from the cups with clean gloves, avoiding the coated side, and placed skin side up onto paper towels labelled with each associated treatment. All the slices were then viewed under the blacklight flashlight in a dim room and the percent cover of Vaseline remaining on each cucumber slice was estimated and recorded. Due to the irregular skin of the cucumber, Vaseline was removed in thin unpredictable streaks, rather than large patches. As a result, using a grid to measure percent coverage would not have been a plausible choice and the percent coverage of each cucumber slice was instead estimated by eye.

The data obtained by each of the four groupmates was then compiled to determine if any cleaner was significantly more effective at removing Vaseline from the cucumber slices. To obtain the mean percent of Vaseline removal, the mean percent cover remaining after cleaning was subtracted from 100%. A one-way ANOVA ($\alpha = 0.05$ as it is standard) was run using GraphPad Prism 9 (version 9.1.0) to determine if any treatments— each with a sample size of 16 — significantly differed in the percent cover of Vaseline remaining. A Tukey Kramer test was then run using RStudio (version 1.3.1093) to determine which cleaners differed significantly in mean percent removal of Vaseline off the cucumber skin. A diagram of the experimental set-up is provided below (Fig. 2).

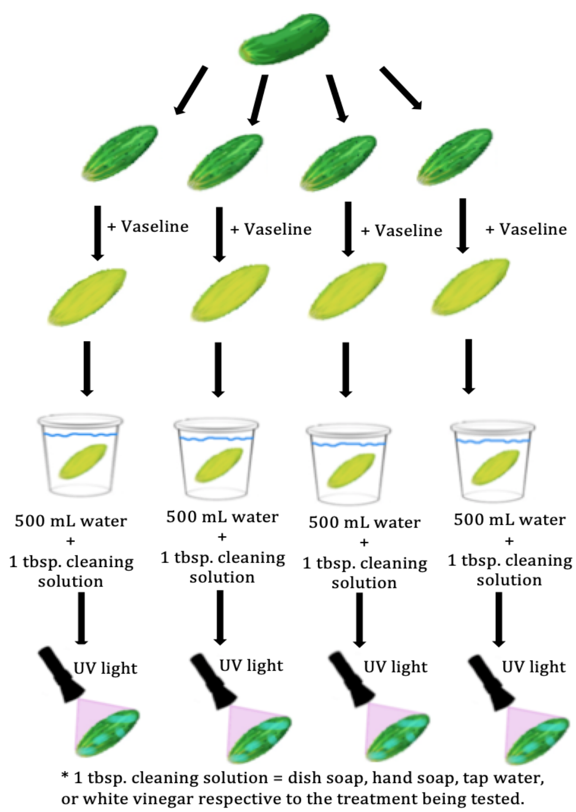


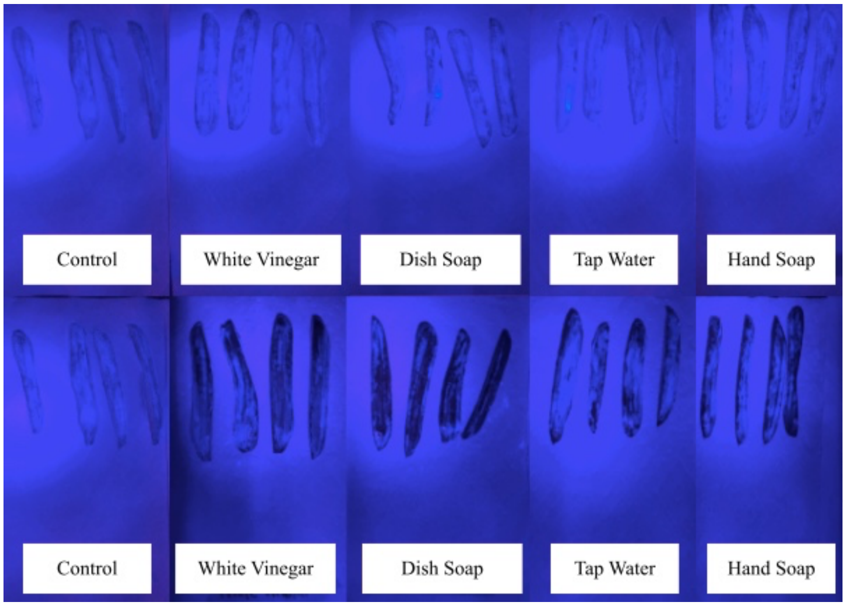
Figure 2. Diagram of the experimental workflow. Methods for each of the 20 mini cucumbers are in order from top to bottom. Slice the mini cucumber into 4 slices and cover each slice with Vaseline. Place the covered slices in cleaning solutions (1tbsp. cleaner mixed with 500mL water) for 5 minutes. Remove the slices from the cleaning solutions and view them under UV light to estimate percent cover of Vaseline remaining on each slice.

Results:

Following the methods described, the data on percent cover of Vaseline remaining after treatment with each cleaner was uploaded onto GraphPad Prism 9 (version 9.1.0) for analysis. A bar graph with standard error (Fig. 2) was created for the mean percent removal of Vaseline. Dish soap and white vinegar appeared to be the two treatment types that removed the highest percent of Vaseline from cucumber skin (36.6% and 32.5%, respectively). Excluding the control group, tap water resulted in the lowest percent Vaseline removal (7.7%). Hand soap appeared to be intermediate, removing on average 10.7% more Vaseline than tap water but still significantly less than dish soap and white vinegar.

A QQ plot was created using the original data, displaying that the data followed a normal distribution with no outliers. The one-way ANOVA presented a P-value of < 0.0001. The Tukey-Kramer test resulted in P-values below the significance level ($\alpha = 0.05$) for all pairs except for dish soap and vinegar ($P=0.725$) and tap water and control ($P=0.139$).

A photo of one trial of the experiment done by a team member is provided in (Fig. 3), displaying the cucumber skin under a blacklight before cleaning (top row) and after cleaning (bottom row). The Vaseline-covered regions in the photos shine bright blue under blacklight whereas the cleaned regions appear as dark black.



Discussion:

In this investigation, we experimentally tested various household cleaners to determine which would be the most effective at removing hydrophobic coatings on produce. It was found that dishwashing soap and white vinegar were the two treatments that resulted in the highest percent removal of Vaseline (36.6% and 32.5% respectively). Hand soap was intermediate (18.4%) and tap water was the least effective, removing only 7.7% of the coating. Statistical tests performed determined statistically significant results, indicating that the majority of cleaners had different efficacies in removing Vaseline. Specifically, the one-way ANOVA test determined a P-value of < 0.0001, thus providing a reason to reject the null hypothesis that all four cleaners — tap water, dish soap, hand soap, and white vinegar — are equally effective at removing Vaseline. Accordingly, we had support for the alternative hypothesis that the percent of Vaseline removed would vary based on the respective properties of each cleaning solution. The Tukey-Kramer test was utilized to determine which treatments differed and ultimately revealed that the effectiveness of all cleaning pairs, excluding two, were significantly different. The test determined the amount of Vaseline removed by tap water was not significantly different from the control, which retained 100% Vaseline, demonstrating that tap water is the least effective treatment as predicted. Though unexpected, the analysis also indicated no significant difference in the effectiveness of dish soap and white vinegar, leading to the conclusion that the two cleaners are equally effective at removing the hydrophobic coating.

Supporting our findings, Achim Losch stated that because water is unable to remove hydrophobic substances, paraffin wax — composed of saturated hydrocarbons — will not be displaced using water alone (1090; Speight). This explains why tap water was the least effective cleaner. Contrarily, dishwashing soap acts as an emulsifier, displacing the oil and grease in paraffin wax, trapping them in micelles and eventually washing them away (Losch 21). Similarly, data from a study conducted by Pandey et al. also revealed dishwashing soap's efficiency at deparaffinization, further justifying its success in removing Vaseline (11). Although hand soap also acts as an emulsifier, it is not an effective degreaser due to its milder properties. This substantiates why hand soap removed on average 18.2% less Vaseline than dishwashing soap (Fig. 3). Finally, despite lacking surfactant properties, white vinegar turned out to be significantly more effective than we initially predicted. This is because white vinegar is composed of acetic acid and previous research indicates that paraffin wax is highly soluble in acetic acid (Arshadi 1). Therefore, the acidic component of white vinegar facilitated the dissolution of the Vaseline, decreasing the percent cover on the cucumber skin. A research study conducted by Omotayo et al. also exemplified vinegar's effectiveness at removing paraffin wax (1). Therefore, based on the aforementioned, our results appear consistent with the findings of other researchers.

Despite experimental results suggesting a statistically significant difference between the four different cleaners, many potential confounding variables might have skewed our data. First, as each team member was responsible for gathering their own supplies and conducting the experiment, a perfectly uniform environment was not plausible. Despite the use of identical blacklight flashlights, estimating the percent cover of Vaseline was highly subjective and prone to error. Additionally, the cucumber grooves made it difficult to estimate the percent cover of Vaseline and ensure consistency throughout every trial. Therefore, the experimental results might have been skewed, depending on whether the particular student had a tendency to underestimate or overestimate the data. One tactic to mitigate subjective data is to take pictures and collaborate with team members to collectively determine the appropriate percent cover estimate of Vaseline. Variation in applied Vaseline thickness is another potential source of discrepancy. This is because thicker Vaseline coats were likely harder to remove and required more time in the cleaning solutions. To prevent this, rather than applying the Vaseline directly, we could melt it and submerge each cucumber slice a fixed number of times, averting the variation in Vaseline coating thickness. Finally, some Vaseline might have been transferred onto our gloves when transporting the cucumber slices, resulting in inaccurate data. One solution would be to use toothpicks to transport the cucumbers, minimizing contact with gloves. Further trials in highly controlled settings should be conducted to increase confidence in our results. As this investigation deemed white vinegar an effective treatment, future studies should investigate the impact of different acid concentrations and pH levels on the dissolution of hydrophobic coatings.

Conclusion:

This research experiment simulated the removal of edible hydrophobic coatings on fresh produce by measuring the percent cover of oil-based Vaseline removed from mini cucumber slices. After investigating which of the four treatment groups performed the best with regard to its Vaseline removal efficiency, it was determined that dishwashing soap and white vinegar were the two most effective cleaners to wash everyday fresh produce for safer ingestion. Although we initially predicted that dishwashing soap would be the most effective, the efficiency of white vinegar in removing the Vaseline coating was not expected. Therefore, the obtained results only partially aligned with our initial prediction. Data analysis using a one-way ANOVA test allowed us to reject the null hypothesis asserting that all four cleaners — tap water, dishwashing soap, hand soap, and white vinegar — are equally effective. Ultimately, our findings indicate that dishwashing soap and white vinegar effectively remove hydrophobic coatings on fresh produce, suggesting these agents should be utilized to prevent potential health risks associated with edible coating.

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References:

Abdelfattah, Ahmed, et al. "Effect of Washing, Waxing and Low-Temperature Storage on the Postharvest Microbiome of Apple." *Microorganisms*, vol. 8, no. 6, 2020, pp. 1-20. doi:10.3390/microorganisms8060944.

An, Kunho, et al. "Quantification of Morpholine in Peel and Pulp of Apples and Oranges by Gas Chromatography−Mass Spectrometry." *Foods*, vol. 9, no. 6, 2020, pp. 1-12. doi:10.3390/foods9060746.

Arshadi, Mohammad Reza. "Separation of n-Paraffin Hydrocarbons by the Urea Adduction Process." *Graduate School of the Oklahoma State University*, 1959, pp. 1-77.

Bekker, Madelyn, et al. "The Benefits of Fischer-Tropsch Waxes in Synthetic Petroleum Jelly." *International Journal of Cosmetic Science*, vol. 35, no. 1, 2012, pp. 99–104. doi:10.1111/ics.12011.

Earley, Brigitt. "Is It Bad to Wash Dishes with Hand Soap? Experts Weigh In." *Apartment Therapy*, 9 Feb. 2019, https://www.apartmenttherapy.com/can-you-use-hand-soap-to wash-dishes-266744. Accessed 4 April 2021.

Flanery, Taylor. "Uses for Vinegar: What Vinegar Can and Cannot Do Around Your Home." *Household Management 101*. https://www.household-management-101.com/uses-for vinegar.html. Accessed 4 April 2021.

Galus, Sabina, et al. “Novel Materials in the Preparation of Edible Films and Coatings—A Review.” *Coatings*, vol. 10, no. 7, 2020, p. 674., doi:10.3390/coatings10070674.

Kilonzo-Nthenge, Agnes, et al. “Efficacy of Home Washing Methods in Controlling Surface Microbial Contamination on Fresh Produce.” Journal of Food Protection, vol. 69, no. 2, 2006, pp. 330–334. doi:10.4315/0362-028x-69.2.330.

Kore, Vijay, et al. “Application of Edible Coatings on Fruits and Vegetables.” *Imperial Journal of Interdisciplinary Research,* vol 3, no. 1, 2016, pp 591-603.

Kumar, Rupak. “Health effect of morpholine based coating for fruits and vegetables.” *International Journal of Medical Research & Health Sciences*. vol 3, no. 9, 2016, pp 32-38.

Lee, Seul Ji, et al. “Distinguishing between Genotoxic and Non-Genotoxic Hepatocarcinogens by Gene Expression Profiling and Bioinformatic Pathway Analysis.” *Scientific Reports*, vol. 3, no. 1, 2013, pp. 1-9. doi:10.1038/srep02783.

Losch, Achim. “Removal of Lubricants: Industrial Cleaners.” *Lubricants and Lubrication*, 3rd ed., vol. 2, edited by Theo Mang and Wilfried Dresel, John Wiley & Sons, pp. 1089-1123.

Pandey, Pinki, et al. “A comparative study to evaluate liquid dish washing soap as an alternative to xylene and alcohol in deparaffinization and hematoxylin and eosin staining.” *Processes*, 2nd ed., Gulf Professional Publishing, 2019, https://doi.org/10.1016/C2015-0-06314-6. Accessed on Apr. 4 2021.

Speight, James G. "Hydrocarbons from crude oil." *Handbook of Industrial Hydrocarbon* to xylene and alcohol in deparaffinization and hematoxylin and eosin staining." *Journal of Laboratory Physicians*, vol. 6, no. 2, 2014, DOI: 10.4103/0974-2727.141504.

"Undeclared Allergens and Acceptability of Post Harvest Protective Coating Treatment."

Canadian Food Inspection Agency, Health Canada. 1 November 2005. inspection.canada.ca/food-safety-for-industry/archived-food-guidance/fresh-fruits-and vegetables/food-safety/protective-coating treatments/eng/ 1374164626560/1374164777040. Accessed on Apr. 4 2021.

Wasilewski, Tomasz, et al. "Improvement in the Safety of Use of Hand Dishwashing Liquids Through the Addition of Hydrophobic Plant Extracts." Journal of Surfactants and Detergents, vol. 19, no. 6, 2016, pp. 1315–1326. doi:10.1007/s11743-016-1868-x. Accessed on Apr. 4 2021.

Appendix:

Table 1. Raw data for percent cover of Vaseline on cucumbers after being submerged into different cleaning solutions (tap water, dish soap, hand soap, vinegar). N = 16 for each treatment.

Control	Tap Water	Dish Soap	Hand Soap	White Vinegar
100	95	60	85	80
100	90	75	90	75
100	90	60	100	75
100	100	80	80	80
100	95	80	85	75
100	90	45	85	90
100	97	60	90	80
100	95	60	90	85
100	90	75	80	70
100	85	55	70	50
100	95	65	70	60
100	90	50	80	50
100	85	70	70	60
100	95	65	80	50
100	90	50	80	60
100	95	65	70	40

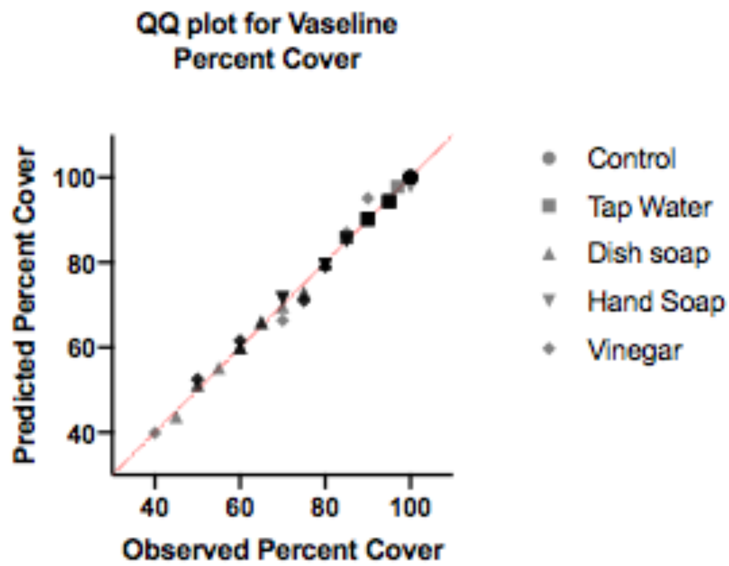

Figure 1. QQ Plot for cucumbers associated with different cleaners (control, tap water, dish soap, hand soap, white vinegar). n=16 for each treatment. Red line shows normal distribution.

Table 2. ANOVA summary for percent cover of Vaseline remaining on cucumber slices after being submerged in different cleaning solutions (control, tap water, dish soap, hand soap, white vinegar). n=16 for each treatment.

ANOVA summary	
F	46.04
P value	<0.0001
P value summary	****
Significant diff. among means (P < 0.05)?	Yes
R squared	0.7106

Sample Calculation for Mean Percent Vaseline Removal:

$$Mean\ Percent\ Vaseline\ Removed = 100\% - Mean\ Percent\ Cover\ of\ Vaseline\ Remaining\ After\ Being\ Washed$$

Example calculation for tap water:

$$Mean\ Percent\ Vaseline\ Removed = 100\% - 92.3\%$$

$$Mean\ Percent\ Vaseline\ Removed = 7.7\%$$

Therefore, tap water removed on average 7.7% of the Vaseline on the cucumbers.